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Genetic Assessment of Indigenous Landraces of *Vigna Mungo* L. and its Evaluation for YMV Resistance

Laxmipreeya Behera; Kailash C Samal; Pavithra BS; Dhaneswar Swain; Arjun M Prusti; Gyana R Rout*

Department of Agriculture, Biotechnology, College of Agriculture, Odisha University of Agriculture & Technology, Bhubaneswar-751003, Odisha, India

***Corresponding Author(s): Gyana R Rout**

Department of Agriculture, Biotechnology, College of Agriculture, Odisha University of Agriculture & Technology, Bhubaneswar-751003, Odisha, India

Email: grrout@rediffmail.com

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Keywords: Vigna mungo landraces; SCAR; SSR; Genetic diversity; MYMV; Marker validation.

Abstract

Blackgram (*V.mungo* L. Hepper) is a major pulse crop and widely cultivated in India and other Asian countries. The yield of black gram is hampered by YMD (Yellow Mosaic Disease), an important destructive disease in pulse crops. Indigenous landraces are highly resistant to various stresses which needed for conservation. The aim of the investigation is to genetic diversity assessment of 27 indigenous landraces of blackgram (*Vigna mungo*) and identifying for MYMV resistance through gene-specific simple sequence repeats and SCAR markers. Twenty-seven landraces and four check genotypes have been evaluated both phenotypic, agronomic traits and molecular analysis. On the basis of morphological and agronomic traits, the 27 landraces and four check genotypes grouped into 4 clusters. Maximum genotypes/landraces (19) present into cluster-II and least in a cluster –IV (01) on the basis of Disc Coefficient analysis. Out of 20 SSR markers, 10 SSR markers and One Scar marker MYMV-583 show linkage to MYMV resistance. On the basis of phenotypic, agronomic traits and DNA based PCR analysis, four genotypes including landraces were resistant to MYMV, nine genotypes moderately resistant, six genotypes moderately susceptible and eleven genotypes high susceptible to MYMV. This study will help for better management of conservation of genetic resources and also for an advance breeding program in black gram.

Introduction

Pulses are the major source of protein. Pulses are also declared as poor man rich protein. India is the largest consumer of pulses in the world. As per statistics, the production of Urdbean (*Vigna mungo*) was the third most important pulse crop followed by green gram and Tur during the year 2018-19. In India and other Asian countries, Urdbean is widely cultivated during kharif, rabi and summer seasons. Urdbean is also very popular because of its suitability in different crop rotation practices and well suitable for cultivation in dry and irrigated conditions. The economic product of it provides significant inexpensive vegetable protein and iron and is an excellent source of essential amino acids to meet out the requirements in daily diets. The crop yield is less because of the non-availability of high yielding and stable varieties.

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The production of grain yield has been hampered by different means such as the selection of genotype and environmental interactions, multiple stresses, lack of screening methods for phenotyping of target traits and low gene pool and genetic diversity. Apart from these constraints, yellow mosaic disease (YMD) is the most prevalent disease contributing reduction of yield [1]. Many efforts have been made to enhance the genetic potential of genotypes through conventional breeding methods to enhance yield. This YMD is caused by a yellow mosaic virus and most prevalent in Asia particularly in Northern, Central and Eastern parts of India [2]. In most of the leguminous crops affected by the YMV which is caused by whitefly (*Bemisia tabaci*) transmitted through gemini virus. Evaluation and exploitation of farmers' varieties for yield and yield attributing traits are also of utmost importance for the development of improved varieties. To avoid the inaccuracies in the field evaluation, selection through molecular markers linked resistance genes is an effective approach for marker-assisted selection (MAS) [3]. Marker-Assisted Selection (MAS) breeding program was not sufficient for agronomically important traits in black gram because the markers were only indirectly associated with targeted traits. It is possible to develop linked markers for targeted agronomic traits including Plant Disease Resistance (R) genes through MAS. In last few decades, the tightly linked markers for Virus Resistance (R) genes have been reported in various plant species [4,5]. Out of different DNA molecular markers, Simple Sequence Repeats (SSRs) have been preferred due to their multi-allelic nature, reproducibility, relative abundance, co-dominant inheritance, and good genome coverage. There are scanty reports that the researchers have been used tightly linked based marker for validation of genotypes for resistance to diseases in different genetic backgrounds [6-9]. Some of the identified markers have also been used in specific mapping populations and therefore, it is necessary to validate these markers in different black gram landraces before adapting MAS of MYMV reaction in black gram. The aim of the study is to analyze the genetic diversity of 27 indigenous landraces and comparing with four check genotypes and also validating these landraces through SSR and SCAR markers for MYMV reaction and breeding program.

Materials and methods

Collection of landraces/genotypes

A set of twenty-seven landraces were collected from different agro-climatic regions of Odisha, India for genetic diversity study and comparing with four check genotypes. Further, these landraces and check genotypes were taken for validation through molecular makers. The seeds were sown in the field with a complete randomized block design and two replications. The experiment conducted in two consecutive summer seasons in the year 2017 and 2018. Each plot composed of 4 m long in two rows. 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. Weeding operations and other recommended packages and practices except for use of insecticides and fungicides during the entire crop growth period.

