Vol 2 | Issue 1 | Pages 35-47

Journal of Plant Biotechnology Research

Research Article

DOI: 10.36959/451/698

Morpho-Genetic Assessment of Greengram [*Vigna radiata* (L.) Wilczek] Landraces of Odisha, India for MYMV Resistance

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Abstract

Green gram (*Vigna radiata L*. Wilczek) is seriously affected by Yellow Mosaic Disease (YMD) in tropical and subtropical countries of the world. The present investigation was carried out to screen the 52 indigenous mungbean germplasms and four breeding genotypes for the identification of Mungbean Yellow Mosaic Virus (MYMV) resistant. Based on twenty-four morpho-agronomic traits, it was observed that IPM-02-3 is highly resistance and five landraces i.e. Nadika local, Makarjhola local, Nayagarh local A, Gania local mixed, Jagasinghpur local-1 are moderately resistance to MYMV. There was a great variation among the landraces with regard to disease incidence and seed weight. Based on MYMV linked SSR markers, out of 56 genotypes including 52 landraces, 05 landraces (Nadika local, Jasinghpur local-1, Gania local mixed, Dhenkhanal 1 and Makarjhola local) are moderately resistant and one breeding genotype (IPM-02-14) act as resistant to MYMV. This study will helpful for conservation and management of genetic resource management of mungbean useful for breeding program.

Keywords

Mungbean, Landraces, SSR markers, MYMV, Molecular analysis

Introduction

Mungbean (*Vigna radiata* L. Wilczek) is widely cultivated throughout the Asia. It is the third most important pulse crop in India and covers more than 14% area under pulse cultivation and productivity rate of 461 kg/ha (NFSM pulses 2018-19) [1]. Mungbean seeds are rich source of minerals like calcium, iron, magnesium, phosphorous, potassium, vitamin A and Vitamin C. Being a 24% of dietary protein with low flatulence, and they are inseparable ingredients in the diets of the Asian population. The worldwide yield performance of mungbean is very low and its production has not considerably increased yet. The low yield is due to the susceptibility to insects and diseases caused by fungus, virus, or bacteria. Mungbean yellow mosaic virus (MYMV) (Begomo virus) is a major exotic disease of mungbean causing leaf discolouration and yield losses [2]. Developing MYMV-resistant mungbean varieties through classical breeding methods remain unsuccessful due to the rapid evolution of new isolates of MYMV and also the complexity mechanism MYMV resistance. Hence, screening based on natural occurrence in the hot spot areas is one of the viable methods and coupled with precision breeding using molecular markers. Genetic diversity among the germplasms, mining the markers linked for resistant gene and the QTL maps through molecular markers, has increased the efficiency in the breeding programs conferring resistance for MYMV [3,4]. Mungbean is drastically affected by the mungbean yellow mosaic virus and powdery mildew [5-7]. Another major disease reducing the crop productivity is the Cercospora leaf spot (CLS). To identify the genotype having a high level of resistant using molecular techniques has been reported [8-10]. Molecular markers are now widely used to identify loci and genome regions in many important crops including legume crops [11,12]. The present study is to identify the mungbean landraces resistant to MYMV by using agro-morphological traits and MYMV linked SSR markers.

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Accepted: May 19, 2021

Published online: May 21, 2021

Citation: Kumari GP, Swain D, Pusti A, et al. (2020) Morpho-Genetic Assessment of Greengram [*Vigna radiata* (L.) Wilczek] Landraces of Odisha, India for MYMV Resistance. J Plant Biotechnol Res 2(1):35-47

Table 1: Indigenous landraces and check genotypes used for experiment.

Sl. No.	Name of Landraces (As per GP number)	Name of Landraces (As per name)	GPS data of collected area
1	CPR BAM GP 324	Daspalla Local Green	Latitude: 20°20'59.99"N Longitude: 84°50'59.99" E
2	CPR BAM GP 359	Saline 7	Latitude: 19°42'59.99"N Longitude: 85°18'60.00" E
3	KVK JAGATSINGHPUR 1	Jagatsinghpur Local 1	Latitude: 20°16'12.00"N Longitude: 86°10'12.00" E
4	CPR BAM GP 327	Gania Local mixed	Latitude: 20°07'48.00" N Longitude: 85°05'60.00"E
5	KVK DHENKHANAL 1	Dhenkhanal 1	Latitude: 20°40'12.00"N Longitude: 85°35'60.00"E
6	CPR BAM GP306	Bilipara Local	Latitude: 20°27'53.89"N Longitude: 85°52'45.37"E
7	CPR BAM GP 354	Saline 2	Latitude: 19°42'59.99"N Longitude: 85°18'60.00"E
8	KVK JHARSUGUDA 1	Jharsuguda 1	Latitude: 21°51'0.00"N Longitude: 84°01'48.00"E
9	CPR BAM GP 297	Paralakhemundi Local	Latitude: 18°46'41.8584''N Longitude: 84°5'37.1436''E.
10	CPR BAM GP 313	Odogaon Local Brown	Latitude: 20°0'17.4852"N, Longitude: 85°0'30.4668"E
11	CPR BAM GP 304	Kamakshya Local	Latitude: 20°40'12.00"N Longitude: 85°35'60.00"E
12	CPR BAM GP 333	Bhapur Local Brown	Latitude: 20°7'37.3008''N Longitude: 85°6'18.2052''E
13	CPR BAM GP 353	Saline 1	Latitude: 19°42'59.99"N Longitude: 85°18'60.00"E
14	KVK MAYURBHANJ 1	Mayurbhanj 1	Longitide: 21°30'0.00"N Latitude: 85°30'0.00"E
15	CPR BAM GP 308	Mahimunda Local	Latitude: 20°42'15.08"N Longitude: 83°29'25.04"E
16	CPR BAM GP 288	Nayagarh Local green	Latitude: 20°7'37.3008''N Longitude: 85°6'18.2052''E
17	CPR BAM GP 305	Nayagarh Local A	Latitude: 20°7'37.3008''N Longitude: 85°6'18.2052''E m
18	CPR BAM GP 345	Hinjili (Durabandha) Local	Latitude: 19°22'48.00"N Longitude: 85°04'12.00"E
19	KVK BARGARH 3	Bargarh 3	Latitude: 21°20'33.00"N Longitude: 83°37'26.04"E
20	KVK BOUDH 2	Boudh 2	Latitude: 20°50'24.00"N Longitude: 84°19'12.00"E
21	CPR BAM GP 274	Makarjhola Local	Latitude: 19°22'48.00"N Longitude: 85°04'12.00"E
22	KVK BARGARH 5	Bargarh local 5	Latitude: 21°20'33.00"N Longitude: 83°37'26.04"E
23	KVK BARGARH 1	Bargarh local 1	Latitude: 21°20'33.00"N Longitude: 83°37'26.04"E

