Identification and validation of new sources of resistance for *Mungbean Yellow Mosaic India Virus* in urdbean [*Vigna mungo* (L.) Hepper]

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ABSTRACT

***Mungbean Yellow Mosaic India Virus* (MYMIV) causes considerable damage to the susceptible urdbean cultivars or landraces wherever they are grown. To manage yellow mosaic disease caused by MYMIV, development of genetically resistant cultivars is strongly advisable. To identify a stable source for MYMIV resistance in urdbean a set of sixty-five advanced urdbean breeding lines/genotypes were screened under field conditions. MYMIV was confirmed by PCR assays in the symptomatic yellow mosaic leaves of urdbean genotypes. Based on the disease reactions genotypes were grouped into different categories. The average disease rating score recorded for test genotypes ranged from 1 to 6.16 as against 8.83 of YMD susceptible check (Co-5). Of the 65 test genotypes, 43 showed resistant reaction (1-2 scale) during all six years of testing against YMD. However, eight of them (IPU 10-1, IPU 96-6, IPU 99-220, IPU 13-7, NP 21, PDU-3, IPU 13-3 and PLU 99-10) consistently showed resistant reaction (disease rating scale 1) with no visible symptoms on leaves indicating that these genotypes have stable resistance for MYMIV. Thirteen genotypes were identified as moderately resistant (disease rating score 3-4). Rest of the genotypes showed moderately susceptible to highly susceptible reactions (disease rating score 5-9). The identified MYMIV resistant urdbean genotypes can be used in yield evaluation trials in breeding programmes.**

Key words: Black gram, Disease resistance, Genetic resistance, MYMIV, Yellow mosaic disease

INTRODUCTION

Urdbean or blackgram [*Vigna mungo* (L.) Hepper] is an important pulse crop grown around the year under different agro-climatic conditions in India and consumed as a source of protein. In India, urdbean occupied 4.11 m hectares with a total production of 2.45 million tonnes and productivity of 596 kg/hectare in 2020-21(Anonymous 2021). Urdbean is affected by several fungal and viral diseases causing substantial production losses (Singh and De 2006). Among viral diseases, yellow mosaic disease (YMD), first described by Williams *et al*. (1968) and Nene (1973) is the most important biotic constraint faced by the crop in all growing areas of the country. Yield losses due to YMD vary from 10-100% and depend on the stage the crop gets infection (Nene 1972; Naimuddin *et al.* 2016). YMD adversely affects not only yield-related parameters like pods/plant, seeds/pod, 1000 seed wt but also the seed colour, texture and size of the seeds (Gurha *et al*. 1982). The disease is commonly characterized by yellow mosaic symptoms in the leaves of affected plants. Symptoms first appear as small yellow flecks on young leaves and subsequently emerging younger leaves exhibit more conspicuous and irregular yellow and green patches alternating with each other. Yellow spots may also be observed on pods and seeds (Williams *et al*. 1968). Generally, two types of symptoms such as yellow mottle and necrotic mottle are observed. These two types of distinct symptoms are infact due to varied reactions of urdbean genotypes to the YMD causing virus (Nair *et al*. 1974). YMD in urdbean is whitefly transmitted and caused by at least three begomovirus species viz., *Mungbean yellow mosaic India begomovirus* (MYMIV), *Mungbean yellow mosaic begomovirus* (MYMV), *Horsegram yellow mosaic begomovirus* (HgYMV), individually or as mixed infection

(Akram *et al.* 2020; Mishra *et al.* 2020).

Managing viral diseases in general and yellow mosaic disease in urdbean, in particular, has always been a challenge before the researchers; however, deployment of resistant cultivars is one of the most cost-effective and environmentally friendly methods to reduce the losses inflicted by YMD. Developing disease-resistant cultivars is a continuous process and a major objective of a crop improvement programme that requires sources of resistance against target disease. Screening of urdbean genotypes to identify the sources of resistance (against YMD) therefore becomes important. Several researchers have screened urdbean genotypes/advanced breeding materials and identified YMD resistant genotypes (Biswas and Verma 2001; Basandrai *et al.* 2003; Kumar and Bal 2012; Gopalaswamy *et al.* 2012; Panigrahi and Baisakh 2013; Bag *et al.* 2014; Peeta Gopi *et al.* 2016; Devi *et al.* 2017; Bhanu *et al.* 2017; Pavishna *et al.* 2019). Present paper deals with the screening against YMD of advanced breeding lines of urdbean developed from such crosses. The identity of the virus prevalent in the disease (YMD) screening nursery was ascertained by PCR assays.