Morphological character as per the DUS descriptors

Phenotypic characteristics were taken as per the DUS guidelines [10]. Thirty days after sowing, whitefly started landing on the plants, the crop has regularly monitored the occurrence of whitefly and development of Yellow Mosaic Disease (YMD). Data on the number of infected plants per experiment were recorded. The disease infection and severity of MYMV was recorded in the next 6 weeks and each plant rated on 1-9 scale as per the guidelines presented in Table 4 [11]. The disease scoring recorded in two growth stages (vegetative and reproductive). Disease index has been calculated as per the formula given below

$$PDI = \frac{\text{Sum of numerical values}}{\text{Total number of leaves examined X maximum grade value}} X100$$

$$MYMV\ reaction\ (\%) = \frac{Number\ of\ plants\ infected}{Total\ number\ of\ plants} X100$$

Isolation and purification of genomic DNA.

Young immature leaves were collected from field-grown plants and kept in -80^{0}C deep freezer till the extraction. Extract of DNA as per the method of Doyle and Doyle [12] with minor changes. The frozen leaf material (about 2 gm) crushed with liquid nitrogen with the pestle to a fine powder and was then transferred to a 50 ml falcon tube containing 15 ml of CTAB extraction buffer (2% CTAB,1.4M NaCl,100 mM Tris HCl (pH 8.0), 20 mM EDTA, 2% PVP, 2% mercaptoethanol). The falcon tubes were incubated at 65^{0}C in a water bath for 3 hours with intermittent shaking. The centrifuge tube was brought to room temperature and added an equal volume of chloroform: Isoamyl alcohol (24:1). Further, the contents were mixed by inverting the tubes gently and centrifuged at 10,000 rpm for 20 min at 4^{0}C. Transferred the supernatant to a fresh tube and added a one-tenth volume of 3M sodium acetate and equal volume of absolute chilled propanol and kept overnight at 4^{0}C to precipitate the DNA. Further, the DNA was precipitated through a centrifuge at 10,000 rpm for 5 min. The DNA pellet washed with 70% (v/v) ethanol. The DNA pellet was dried and dissolved in 200 µl of Tris-EDTA buffer (pH 8.0) for purification. To remove the RNA from the sample, 5 µl of RNase (10 mg /ml) (Sigma, USA) was added in the DNA sample and incubated at 37^{0}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, one-tenth volume of 3 M sodium acetate and an equal volume of absolute chilled ethanol were added and centrifuge at 10000 rpm for 15 min. 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) gel-electrophoresis stained with ethidium bromide by comparing the standard DNA (λ DNA digested by Hind-III, Bangalore Genei, India).

PCR for gene specific primer

SSR analysis

The reaction mixture composition and condition of the thermocycler need proper PCR amplification. Before starting to PCR amplification, a gradient set up was made with a different annealing temperature of the primers ± 2°C. This range can then be further modified to polish up the assay. This step is combined with different primer concentrations and combinations to standardize both annealing temperature and primer concentration. The amplification was performed in programmable gradient thermal cycler (Bio-Rad, USA) with an initial denaturation at 94^{0}C for 3 min followed by 35 cycles of denaturation at 94^{0}C for 1 min, annealing at gradient temperature for 1 min and extension at 72^{0}C for 2 min. The final extension was made for 7 min at 72^{0}C. The gel electrophoresis was made with the amplified products

in a 1.5% (w/v) agarose gel. The size of amplification by comparing with the molecular marker (Bangalore Genei, India).

SCAR analysis

For SCAR analysis, the reaction mixture contained 25 ng template DNA, 2 mM $MgCl_2$, 200 mM each dNTPs, 10 pmol primer, 10 mM Taq polymerase buffer and 1 unit of Taq DNA polymerase (Bangalore Genei, India) and the volume makes up to 25 µl by using nucleic acid-free water. PCR was made in a thermocycler (Bio-Rad, USA) under the program with a first denaturation at 94^0C for 3 min followed by 35 cycles of denaturation at 94^0C for 1 min, annealing at a gradient temperature for 1 min and extension at 72^0C for 2 min. The last extension was made for 7 min at 72^0C. Five SCAR primers were selected based on the clarity of DNA amplicons and the scalability of banding patterns. Electrophoresis was made with 2% (w/v) agarose at 4 V/cm. The identified band Sizes were made on comparing with a medium-range DNA ruler (Bangalore Genei, India) and visualized through GEL-DOC systems (Bio-Rad, USA).

Statistical analysis

To ascertain the utility of observations based on data obtained from morphological and agronomic traits as per the DUS descriptors was analyzed. The resultant matrix was subjected to generate a dendrogram using software program NTSYS pc Ver 2.2. Exeter Software, New York to estimate the genetic dissimilarity between genotypes [13].