24	KVK SUNDARGARH 2	Sundargarh	Latitude: 22°07'0.01"N Longitude: 84°01'59.99"E
25	KAMDEV	Check	Latitude: 19°22'48.00"N Longitude: 85°04'12.00"E
26	IPM 02-03	Check	Latitude: 19°22'48.00"N Longitude: 85°04'12.00"E
27	IPM 02-14	Check	Latitude: 19°22'48.00"N Longitude: 85°04'12.00"E
28	OBGG 52	Check	Latitude: 19°22'48.00"N Longitude: 85°04'12.00"E
29	CPR BAM GP 283	Banpur Local green	Latitude: 19°46'48.00"N Longitude: 85°10'48.00"E
30	CPR BAM GP 321	Nuagaon Local Brown	Latitude: 19°22'48.00"N Longitude: 85°04'12.00"E
31	KVK SUNDARGARH II-1	Sundargarh local -1	Latitude: 22°07'0.01"N Longitude: 84°01'59.99"E
32	KVK NAYAGARH 6	Nayagarh 6	Latitude: 20°7'37.3008''N Longitude: 85°6'18.2052''E m
33	CPR BAM GP 344	Digapahandi Local	Latitude: 19°22'21.90"N Longitude: 84°34'18.62"E
34	CPR BAM GP 273	Berhampur Local 2	Latitude: 19°19'12.00"N Longitude: 84°46'48.00"E
35	KVK BOLANGIRI 1	Bolangiri 1	Latitude: 20°42'15.08"N Longitude: 83°29'25.04"E
36	CPR BAM GP 290	Hinjili Local	Latitude: 19°22'48.00"N Longitude: 85°04'12.00"E
37	CPR BAM GP 258	Kantapada Local green	Latitude: 20°27'53.89"N Longitude: 85°52'45.37"E
38	KVK KENDRAPARA 1	Kendrapara 1	Latitude: 20°27'53.89"N Longitude: 85°52'45.37"E
39	CPR BAM GP 302	Kalahandi Local 1	Latitude: 20°6'11.8728''N Longitude: 83°9'53.6472''E
40	CPR BAM GP 280	Purusottampur Local	Latitude: 19°31'12.86"N Longitude: 84°53'6.50"E
41	KVK SAMBALPUR 2	Sambalpur 2	Latitude: 21°23'59.99"N Longitude: 83°52'59.99"E
42	CPR BAM GP 331	Bhapur Local mixed	Longitude: 20°7'37.3008''N Latitude: 85°6'18.2052''E.J
43	CPR BAM GP 293	Nadika Local	Latitude: 22°13'48.0000''N Longitude: 84°27'35.9964''E
44	CPR BAM GP 259	Kantapada Local brown	Latitude: 20°27'53.89"N Longitude: 85°52'45.37"E
45	CPR BAM GP 334	Bhapur Local Black	Latitude: 20°7'37.3008''N Longitude: 85°6'18.2052''E m
46	CPR BAM GP 267	Jharsuguda Local green small	Latitude: 21°51'0.00"N Longitude: 84°01'48.00"E
47	KVK BHANJANAGAR	Bhanjanagar	Latitude: 19°55'37.88"N Longitude: 84°34'55.24"E
48	CPR BAM GP 317	Aska Local Brown	Latitude: 19°55'37.88"N Longitude: 84°34'55.24"E
49	CPR BAM GP 346	Labanyagarh local	Latitude: 19°55'37.88"N Longitude: 84°34'55.24"E

50	CPR BAM GP 285	Charpalli Local	Latitude: 19°55'37.88"N Longitude: 84°34'55.24"E
51	KVK NAYAGARH 4	Nayagarh 4	Latitude: 20°7'37.3008''N Longitude: 85°6'18.2052''E
52	KVK BARGARH 4	Bargarh 4	Latitude: 21°20'33.00"N Longitude: 83°37'26.04"E
53	CPR BAM GP 266	Badamba Local	Latitude: 20°27'53.89"N Longitude: 85°52'45.37"E
54	CPR BAM GP 268	Jharsuguda Local brown	Latitude: 21°51'0.00"N Longitude: 84°01'48.00"E
55	CPR BAM GP 311	Odogaon Local Mixed	Latitude: 20°7'37.3008''N Longitude: 85°6'18.2052''E.
56	CPR BAM GP 357	Saline 5	Latitude: 19°42'59.99"N Longitude: 85°18'60.00"E

Figure 1: Mungbean landraces collected from different geographical regions the state of Odisha, India.