MATERIALS AND METHODS

Urdbean genotypes

The urdbean genotypes (n=65) and two susceptible checks, Co-5 and MDU-1were screened against yellow mosaic disease. All the genotypes designated with initials of IPU were developed at ICAR-Indian Institute of Pulses Research (ICAR-IIPR, Kanpur) using the pedigree method of breeding. The other genotypes used are released cultivars or germplasm lines from other research centers. To further enhance the resistance level of urdbean genotypes, crosses were made involving YMD resistant and agronomically superior genotypes at ICAR-IIPR Kanpur. The genotypes with initials of IPU are the product of crosses made to improve seed yield and related traits besides disease and insect-pest resistance. Many of these genotypes are currently under multi-location evaluation in All India coordinated trials or state adaptive trials and have the potential to be released as a variety. All the test genotypes were evaluated continuously for 6 years (2015-2020) during *Kharif* season for their reaction to yellow mosaic disease caused by *Mungbean yellow mosaic India virus*.

Growing of genotypes

All the genotypes were grown during *Kharif* 2015, 2016, 2017, 2018, 2019 and 2020 in the field at Main Campus, ICAR-IIPR Kanpur (26.4499° N, 80.3319° E). Each genotype was sown in 2 rows of 4 meter length every year alternated by the infector rows of susceptible genotypes (Co5 or MDU-1) and the experiment was replicated twice in test years (2015-2020) following row-to-row and plant-to-plant spacing of 30 x10 cm approximately. Some years, these genotypes were grown in a flat and some years on ridges to avoid water stagnation due to heavy rains. The infector-row technique (Fig. 1) is known to ensure

Fig. 1. Field view of the screening using infector row technique (A); severe symptoms (indicated by arrows) of YMD on susceptible check (cultivar Co-5) used as infector row and resistant genotypes with no visible symptoms of YMD (B); Gel photographs showing presence of bands (~1kb) amplified from 10 randomly selected infected samples (lane 3-12) of urdbean genotypes subjected to PCR using MYMIV specific primers during different growing years (2015-2020); Lane M= 1 kb DNA ladder (Thermo Scientific), Lane 2= Positive control and Lane 3= Negative control in each gel.

proper exposure of the test genotype against yellow mosaic disease-causing viruses which are harboured by the susceptible genotype (infector-row) and the virus inoculum is spread to the genotypes by the whitefly vector of YMD. Recommended agronomic and cultural practices were adopted to raise the crop. However, no herbicide, insecticide and fungicide were sprayed. The need-based weeding was done manually.

Observation of disease data

The YMD data were recorded on a 1-9 scale as followed in the All Indian Coordinated Research Project on MULLaRP (Anonymous 2021). Final observations of disease were recorded when the crop was 50-60 days old and used for the determination of genotypes reaction. Finally, the average data of all the years of disease rating score were used to categorize the genotypes.