Results and discussion

The generation of breeding material is of utmost importance for crop improvement programs. The indigenous landraces of Vigna mungo are the main sources for promoting the resistant genes for crop breeding network. Genetic diversity study of different gene pools can increase productivity and resistance to different stresses. Phenotypic and agronomic traits of indigenous landraces along with four check genotypes were evaluated under field conditions and presented in Table 1. These characters have been made through DUS (Distinctness, Uniformity and Stability) guidelines [10]. On the basis of phenotypic characteristics, seven landraces showing variability on the basis of the absence of anthocyanin content in hypocotyls, leaf shape, long plant height, brown and mottled seed color, seed shape and large seed size. The highest yield (1282 kg/ha) was recorded in 'Kantapada Local-1' whereas the lowest yield was recorded in 'Bakuribiri Jajapur Local' (948 kg/ha) (Table 2). The cluster analysis was made on the basis of phenotypic and agronomic traits and it was noted that the twenty-seven landraces and four check genotypes are making four clusters at 10.42 dis-similarity coefficient (Figure 1). The cluster I having five genotypes i.e. three landraces and two check genotypes (OBG-31 & PU-31). These two check genotypes were moderate to highly resistant to MYMV. The cluster-II having twenty-one genotypes including nineteen indigenous landraces and two check genotypes. These two check genotypes are moderately resistant (Ujala) and moderately susceptible (Prasad) to MYMV. Cluster-III signifies four landraces that are moderate to highly susceptible to MYMV. Cluster- IV having only one landrace (Kanas (Student) local was fully susceptible to MYMV (Table 3). The maximum dissimilarity (3.55) was observed between two landraces i.e. 'Semiliguda Local' and 'Sambalpur Local'. The phenotypic and agronomic variation among the black gram and green gram genotypes have been reported [14,15]. 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 scoring was recorded both the season periodically using a disease rating scale (0 to 9) (Table 4). With respect to YMD infection in two seasons, the landraces were graded according to the 0-9 arbitrary scale. It was also indicated that MYMV infection was more prominent in the reproductive stage as compared to the vegetative stage. The experiment revealed that all the twenty-seven landraces and four check genotypes were classified into five categories i.e. one genotype (PU-31) highly resistance, four resistance (Monika, VI X UR, WBG-26 and WBG-108), nine moderately resistance (OBG-31, Ujala, Sambalpur local, Puri-local, Jagatsinghpur local, Mohana local (black), Bhawanipatna local, Jashipur local, Similiguda local), six moderately susceptible (Prasad, Kantapada local, Mahuri, Dharakote local, Kothagarha local, Nabina) and rest eleven were highly susceptible (Table 4). Murugesan et al. [16] reported a simple correlation and regression analysis of whitefly population and yellow mosaic disease with weather factors. They also noted that a positive correlation of the whitefly population in 20 to 30 days of crop and disease incidence at 45 days old crop with maximum temperatures. They also noted that the maximum temperature alone was the important variable in predicting whitefly population on the basis of the partial regression coefficient analysis. The present study revealed that the majority of landraces (about 35.48 %) was included under highly susceptible and moderately susceptible (19.3%) group. The medium resistance was 29.03%. The percentage of resistant and highly resistant landraces were 12.90% and 3.2% respectively.

Validation of blackgram landraces through SSR and SCAR markers

Various efforts have been made since the last decade to identify the germplasms against YMV and to know the mechanism of the movement of pathogens into the cell machinery for their survival. However, very scanty reports are available [17-22]. The information related to genetic tools to clone and characterize R genes were not fully standardized in legume crops particularly black gram. The present investigation has to assess the genetic diversity of the 27 indigenous landraces collected from different agro-climatic regions of Odisha, India and to validate through a linked marker which is a prerequisite for MAS for the genetic improvement program. Marker validation is a process to study the behavior of linked markers and the associated polymorphism in different genetic backgrounds. Seven markers have been identified as linked to YMV resistance in black gram by other researchers [23-26]. In the present study, 20 linked markers were tested for validation resistance to MYMV. Among 20 markers, 10 SSR markers amplified in the range of 136 to 164 bp and five SCAR markers amplified at 583bp region which helps to distinguish between resistant and susceptible genotypes (Table 5). Primer MYMV-583 produced one amplicon at 583 bp in most of the tested landraces. From the gel photographs, it was observed that the landraces i.e. Aska Local, Badamba Local-2, Similiguda local, Sambalpur Local, Puri local-2, Nayagarh local-1, Bolangiri local, Kantapada Local-1, Kothagarha Local, Mohana, Nayagarh Local-2, Bhawanipatna Local, Golapali Local, Dharakote Local, Nabina, Kanas (Student) Local, VI X UR, WBG-26, WBG-108 along with two check genotypes i.e. PU-31, Prasad showed amplification at 583bp (Figure 2A & B). Primers DMB-SSR 125 and DMB-SSR 130 showed the Rf value range from 0.775 to 0.881 and it amplified between the range of 136.1 to 164.2 bp. DMB-SSR 125 amplified two alleles in four landraces i.e. Jagatsinghpur Local, Kothagarha Local, VI X UR, WBG-26. The SSR marker am-

plified 136bp fragment linked to the MYMV resistant to check genotypes i.e.PU-31, Prasad as well as landraces i.e. Puri Local-2, Jashipur local, Jagatsinghpur Local, Sambalpur Local, Nabina, Monika, VIXUR, WBG-108, and WBG-26. Another amplicon at 164 bp linked to susceptibility to MYMV (Table 6). The primers SSR-VR078 & SSR-VR044 produced the Rf value ranged from 0.785 to 0.895 and from 0.794 to 0.881 respectively. Both the primers amplified single band at 136bp in most of the landraces as well as check genotypes (Figure 2A-B). Kang et al. [27] reported that more than 80% of viral resistance is monogenically controlled by host's resistant factors. It was also noted that in most of the cases, the R genes derived from monogenic dominant resistance plants [5,28,29] successfully developed of resistance linked molecular markers from consensus motifs of other resistance (R) gene or R gene homologous sequences. The disease resistance (R) genes have been isolated and characterized [26]. The experimental data clearly indicate that he SCAR marker MYMV-583 is completely linked to MYMV resistance and shows amplicon at 583bp (Figure 2B). The similar response have been achieved by another researcher [24].