Materials and Methods

Collection of genetic materials

A set of 52 mungbeans (*Vigna radiata*) landraces and four breeding genotypes were collected from the Centre for Pulses Research, Odisha University of Agriculture & Technology, Berhampur (Table 1). All the landraces were derived from different agro-climatic regions of Odisha, India (Figure 1) for genetic assessment study. Augmented design was adapted for the field experiment. The seeds were grown in the six rows of with 30 × 10 cm spacing with three replications. The experiment conducted in two consecutive summer seasons in the year 2017 and 2018. The rows distance was 30 cm apart and plant to plant distances were kept 10 cm. Two rows of the susceptible check are raised all around the experimental plot to attract whitefly and enhance infection of MYMV under field conditions. Insecticides and fungicides have not applied during the entire crop growth period. The weeding operation and other recommended packages and practices have been adapted.

Collection of phenotypic traits

Distinctness, uniformity and stability (DUS) guidelines [13] were adopted to collect the phenotypical traits after 30-d of sowing. The occurrence of whitefly and development of yellow Mosaic Disease (YMD) were regularly monitored in the crop. The disease infection and severity of MYMV was recorded and each plant rated on a 1 to 9 scale as per the guidelines

Table 2: Scale 1 to 9 used for MYMV reaction.

Grade	Description	Reaction
1	No visible symptoms on leaves	Free
2	Small yellow specks with restricted spread covering 01-5% leaf area	Highly resistant (HR)
3	Mottling of leaves covering 6-10% leaf area	Resistant (R)
4	Yellow mottling covering 11-15 % leaf area	Moderately resistant (MR)
5	Yellow mottling and discolouration of 15-20% leaf area	Moderately resistant (MR)
6	Yellow colouration of 21-30% leaves and yellow pods	Susceptible (S)
7	Pronounced yellow mottling and discolouration of leaves and pods, reduction in leaf size and stunting of plants covering 30-50% of foliage	Susceptible (S)
8	Severe yellowing discolouration of leaves covering 50-75% of foliage, stunting of plants and reduction in pod size	Highly Susceptible (HS)
9	Severe yellowing of leaves covering above foliage, stunting of plants and no pod formation	High Susceptible (HS)

presented in Table 2 [14]. The disease scoring recorded in two growth phases (vegetative and reproductive). Disease index has been calculated as per the formula is given below:

PDI = Sum of the numerical values/Total number of leaves examined × maximum grade value × 100

MYMV reaction (%) = Number of plant infected/Total number of plants × 100

Isolation and purification of genomic DNA

Young immature leaves were collected from field-grown plants and kept in -80 °C deep freezer till the extraction. Extraction of DNA was carried out as per the method of Doyle and Doyle [15]. Genomic DNA was isolated by using CTAB extraction buffer. The DNA pellet was washed with 70% (v/v) ethanol and dried properly. DNA pellet was properly dissolved with 250 µl of Tris-EDTA buffer (pH 8.0). To remove the RNA from the extracted sample, 5 µl of RNase (10 mg/ml) (Sigma, USA) was added in the DNA sample and incubated in the water bath at 37 °C for 1h. The DNA was further purified by extracting twice with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1). For precipitation of DNA, the one-tenth volume of 3M sodium acetate and an equal volume of absolute chilled ethanol were added for precipitation. The purified DNA pellet was collected and dissolved in 200 µl TE buffer at room temperature. DNA concentration was determined through 0.8% (w/v) agarose gel-electrophoresis stained with ethidium bromide by comparing the standard DNA (λ DNA digested by Hind-III) (Bangalore Genei, India).

PCR analysis

A gradient set up was made with a different annealing temperature of the synthesis primers. The range of annealing temperature and primer concentration can also further modified to get scorable amplification. The amplification was performed in a programmable gradient thermal cycler (Bio-Rad, USA) with an initial denaturation at 94 °C for 3 min followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at a gradient temperature for 1 min, and extension at 72 °C for 2 min. The final extension was made for 10 min at 72 °C. The gel electrophoresis was run with the amplified products in a 1.5% (w/v) agarose gel electrophoresis. The size of amplification was determined with the DNA ruler.

Statistical analysis

To ascertain the utility of observations based on data obtained from phenotypical and agronomic traits as per the DUS descriptors was analyzed. The resultant matrix was subjected to generate a dendrogram using the software program NTSYS pc Ver 2.2. Exeter Software, New York to estimate the genetic dissimilarity between genotypes [16].

Results and Discussion

Fifty-two indigenous landraces and four breeding mungbean genotypes were evaluated under the natural condition in two conjugative years. The present results highlight the report of both morphological and agronomic traits of 56 genotypes including 52 indigenous landraces (Table 3). On the basis of the phenotypic characteristics such as plant height, growth habit, time of flowering, stem color, stem pubescence, leaf vein color, pod pubescence, pod position, pod length, seed luster and 100 seed weight and size of the seeds were greatly varied among the tested germplasms. The major phenotypic and agronomic traits such as seed color, seed lusture and seed weight and MYMV reaction were varied. It was observed that out of the tested germplasms screened, 34 are susceptible, 11 highly susceptible, 02 moderately susceptible, 02 resistant and 07 moderately resistant to MYMV (Table 4). The results implied that some of the indigenous landraces were higher yield (100g seed weight) and variation in degrees of MYMV reaction. These landraces include Sundargarh local-1 (4.3 g/100 seed weight), Nayagarh local green (3.7g), Nayagarh local A (3.1g), Bilipara local (3.4g), Banpur local green (3.1g), Saline 5 (3.0g.) and four breeding genotypes (Table 4). The lowest yield was observed in 'Jarsuguda local green' (2.3g/100 seed weight). On the basis of Disc Similarity Coefficient analysis, a cluster was made using 24 morpho-agronomic traits among 52 indigenous landraces and four breeding genotypes. It showed that there are two major clusters (I & II) among 56 tested germplasms (Figure 2). The first major cluster (I) having 52 genotypes with 44% similarity with cluster-II having four genotypes (V2, V9, V11, and

Table 3: DUS characterization of 52 indigenous landraces and four breeding genotypes of *Vigna radiata* (L.) Wilczek.