Diagnosis of viruses involved in causing YMD

The identity of the virus causing yellow mosaic disease in urdbean during the growing seasons was confirmed by PCR assays. Every year leaves of ten genotypes samples showing yellow mosaic symptoms were collected randomly at the crop stage of 30-45 days and used to detect the virus involved. The total DNA from the collected leaves was extracted using DNeasy Plant Mini Kit (Qiagen Inc., USA), following the manufacturer's instructions. A set of four primer pairs (NM1/NM2, MYMV-CP-F/MYMV-CP-R, HgYMV-CP-F/HgYMV-CP-R and DoYMV-CP-F/DoYMV-CP-R) specific to detect MYMIV, MYMV, HgYMV and DoYMV were used to amplify the coat protein region of targeted viruses (Table 1). The polymerase chain reaction mix (total volume of 25 µl for each reaction) was prepared using Dream Taq Green Master Mix 2x (Fermentas) as per instructions given by the manufacturer. The reaction was carried out in an automated Mastercycler ProS (Eppendorf AG, Germany) as per the following thermal regimes: Initial denaturation at 94 °C for 3 minutes followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 54 °C for 1 minute, extension at 72 °C for 1 min and a final step at 72 °C for 10 min. The PCR products were resolved in 1% agarose gel prepared in 1xTAE buffer and photographed.

RESULTS AND DISCUSSION

Based on average disease rating, eight genotypes (IPU 10-1, IPU 96-6, IPU 99-220, IPU 13-7, NP 21, PDU-3, IPU 13-3 and PLU 99-10) were categorized as resistant (disease rating scale 1) as these genotypes did not show any visible symptoms of YMD at any growth stage of the plants during the six years of testing (Table 2). Another 35 genotypes (IPU 12-4, UPU 85-15, IPU 99-200, UH 84-4, IPU 10-16, UH 84-01, PLU 570,

Table 1. Detection of the virus involved in causing yellow mosaic disease and the primers used

Year	Number of samples tested	Result of PCR assays using primer pairs specific			
		MYMV	MYMIV	HgYMV	DoYMV
2015	10	All -ve	All +ve	All -ve	All -ve
2016	10	All -ve	All +ve	All -ve	All -ve
2017	10	All -ve	All +ve	All -ve	All -ve
2018	10	All -ve	All +ve	All -ve	All -ve
2019	10	All -ve	All +ve	All -ve	All -ve
2020	10	All -ve	All +ve	All -ve	All -ve

Details of the primers used to detect the viruses causing yellow mosaic disease				
Primer ID	**Sequences 5′…3′**	**Annealing temperature**	**Expected amplicon size in base pair**	**Virus to be identified**
NM1	GTATTTGCAKCAWGTTCAAGA	54 °C	~1000 bp	MYMIV
NM2	AGGDGTCATTAGCTTAGC			
MYMV -CP-F	ATGGG KTCCGTTGTATGCTTG	54 °C	~1000 bp	MYMV
MYMV -CP-R	GGCGTCATTAGCATAGGCAAT			
HgYMV -CP-F	ATGCTTGCAATTAAGTACTTGCA	54 °C	~1000 bp	HgYMV
HgYMV -CP-R	TAGGCGTCATTAGCATAGGCA			
DoYMV -CP-F	CTGTGAAATTTGTGCAGG	54 °C	~1000 bp	DoYMV
DoYMV -CP-R	TACGCGGTTGCGAATATGTAT			

Table 2. Categorization of urdbean genotypes based on disease rating score of yellow mosaic disease

*Average disease rating score	Per cent disease severity range	Genotypes	Reaction
1	0	IPU 10-1, IPU 96-6, IPU 99-220, IPU 13-7, NP 21, PDU-3, IPU 13-3, PLU 99-10	R
1.16-2	0.1 to 5	IPU 12-4, UPU 85-15, IPU 99-200, EH 84-4, IPU 10-16, UH 84-01, PLU 570, IPU 99-336, IPU 13-6, IPU 13-01, IPU 99-218, IPU 99-204, IPU 10-117, IPU 99-31, IPU 2-33, IPU 99-4, IPU 99-1, UH 84-04, IPU 12-29, IPU 99-211, IPU 99-45, PLU 648, NHKD-31, IPU 13-5, IPU 12-19, IPU 88-31, IPU2-37, V 3108, IPU 96-3, IPU 13-10, PLU 96-06, IPU 6-2, IPU 11-6, EH 82-2, IPU 83-2	
2.16-3	5.1 to 10	IPU 11-1, IPU 12-9, IPU 12-21, IPU 99-62, IPU 96-1, VBN 7, IPU 367, IPU 9-16, IPU 99-40, UPU 85-86	MR
3.83-4	10.1 to 15	PLU 856, PLU 557, IPU 2-43	
4.16-4.33	15.1 to 30	PLU 62, UH 82-23	MS
5-6	30.1 to 50	IPU 13-8, IPU 99-222, PLU 710, KARS 159, IPU 12-3	S
6.16	50.1 to 75	IPU 13-4	HS
8.0	75.1 to 90	Nil	
8.66-8.83	>90.1	MDU-1, PLU 285, Co-5	