It is concluded that there are significant differences among the landraces with regard to yield attributing traits and length of pods. The landraces like 'Puri Local-2', 'Jashipur local', 'Nabina', 'Monika', VIXUR', 'WBG-108, and 'WBG-26, were resistant to MYMV as comparable to check genotype i.e. 'PU-31' and 'Prasad'. The landrace like 'Sambalpur Local' and Jagatsinghpur Local' were found moderately resistant on accordance with phenotypic and molecular analysis. The present assessment revealed that both SSR and SCAR markers are efficient and widespread genotyping of MYMV-reaction in a PCR based marker technique. This study will help the breeder to screen the high yield genotype with resistance to MYMV reaction for an advance breeding program in black gram. These indigenous lines (landraces) may be considered further as a resistant donor for MAS.

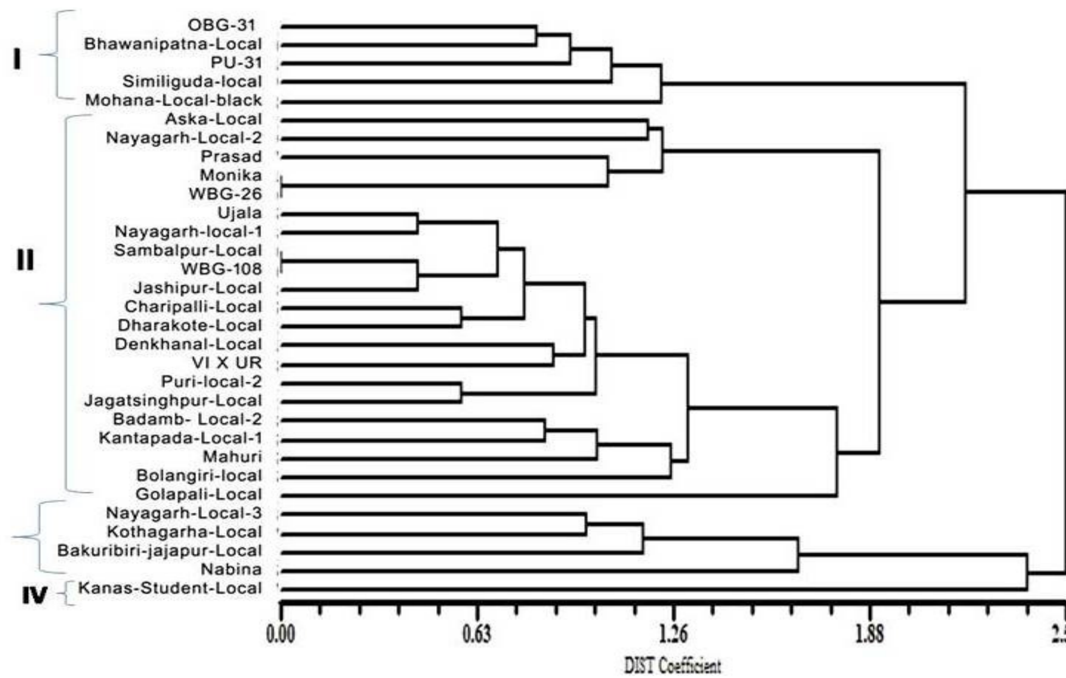
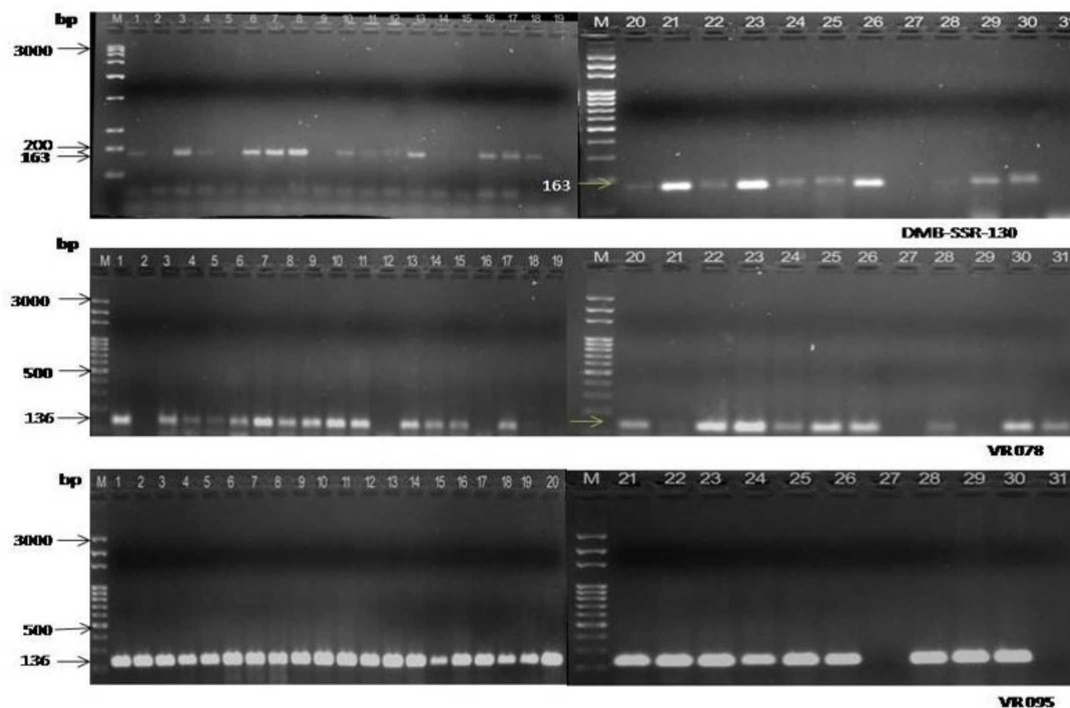