Sl. No	Characteristics	States	Note	Number of entries	State of Observation
1	Hypocotyl anthocyanin colouration	Absent	1	0	Cotyledons unfolded
		Present	9	56	
2	Time of flowering	Early (< 40-50 days)	3	41	50% plants with at least one open flower
		Medium (40-50 days)	5	12	
		Late (> 50 days)	7	3	
3	Growth habit	Erect	3	3	50% flowering
		Semi erect	5	10	
		Spreading	7	43	
4	Plant habit	Determinate	1	15	50% flowering
		Indeterminate	3	41	
5	Stem colour	Green	1	44	50% flowering
		Green with purple	2	6	
		purple	3	6	
6	Stem pubescence	Absent	1	10	50% flowering
		Present	9	46	
7	Leaflet lobes (Terminal)	Absent	1	0	50% flowering
		Present	9	56	
8	Leaf shape (Terminal)	Deltoid	1	0	50% flowering
		Ovate	2	56	
		Lanceolate	3	0	
		Cunate	4	0	
9	Leaf Colour	Green	1	53	50% flowering
		Dark green	2	3	
10	Leaf:Vein colour	Green	1	30	50% flowering
		Greenish purple	2	11	
		Purple	3	15	
11	Petiole colour	Green	1	6	50% flowering
		Green with splashes	2	32	
		Purple	3	18	
12	Leaf size (at 5th node from the base)	Small	3	12	50% flowering
		Medium	5	14	
		Large	7	30	
13	Flower colour of petal (std)	Yellow	3	40	50% flowering
		Light yellow	5	16	
14	Colour of premature pod	Green	1	38	Fully developed green pods
		Green with pigmented suture	2	18	
15	Pod pubescence	Absent	1	8	Fully developed green pods
		Present	9	48	
16	Pod position	Above canopy	1	22	Fully developed green pods
		Intermediate	2	15	
		Not visible	3	19	

17	Plant height	Short (< 50 cm)	3	12	Fully developed green pods
		Medium (50-70 cm)	5	16	
		Long (> 70 cm)	7	28	
18	Pod colour	Brown	1	51	Harvest maturity
		Black	2	05	
19	Pod curvature of mature pod	Straight	1	12	Harvest maturity
		Curved	3	44	
20	Pod length (mature pod)	Short (< 8 cm)	3	16	Harvest maturity
		Medium (8-10 cm)	5	32	
		Long (> 10 cm)	7	08	
21	Seed colour	Yellow	1	0	Mature seeds
		Green	2	22	
		Mottled	3	28	
		Black	4	06	
22	Seed lusture	Shiny	1	20	Mature seeds
		Dull	2	36	
23	Seed shape	Oval	1	56	Mature seeds
		Drum shaped	3	0	
24	100 seed weight (g): Size	Small (< 3 g)	3	40	Mature seeds
		Medium (3-5 g)	5	16	
		Large (> 5 g)	7	0	

Table 5: MYMV linked - SSR markers used for the validation of 52 indigenous landraces and 4 breeding genotypes of mung bean.

Sl. No.	Name of Primer	Sequence (5'-3')	Tm	Detection Band size (bp)
1	DMB SSR 125	F: AAAAGAGTGACAGAGGTGGAAA	59	220,240
		R: ACATGCACATTCTGAACCACAT		
2	DMB SSR 130	F: ATCCAAGAGCATTTGAACTTCC	64	190
		R: CAACTGCAAATGACGTGAAGAT		
3	DMB SSR 158	F: TGGAAAATTTGCAGCAGTTG	59	220
		R: ATTGATGGAGGGCGGAAGTA		
4	VR 095	F: GAAATGGGAGTTCAAAGAGGAA	58	120
		R: TGGAGAAGTCTGGAAGAGAACC		
5	VR 044	F: CCCATGAAGGTATGAGACAACA	60	150
		R: GACTGAGAAAGAGAGAGAAGCATTT		
6	VR 078	F: CATGTGGCAACGCAGAAG	60	130
		R: TCAACTTATTCCTCCTTTCTCTCAC		
7	DMB -SSR 080	F: CGAGGCAGAGAAACCTTAAGAA	58	130
		R: GCTCGATACTCTTGGGTTGAA		
8	YMV1	F:GAGAGAGAGAGAGAGAACTTTG	54	100,200
		R;GAGAGAGAGAGAGAGACAGGA		
9	DMB SSR 160	F: GGTGGATCAAATCCATTTTAGG	57	220
		R: ACAGATCACATAGCAACCAAACA		
10	DMB- SSR 220	F: AAAGAGCCCAGATTTGAAGCTA	56	180
		R: GGGAATCAATACATGAAACCAAC		