*Average disease rating score based on the disease data recorded consecutively for 6 years

IPU 99-336, IPU 13-6, IPU 13-01, IPU 99-218, IPU 99-204, IPU 10-117, IPU 99-31, IPU 2-33, IPU 99-4, IPU 99-1, UH 84-4, IPU 12-29, IPU 99-211, IPU 99-45, PLU 648, NHKD-31, IPU 13-5, IPU 12-19, IPU 88-31, IPU2-37, V 3108, IPU 96-3, IPU 13-10, PLU 96-06, IPU 6-2, IPU 11-6, UH 82-2 and IPU 83-2) had a disease rating score between 1.16-2.0 and were also considered resistant as per illustration of disease rating score. Thirteen genotypes (IPU 11-1, IPU 12-9, IPU 12-21, IPU 99-62, IPU 96-1, VBN 7, IPU 367, IPU 9-16, IPU 99-40, UPU 85-86, PLU 856, PLU 557 and IPU 02-43) were categorized as moderately resistant with a disease score of >2 to 4. Two genotypes (PLU 62 and UH 82-23) were found moderately susceptible (disease rating score >4 to 5), 5 genotypes (IPU 13-8, IPU 99-222, PLU 710, KARS 159 and IPU 12-3) were susceptible (disease rating >5 to 6) and one genotype (IPU 13-4) was highly susceptible (disease rating > 6). Genotypes used as a susceptible check and one test genotype PLU 285 also showed highly susceptible reaction with an average disease rating score of 8.66 to 8.83 (Table 2). Genotypes with a high level of resistance can be exploited as sources of resistance in breeding urdbean for YMD (caused by MYMIV) resistance. Some of the advanced breeding lines can be evaluated under the AICRP programme and utilized as YMD resistant cultivars.

Since different viruses are involved in casing YMD in urdbean (Malathi and Jones 2008; Naimuddin *et al.* 2016; Mishra *et al.* 2020), it is important to ascertain the identity of the virus causing YMD in a screening nursery at a location, as it would be more meaningful to report sources of resistance against the virus rather than disease. Product of PCR assays performed using specific primers to ascertain the identity of the virus prevalent in the disease nursery analyzed in agarose gel electrophoresis revealed the presence of an amplicon of the expected size (1000 bp) with primer pairs specific to MYMIV (Fig. 1) and indicated that the virus causing YMD in disease nursery was MYMIV every year. No band was observed in products of PCR with MYMV, HgYMV and DoYMV specific primers indicating a negative reaction (Table 1). Most of the workers (Basandrai *et al.* 2003; Bhanu *et al.* 2017; Devi *et al.* 2017; Gopalaswamy *et al.* 2012; Kumar and Bal 2012) have identified and reported YMD resistant genotypes of urdbean without ascertaining the identity of the causal virus responsible for YMD at the place of screening. However, at present, it is unequivocally proved that more than one virus causes YMD in urdbean at different places and therefore while reporting the sources of resistance against YMD, the identity of the causal virus should be mandatory.

It is concluded that a high level of field resistance is present in urdbean genotypes against MYMIV and these genotypes can be evaluated for resistance against other YMD causing viruses such as MYMV and HgYMV, mostly prevalent in the southern part of

the country to identify the multi-virus resistant genotypes among them. While evaluating for the other YMD causing viruses, the identity of the virus should be ascertained unequivocally. Further, these genotypes may be used in a breeding programme to improve the resistance level of the high yielding urdbean cultivars.