Figure 1: Cluster analysis of 27 indigenous landraces and four check genotypes of *Vigna mungo* based on phenotypic and agronomic traits.



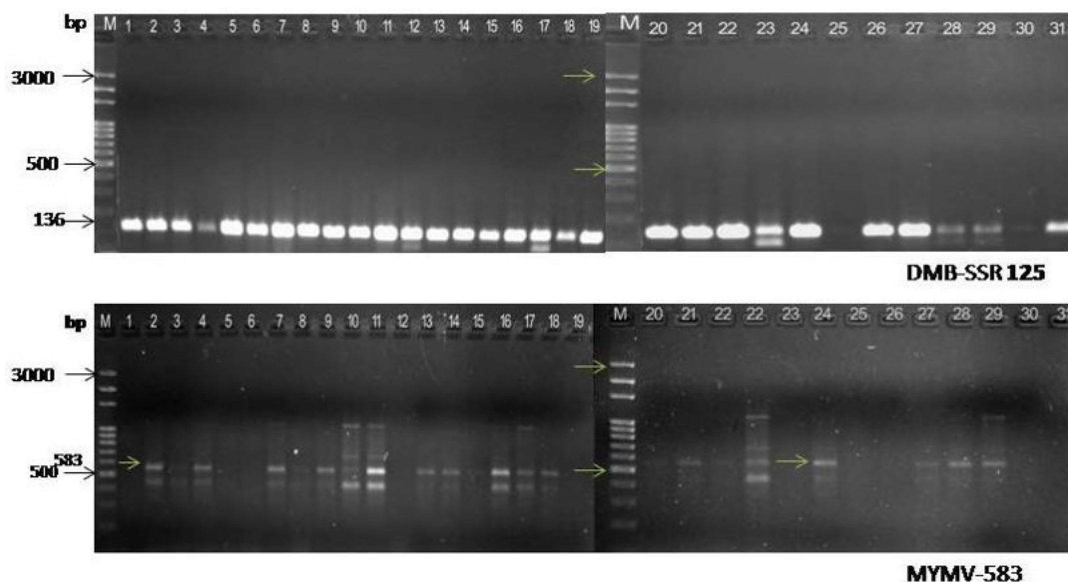


Figure 2: **(A-B)** The DNA profile of 27 landraces and four check genotypes of *Vigna mungo* through SSR and SCAR linked markers resistant to MYMV.

Table 1: Characterization of *Vigna mungo* L. landraces based on Distinctness, Uniformity and Stability guidelines [10].

Sl No.	Characteristics	States	Note	No of Entries	% of Entries
1	Hypocotyls: Anthocyanin coloration	Absent Present	1 9	0 31	0 100
2	Time of flowering	Early(<40 days) , Medium(40-50days), Late(>50days)	3 5 7	20 4 7	64.51 12.09 22.58
3	Plant: Growth habit	Erect , Semi erect , Spreading	3 5 7	4 15 12	12.09 48.38 38.70
4	Plant: Habit	Determinate, Indeterminate	1 3	13 18	41.93 58.06
5	Stem: Colour	Green Green with purple splashes Purple(with green splashes) Purple	1 2 3 4	2 11 9 9	6.45 35.48 29.03 29.03
6	Stem: Pubescence	Absent Present	1 9	4 27	12.09 87.09
7	Leaf: Shape (terminal)	Deltoid Ovate-2 Lanceolate Cuneate	1 2 3 4	7 20 4 0	22.58 64.51 12.09 0
8	Foliage color	Green Dark green	1 2	12 19	38.70 61.29
9	Leaf: Vein color	Green Purple	1 2	25 6	80.60 19.35
10	leaf pubescence	Absent Present	1 9	25 6	80.60 19.35
11	Petiole: Color	Green Green with purple splashes Purple	1 2 3	3 8 20	9.67 25.80 64.51
12	Pod: Intensity of green color of pre mature-pods	Yellowish Green Green Dark green	3 5 7	21 8 2	67.74 25.80 6.45
13	Pod pubescence	Absent Present	1 9	4 27	12.09 87.09