11	VR 0148	F: CCGTTGTTGTTGCTGTTGTG R: GAGCTTGCTAACCCTCTCCAAT	58	180
12	DMB- SSR 165	F: TGGGACTAAACCACACTTTC R: GAACTATGAAGGTTTCACAGAAA	61	220
13	VR 0226	F: GACTAGGCGCTGGGAAAA R: GCTTCTCTTTCTTGCATTCATC	60	180
14	VR 0238	F: ATTCTCTGCCTGCCATTTT R: ACGATTGTGTTTGTTGATGC	58	190
15	VR 040	F: TGACAACATGGGAAGAAGAAGA R: ACACCAACACAAAAGCAAACAC	60	280
16	DMB SSR 151	F: AATGAAGGCTTGTCAAATCCA R: TTATTTCACCTTGGCTGGATCA	58	220
17	VR 0216	F:TTCCCTGTGTCCTTATATGTCC R:GAGGATAGTGAATTTTGAAGGC	60	200
18	VR 169	F:GGAAGATAGCGGAGATGAAG R:CACCATACACCATAACATTCCTG	60	190

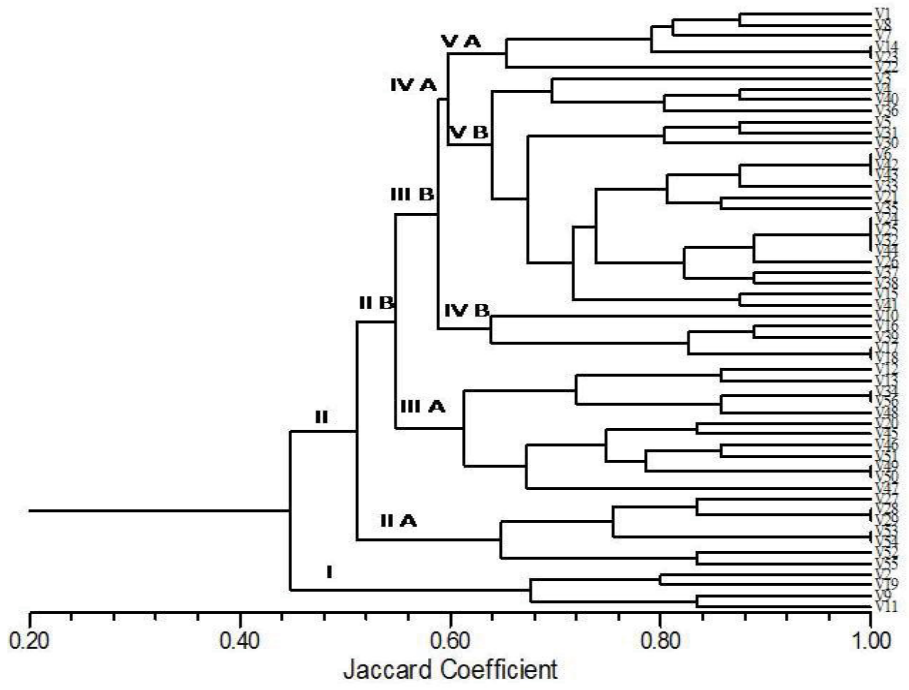

Figure 2: Cluster analysis of 52 indigenous landraces and four breeding genotypes of mungbean (*Vigna radiata*) based on phenotypic and agronomic traits.

V19). The second major cluster (II) further divided into two sub-major clusters i.e. II A & II B. Sub major cluster-IIA having only 07 genotypes and the second sub major cluster (II B) with45 genotypes with 50% similarity. Further, cluster-IIB divided into two minor clusters i.e, III A and III B. Cluster III A having 12 genotypes with 56% similarity with cluster III B (33 genotypes). Minor cluster-III B further divided into two sub minor clusters i.e. IV A and IV B. Cluster IVA consisted of five genotypes and cluster IVB having 28 genotypes with 58% similarity. Cluster IV B further divided into two clusters i.e. VA and VB. Cluster VA having 22 genotypes and VB with 10 genotypes having 64% similarity. The highest similarity (100%) was observed between V53 & V54, V28 & V29, V24 & V25, V42, V43 & V6 and V14 & V21 (Figure 2). The phenotypic and agronomic variation among the greengram genotypes has been reported [17,18].

Further, these landraces were assessed by using MYMV disease incidence on the basis of the rating scale. All genotypes were predicted from disease reaction with respect to R loci. The experiment was conducted both in Kharif and

Table 4: Phenotypical and agronomic traits of indigenous *Vigna radiata* (L.) Wilczek landraces and breeding genotypes.

Sl. No.	Indigenous landrace/check genotypes	Colour of mature pod	Seed colour	Seed lusture	100 seed weight (g.):size	Grade	MYMV reaction
1	Daspalla Local Green	Brown	Green	Dull	2.5	7	S
2	Saline 7	Brown	Green	Dull	2.6	9	HS
3	Jagatsinghpur Local 1	Black	Mottled	Dull	2.9	5	MR
4	Gania Local mixed	Brown	Mottled	Dull	2.4	5	MR
5	Dhenkhanal 1	Brown	Mottled	Dull	2.8	5	MR
6	Bilipara Local	Brown	Black	Shiny	3.4	6	S
7	Saline 2	Brown	Green	Dull	2.8	7	S
8	Jharsuguda 1	Brown	Mottled	Shiny	3.3	8	HS
9	Paralakhemundi Local	Brown	Mottled	Dull	3.1	7	S
10	Odogaon Local Brown	Brown	Mottled	Shiny	2.6	9	HS
11	Kamakshya Local	Brown	Green	Dull	2.6	6	S
12	Bhapur Local Brown	Brown	Mottled	Dull	2.6	7	S
13	Saline 1	Brown	Mottled	Dull	2.9	8	HS
14	Mayurbhanj 1	Brown	Mottled	Shiny	2.4	7	S
15	Mahimunda Local	Brown	Green	Dull	2.4	9	HS
16	Nayagarh Local green	Brown	Mottled	Dull	3.7	7	S
17	Nayagarh Local A	Brown	Green	Dull	3.1	4	MR
18	Hinjili (Durabandha) Local	Brown	Mottled	Dull	2.8	7	S
19	Bargarh 3	Black	Mottled	Dull	2.7	7	S
20	Boudh 2	Brown	Green	Dull	3.0	5	S
21	Makarjhola Local	Brown	Green	Shiny	2.4	4	MR
22	Bargarh local 5	Brown	Green	Shiny	2.4	8	S
23	Bargarh local 1	Black	Mottled	Dull	2.5	8	S
24	Sundargarh	Black	Mottled	Dull	3.0	7	S
25	KAMDEV	Brown	Green	Shiny	3.9	7	S
26	IPM 02-03	Brown	Green	Shiny	3.7	4	MR
27	IPM 02-14	Brown	Green	Shiny	3.3	5	R
28	OBGG 52	Brown	Green	Shiny	3.3	3	R
29	Banpur Local green	Brown	Mottled	Dull	3.1	6	S
30	Nuagaon Local Brown	Brown	Mottled	Dull	2.8	7	S
31	Sundargarh Iocal-1	Black	Green	Shiny	4.3	8	HS
32	Nayagarh 6	Brown	Mottled	Dull	3.5	6	S
33	Digapahandi Local	Brown	Mottled	Dull	2.6	7	S
34	Berhampur Local 2	Brown	Mottled	Dull	2.9	8	HS
35	Bolangiri 1	Brown	Mottled	Dull	2.5	6	S
36	Hinjili Local	Brown	Mottled	Dull	2.4	7	S
37	Kantapada Local green	Brown	Green	Shiny	2.8	6	S
38	Kendrapara 1	Brown	Green	Shiny	2.7	7	S
39	Kalahandi Local 1	Brown	Green	Shiny	2.6	7	S
40	Purusottampur Local	Brown	Green	Shiny	2.8	8	HS
41	Sambalpur 2	Brown	Green	Dull	2.8	6	S
42	Bhapur Local	Brown	Green	Dull	2.7	7	S
43	Nadika Local	Brown	Green	Dull	3.0	5	MR

44	Kantapada Local brown	Brown	Mottled	Dull	2.8	9	HS
45	Bhapur Local Black	Brown	Green	Dull	2.9	8	HS
46	Jharsuguda Local green small	Brown	Mottled	Dull	2.3	7	S
47	Bhanjanagar	Brown	Mottled	Dull	2.8	6	S
48	Aska Local Brown	Brown	Mottled	Dull	2.7	7	S
49	Labanyagarh local	Brown	Mottled	Dull	2.4	6	S
50	Charpalli Local	Brown	Black	Shiny	2.7	8	HS
51	Nayagarh 4	Brown	Black	Shiny	2.6	6	MS
52	Bargarh 4	Brown	Mottled	Dull	2.4	5	S
53	Badamba Local	Brown	Green	Dull	2.4	6	S
54	Jharsuguda Local brown	Brown	Mottled	Shiny	2.7	6	S
55	Odogaon Local Mixed	Brown	Mottled	Dull	2.9	7	S
56	Saline 5	Brown	Green	Shiny	3.0	6	MS

R: Resistant; HR: Highly Resistant; MR: Moderately Resistant; MS: Moderately Susceptible; HS: Highly Susceptible; S: Susceptible; MYMV: Yellow mosaic Virus.

Rabi seasons for YMD incidence during the vegetative and reproductive phase. Gupta, *et al.* [19] reported that the appearance of MYMV incidence in mungbean under natural condition is dependent on the virus vector population and climatic conditions including temperature. The MYMV disease scoring was recorded as per the data 1 to 9 arbitrary scale. It was observed that MYMV infection was maximum visibility in the reproductive stage as compared to the vegetative stage. On the basis of disease scoring, the 52 indigenous landraces and four breeding genotypes were classified into five categories i.e. highly resistance (IPM-02-3), resistance (IPM-02-14 & OBGG-52), moderately resistance (Nadika local, Makarjhola local, Nayagarh local A, Gania local mixed, Jagasinghpur local 1), thirty-five were susceptible, two moderately susceptible (Nayagarh 4 , saline 5) and highly susceptible (Charpalli local, Bhapur local Black, Kantapada local brown, Purusottampur local, Berhampur local 2, Sundargarh local 1, Mahimunda local, Saline 1, Odogaon local Brown, Jharsuguda 1, Saline 7). There was positive correlation of the whitefly population in 20-30 days of crop growth and occurrence of disease incidence at 45-days-old crop with maximum temperatures [20]. Majority of indigenous landraces (about 62.5%) were included under susceptible, 3.5% moderately susceptible and 19.7% highly susceptible group.