14	Peduncle length	Small(<5cm) Medium(5-10cm) Long(>10cm)	3 5 7	9 21 1	29.03 67.74 3.33
15	Pod length	Small(<5cm) Medium(5-7cm) Long(>7cm)	3 5 7	23 6 2	74.19 19.35 6.45
16	Pod: Color of mature pod	Buff(off white) Brown Black	1 2 3	4 6 21	12.90 19.35 67.74
17	Plant height	Short(<45cm) Medium(45-60cm) Long(>60cm)	3 5 7	30 1 0	96.77 3.33 0
18	Seed: Color	Green Greenish brown Brown Black Mottled	1 2 3 4 5	1 1 0 29 0	3.33 3.33 0 93.54 0
19	Seed: lusture	Shiny Dull	1 2	1 30	3.33 96.77
20	Seed: Shape	Globose Oval Drum shaped	1 2 3	15 16 0	48.38 51.61
21	Seed: Size(weight of 100 seeds)	Small(<3g) Medium(3-5g) Large(>5g)	3 5 7	15 16 0	48.38 51.61 0

Table 2: Phenotypical and agronomic traits of *Vigna mungo* (L.) Hepper indigenous landraces and check genotypes.

Ref. No.	Landrace/ check variety	Color of mature pod	Seed color	Seed lusture	Seed size	Yield (Kg/ha)
V1	OBG-31 (check)	Brown	Black	Dull	Medium	1234
V2	Aska Local	Black	Black	Dull	Small	957
V3	NayagarhLocal-3	Brown	Greenish brown	Dull	Medium	1270
V4	PU-31 (check)	Black	Black	Dull	Medium	1047
V5	Ujala (check)	Black	Black	Dull	Medium	1104
V6	Charipalli Local	Brown	Black	Dull	Small	1018
V7	Prasad (check)	Black	Black	Dull	Medium	1166
V8	Badamba Local-2	Brown	Black	Dull	Small	1132
V9	Similiguda local	Black	Black	Dull	Small	996
V10	Sambalpur Local	Black		Dull	Medium	1249
V11	Puri local-2	Black	Black	Dull	Medium	1213
V12	Jagatsinghpur Local	Black	Black	Dull	Medium	1218
V13	Nayagarh local-1	Black	Black	Dull	Medium	1156
V14	Denkhanal Local	Brown	Black	Dull	Small	1023
V15	Bolangiri local	Black	Black	Dull	Small	1203
V16	Kantapada Local-1	Black	Black	Dull	Small	1282
V17	Kothagarha Local	Buff(off white)	Black	Dull	Medium	1258
V18	Mohana Local(black)	Black	Black	Dull	Small	987
V19	Nayagarh Local-2	Buff(off white)	Black	Dull	Small	1067
V20	Bhawanipatna Local	Black	Black	Dull	Medium	1110
V21	Golapali Local	Brown	Black	Dull	Small	1021
V22	Dharakote Local	Brown	Black	Dull	Small	982
V23	Jashipur Local	Black	Black	Dull	Medium	1069
V24	Monika	Black	Black	Dull	Medium	1057
V25	Nabina	Black	Black	Dull	Medium	1153
V26	Mahuri	Black	Black	Dull	Small	1263

V27	Kanas (Student) Local	Black	Black	Shiny	Small	1142
V28	VI X UR	Buff(off white)	Black	Dull	Small	1052
V29	WBG-26	Black	Black	Dull	Medium	976
V30	WBG-108	Black	Black	Dull	Medium	1023
V31	Bakuribiri jajapur Local	Buff(off white)	Green	Dull	Small	948

Table 3: Cluster mean of quantitative traits for agro-morphological variation of indigenous landraces and check variety of *Vigna mungo* L.

Clusters	Distribution	DF	PH	NPP	LP	NSP	TW	Landraces/ Check Genotypes
Cluster –I	5	38	38.32	19.4	4.52	4.2	3.42	OBG-31 (check), PU-31 (check), Similiguda local, Mohana Local(black), Bhawanipatna Local,
Cluster-II	21	41.1	36.13	25.55	5.14	4.75	3.18	Aska Local, Charipalli Local, Prasad,Ujala, Badamba Local-2, Puri local-2, Bolangiri local, Nayagarh local-1, Denkhanal Local, Jagatsinghpur Local, Kantapada Local-1, Nayagarh Local-2, Golapali Local, Dharakote Local, Jashipur Local, Monika, Mahuri, Sambalpur Local, VI X UR, WBG-26, WBG-108,
Cluster –III	4	54	39	19.25	4.62	4.25	4.4	Nayagarh local -3, Kothagarha Local, Nabina, Bakuribiri jajapur Local,
Cluster –IV	1	54	41.9	20	5	6.7	4.4	Kanas (Student) Local

DF: Days to 50% flowering; PH: Plant height (cm); NPP: Number of pods/ plant; LP: Length of pods (cm); NSP: Number of seeds per pod; TW: 100-seed weight (g).