Validation of germplasms through MYMV linked SSR markers

Validation of MYMV linked marker with the desired trait is an essential requirement for MAS in an advance breeding program [21,22]. They reported that the validation of the marker would help the identification of the resistant genotypes in the mapping population for breeding purposes. Various researchers have undergone research to know the molecular mechanism linked to MYMV for identification of germplasms and the movement of pathogens into the cell machinery for their survival. However, very scanty publications on molecular characterization of MYMV in mungbean genotypes [23-27]. The information related to genetic tools to clone and characterize R genes was not fully standardized in legume crops. About twenty selected markers have been used to identify the mungbean resistant to MYMV [19,28]. Out of 20 SSR markers, 16 markers amplified in the range of 120 to 280 bp which able to distinguish between resistant and susceptible genotypes (Table 5). Primer DMB-SSR-158 produced one amplicon at 220 bp in most of the landraces including breeding genotypes. Based on the amplification, it was observed the landrace i.e. Nadica local was moderately resistant to MYMV. Primer DMB-SSR-130 produced one amplicon at 190 bp in most of the selected landraces. Based on the amplification, it was observed that four landraces (Jasinghpur local-1, Gania local mixed, Dhenkhanal 1 Makarjhola local) were moderately resistant and check genotype IPM-02-14 as resistant to MYMV (Figure 3A). The primer DMB-SSR-125 generated two amplicons at 220 bp and 240 bp. However, 240 bp amplicon indicates in 12 landraces. Primer VR-044 and VR-078 showed one amplicon each at 150 bp and 130 bp respectively in all the landraces and breeding genotypes. Kang, *et al*. [29] reported that about 80% of viral resistance is monogenically controlled by the host's resistant factors. In most of the cases, the R genes derived from monogenic dominant resistance plants successfully linked to molecular markers from consensus motifs of other resistance (R) gene or R gene homologous sequences as reported earlier [21,30,31]. The primers like DMB-SSR-130, DMB-SSR-158, DMB-SSR-125, VR-044, and VR-078 were completely linked to MYMV resistance and shows amplicon at 190 bp, 220 bp, 240 bp, 150 bp, and 130 bp respectively (Figure 3B).

Conclusions

It is concluded that the screening of indigenous landraces including breeding genotypes had significant differences with regard to yield attributing traits and phenotypic characteristics. Based on the morpho-agronomic screening of 56 genotypes including 52 landraces, IPM-02-14, OBGG-52 was resistant, IPM-02-3 highly resistance, and five moderately resistance (Nadika local, Makarjhola local, Nayagarh local A, Gania local mixed, Jagasinghpur local 1) to MYMV. On the basis of MYMV linked SSR markers, five mungbean landraces (Nadica

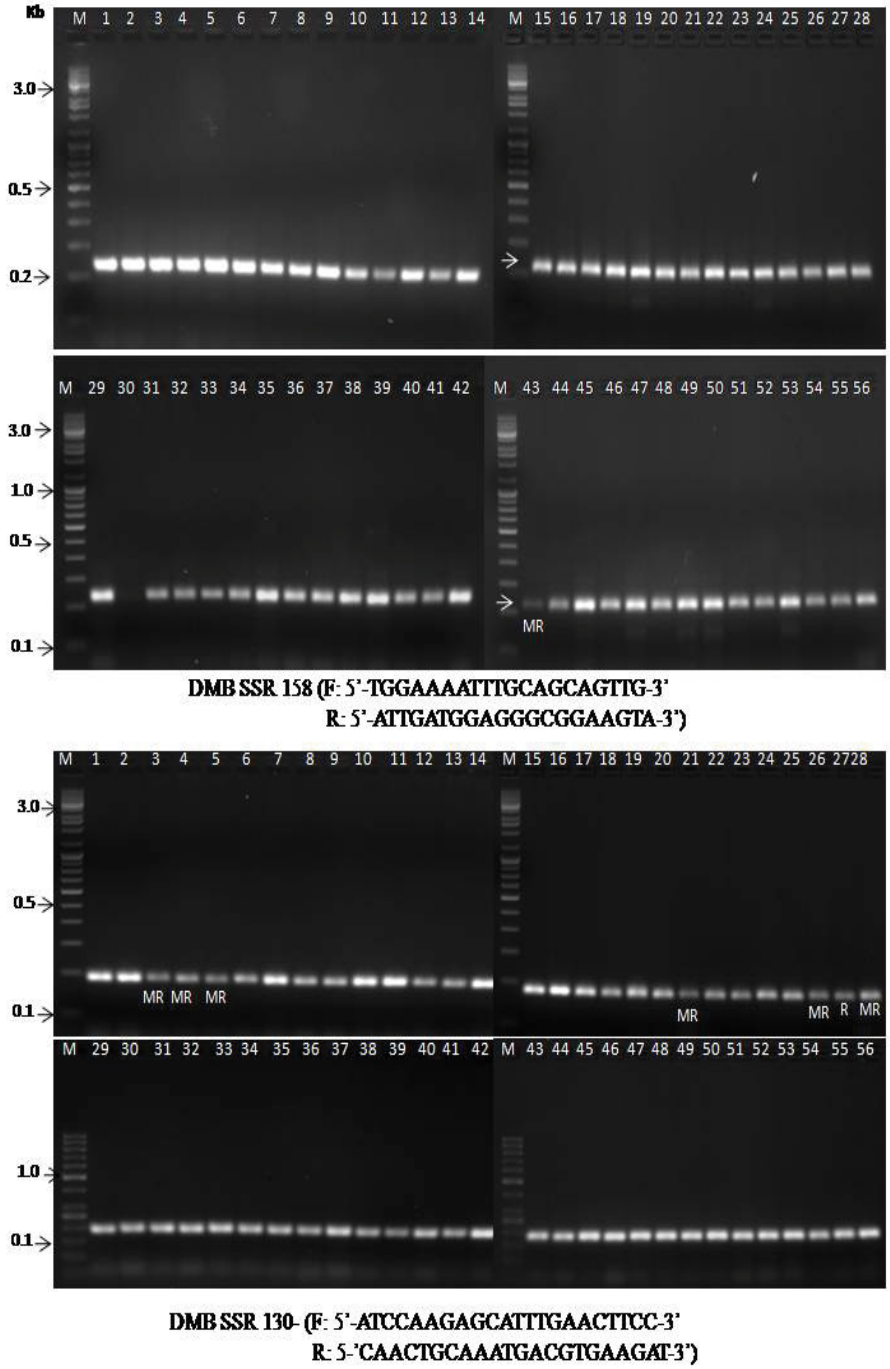

Figure 3A: DNA amplification of 52 landraces and four breeding genotypes of *Vigna radiata* using MYMV linked- SSR markers (DMB SSR-158 and DMB SSR-130).