Table 4: Assessment of disease scoring and MYMV reaction of 27 indigenous landraces and 04 check genotypes of *Vigna mungo* L.

Sl. No	Genotypes	Disease Score	Identifying Grade		Genotypes	Disease Score	Grade
V1	OBG-31 (check)	12.6	MR	V17	Kothagarha Local	28.02	MS
V2	Aska Local	89.56	HS	V18	Mohana Local(black)	12.37	MR
V3	NayagarhLocal-3	68.72	HS	V19	Nayagarh Local-2	78.64	HS
V4	PU-31 (check)	3.87	HR	V20	Bhawanipatna Local	14.21	MR
V5	Ujala (check)	13.2	MR	V21	Golapali Local	88.21	HS
V6	Charipalli Local	71.24	HS	V22	Dharakote Local	23.56	MS
V7	Prasad (check)	17.62	MS	V23	Jashipur Local	11.35	MR
V8	Badamba Local-2	85.74	HS	V24	Monika	7.02	R
V9	Similiguda local	17.6	MR	V25	Nabina	27.86	MS
V10	Sambalpur Local	11.54	MR	V26	Mahuri	26.54	MS
V11	Puri local-2	13.21	MR	V27	Kanas (Student) Local	85.61	HS
V12	Jagatsinghpur Local	11.51	MR	V28	VI X UR	6.32	R
V13	Nayagarh local-1	87.26	HS	V29	WBG-26	6.21	R
V14	Denkhanal Local	79.25	HS	V30	WBG-108	7.27	R
V15	Bolangiri local	69.23	HS	V31	Bakuribiri jajapur Local	67.89	HS
V16	Kantapada Local-1	26.34	MS				

R: Resistant; HR: Highly Resistant: MR: Moderate Resistant; MS: Moderate Susceptible; HS: Highly Susceptible.

Table 5: A Molecular markers linked to MYMV resistance used for validation study.

Primers	Forward Sequence (5' To 3')	Reverse Sequence (5' To 3')	Annealing temperature	Expected product size (bp)
MYMV-583	GTGATGCACACGGTTACGGT	GGTGACGCAGTCCATACAAATTT	50	583
YMV	GAGAGAGAGAGAGAGACAAAG	GAGAGAGAGAGAGAGACAGGA	54	1357
VR0135	GCCCAGATTTGTTCATCCTAGA	ACTGTTTTGAGTGGGGAAAAGA	60	136/164

VR095	GAAATGGGAGTTCAAAGAGGAA	TGGAGAAGTCTGGAAGAGAACC	59.2	136/164
VR078	CATGTGGCAACGCAGAAG	TCAACTTATTCCTCCTTTCTCTCAC	60	136/164
VR044	CCCATGAAGGTATGAGACAACA	GACTGAGAAAGAGAGAGAAGCATTT	60	136/164
DMB-SSR130	CAACTGCAAATGAGGTGAAGAT	ATCCAAGAGCATTTGAACTTCC	63.5	136/164
DMB-SSR125	AAAATGAGTGACAGAGGTGGAAA	ACATGCACATTCTGAACCACAT	59	136/164
SCAR-U223a7	TTGCAGTATGAAAGCATCGTG	AGCCTTCAAATTCATGAGCG	51.5	588
SCAR-U11a4	AGGCAGAGGGGGAAGAAAGAA	ACCCAGAGCTTCCTGGATAA	52	582
SCAR-V02a3	CCAAGAAAGCTTCACAACGTCA	TGGTGAATGAATTGTCCGTAGG	50	
DMB-SSR080	CGAGGCAGAGAAACCTTAAGAA	GCTCGATACTCTTGGGTTGAA	60	136/164
DMB-SSR151	AATGAAGGCTTGTCAAATCCA	TTATTTCACCTTGGCTGGATCA	60	136/164
DMB-SSR158	TGGAAAATTTGCAGCAGTTG	ATTGATGGAGGGCGGAAGTA	63.9	136/164
DMB-SSR160	GGTGGATCAAATCCATTTTAGG	ACAGATCACATAGCAACCAAACA	62.4	136/164

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References

1. Marimuthu T, Subramanian CL, Mohan R. Assessment of yield losses due to yellow mosaic infection in mungbean. Pulse Crop News Letter. 1981; 1: 104-110.
2. Usharani KS, Surendranath B, Haq Q, etc. Infectivity analysis of a soybean isolate of Mungbean yellow mosaic India virus by agroinoculation. Journal of General Plant Pathology. 2005; 71: 230-237.
3. Tanksley SD, Young ND, Paterson AH, etc. RFLP mapping in plant breeding: new tools for old science. Bio/Technol. 1989; 7: 257-264.
4. Bagge M, Xia X, Lubberstedt L. Functional markers in wheat. Current Opinion in Plant Biology. 2007; 10:211-216.
5. Maiti S, Basak J, Kundagrami S, etc. Molecular marker-assisted genotyping of mungbean yellow mosaic India virus resistant germplasms of mungbean and urdbean. Molecular Biotechnol. 2011; 47: 95-104.
6. Selvi R, Muthiah AR, Manivannan N, etc. Tagging of RAPD marker for MYMV resistance in mungbean [Vigna radiata (L.) Wilczek]. Asian Jour Plant Sci. 2006; 5: 277-280.
7. Chen HM., Ku HM., Schafleitner R, etc. The major quantitative trait locus for mungbean yellow mosaic Indian virus resistance is tightly linked in repulsion phase to the major bruchid resistance locus in a cross between mungbean [Vigna radiata (L.) Wilczek] and its wild relative Vigna radiata ssp. sublobata. Euphytica. 2013; 192: 205-216.
8. Dhole VJ, Reddy KS. Genetic analysis of resistance to mungbean yellow mosaic virus in mungbean (Vigna radiata). Plant Breeding. 2012; 131: 414-417.
9. Dhole VJ, Reddy KS. Development of a SCAR marker linked with a MYMV resistance gene in mungbean (Vigna radiata L. Wilczek). Plant Breeding. 2013; 132: 127-132.
10. PPV & FRA. Protection of plant varieties and farmer's rights Authority , Guidelines for the conduct of test for the Distinctness, Uniformity and Stability on Blackgram (Vigna mungo L.) Hepper, Plant Variety Journal of India. 2007; 1: 4-6.
11. Singh G, Sharma YR, Kaur L. Method of rating mungbean yellow mosaic virus of mungbean and urdbean. Plant Disease Research. 1992; 7: 1-6.
12. Doyle JJ, Doyle JL. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bulletin. 1987; 19:11-15.
13. Rohlf FJ. NTSYSpc (Numerical Taxonomy & Multivariate Analysis System). Version 2.2, Exeter Software, Applied Biostatistics Inc., New York. 2005.
14. Abna F, Golam F, Bhassu S. Estimation of genetic diversity of mungbean (Vigna radiata (L.) Wilczek) in Malaysian tropical environment. Afr. Jour. Microbiol. 2012; 6: 1770-1775.
15. Gadakh SS, Dethe AM, Kathale MN, etc. Genetic diversity for yield and its component traits in green gram [Vigna radiata (L.) Wilczek]. Journal of Crop and Weed. 2013; 9: 106-109
16. Murugesan S, Chelliah S. Influence of sowing time on the incidence of the vector Bemisia tabaci (Genn.) and the yellow mosaic disease of greengram. Madras Agric Jour. 1977; 64: 128-130
17. Gupta OM. Resistance to Mungbean yellow mosaic virus, phenotypic characters and yield components in Urdbean. Indian Phytopathology. 2003; 56: 110-111.
18. Shivaprasad P, Thillaichidambaram P, Balaji V, etc. Expression of full length and truncated Rep genes from mungbean yellow mosaic virus-Vigna inhibits viral replication in transgenic tobacco, Virus Genes. 2006; 33: 365-374.
19. Gupta S, Kumar S, Singh RA, etc. Identification of a single dominant gene for resistance to mungbran yellow mosaic virus in blackgram. SABRAO Jour. Breeding & Genetics. 2005 37:85-89.
20. Gupta SK, Souframanien J, Gopalakrishna T. Construction of a genetic linkage map of black gram based on molecular markers and comparative studies. Genome. 2008; 51: 628-637.
21. Maheshwari R, Panigrahi G, Angappan J. Molecular characterization of distinct YMV (yellow mosaic virus) isolates affecting pulses in India with the aid of coat protein gene as a marker for identification. Molecular Breeding Reports. 2014; 41:2635-2644.
22. Shanmugapriya G, Das SS, Veluthambi K. Transgenic tobacco plants expressing siRNA targeted against the mungbean yellow mosaic virus transcriptional activator protein gene efficiently block the viral DNA accumulation, Virus Disease. 2015; 26: 55-61.

23. Basak J, Kundagramy S, Ghose TK, etc. Development of yellow mosaic virus (YMV) resistance linked DNA marker in Vigna mungo from population segregating for YMV reaction. Molecular Breeding. 2005; 14: 375-383.

24. Souframanien J, Gopalakrishna T. ISSR and SCAR markers linked to the mungbean yellow mosaic virus (MYMV) resistance gene in blackgram [Vigna mungo (L.) Hepper]. Plant Breeding. 2006; 125: 619-622.

25. Prasanthi L, Reddy BVB, Gettha B, etc. Molecular markers for screening yellow mosaic disease resistance in black gram [Vigna mungo (L.) Hepper]. Electronic Jour. Plant Breeding. 2013; 4: 1137-1141.

26. Gupta S, Gupta DS, Anjum TK, etc. Inheritance and molecular tagging of MYMIV resistance gene in black gram (Vigna mungo L. Hepper). Euphytica. 2013; 193:27-37.

27. Kang BC, Yeam I, Jahn MM. Genetics of plant virus resistance. Annual Review of Phytopathology. 2005; 43: 581-621.

28. Diaz-pendon JA, Truniger V, Nieto C, etc. Advances in understanding recessive resistance to plant virus. Molecular Plant Pathology. 2004; 5:223-233.

29. Maule AJ, Caranta C, Boulton MI. Sources of natural resistance to plant viruses:status and prospects. Molecular Plant Pathology. 2007; 8:223-231.