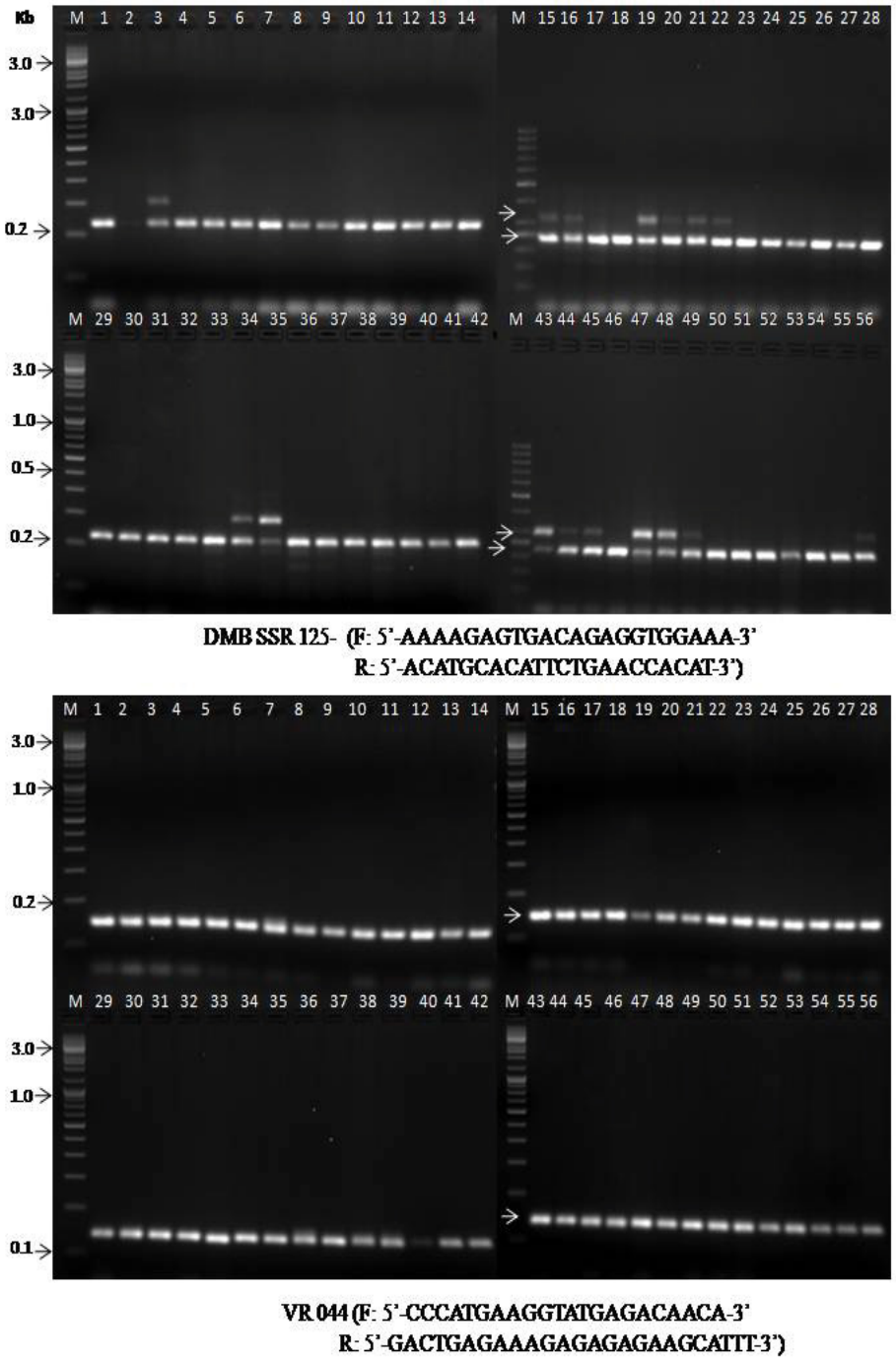

Figure 3B: DNA amplification of 52 landraces and four breeding genotypes of *Vigna radiata* using MYMV linked- SSR markers (DMB SSR-125 and VR-044).

local, Jasinghpur local 1, Gania local mixed, Dhenkhanal 1 and Makarjhola local) were moderately resistant and breeding genotype IPM-02-14 as resistant to MYMV. The present investigation revealed that both morpho-agronomic screening and SSR based linked markers are efficient for the identification of MYMV-resistant genotype which will helpful for an advanced breeding program in mungbean. Identified landraces shall be considered further as a resistant donor for MAS.

Acknowledgements

The authors wish to acknowledge to Department of Biotechnology, Ministry of Science and Technology, Govt. of India, New Delhi for PG-HRD teaching program and Department of Science & Technology for proving the FIST grant for development of infrastructure.

Conflict of Interest

The authors declare that they have no conflict of interest.

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DOI: 10.36959/451/698