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REFERENCES

Akram M, Naimuddin K, Pratap A, Muin A, Ahmad S, Agnihotri AK, Shinde P, Saable PR and Singh NP. 2020. Molecular characterization of begomoviruses causing yellow mosaic disease in *Vigna stipulacea* and evidence of recombination. Journal of Food Legumes **33**(4):232-244.

Anonymous 2021. Project coordinator report: virtual group meet on mungbean and urdbean, May 27–28, 2021. AICRP on MULLaRP, ICAR-IIPR, Kanpur, Annexure VI, page 38.

Bag MK, Gautam NK, Prasad TV, Pandey S, Dutta M and Roy A. 2014. Evaluation of an Indian collection of blackgram germplasm and identification of resistance sources to *Mungbean Yellow Mosaic Virus*. Crop Protection **61**:92-101.

Basandrai D, Basandrai AK, Singh I and Kalia V. 2003. Multiple disease resistance against anthracnose, Cercospora leaf spot, powdery mildew and mungbean yellow mosaic virus in blackgram (*Vigna mungo*). Journal of Mycology and Plant Pathology **33**:56–58.

Bhanu AN, Singh MN and Srivastava K. 2017. Screening mungbean [*Vigna radiata* (L.) Wilczek] genotypes for mungbean yellow mosaic virus resistance under natural conditions. Advances in Plant & Agricultural Research **7** (6):417-420. DOI: 10.15406/apar.2017.07.00276

Biswas KK and Varma A. 2001. Evaluation of resistance in blackgram (*Phaseolus mungo*) to variants of mungbean yellow mosaic geminivirus. Indian Journal of Agricultural Sciences **71**:215–218

Devi CH, Kumari VP, Devi PHS and Sinha B. 2017. Screening of blackgram genotypes for molecular variability in reaction to Mungbean yellow mosaic disease. Indian Phytopathology **70** (3) : 353-358. DOI 10.24838/ip.2017.v70.i3.74242

Gopalaswamy SVS, Ramana MV and Krishna YR. 2012. Screening of blackgram entries for resistance to insect pests and diseases under rice fallows. Annals of Plant Protection Sciences **20**:237-238.

Gurha SN, Misra DP and Kamthan KP. 1982. Studies on some aspects of yellow mosaic disease of black gram (*Vigna mungo* (L.) Hepper). Madras Agricultural Journal **69**:435- 438.

Kumar A and Bal RS. 2012. Screening of blackgram germplasm against MYMV and Cercospora leaf spot diseases. Trends in Biosciences **5**:127-128.

Malathi VG and John P. 2008. Geminiviruses infecting legumes. In: Characterization, Diagnosis & Management of Plant Viruses, Volume 3: Vegetables and Pulse Crops (G.P. Rao, P. Lava Kumar and Ramon J. Holguin-Pena, Ed.), Stadium Press LLC, Texas, USA. Pp 97-123.

Mishra GP, Dikshit HK, Ramesh SV, Tripathi K, Kumar RR, Muraleedhar A, Singh A, Roy A, Priti, Kumari N, Dasgupta U, Kumar A, Shelly P and Nair RM. 2020. Yellow Mosaic Disease (YMD) of Mungbean (*Vigna radiata* (L.) Wilczek): Current Status and Management Opportunities. Frontiers in Plant Science **11**:918: DOI=10.3389/fpls.2020.00918

Naimuddin K, Akram M and Singh NP. 2016. Yellow mosaic of mungbean and black gram: current status and future strategies. Journal of Food Legumes **29**(2): 77-93

Nair NG and Nene YL. 1974. Studies on yellow mosaic of blackgram (*Phaseolus mungo* L.) caused by mungbean yellow mosaic virus. Factors influencing transmission and symptom expression. Indian Journal of Farm Sciences **2**:42-47.

Nene YL. 1972. A survey of viral disease of pulse crops in Uttar Pradesh. G.B. University of Agriculture and Technology Research Bulletin No 4, pp: 191

Nene YL. 1973. Viral diseases of warm weather pulse crops in India. Plant Disease Reporter **57**:463–467.

Panigrahi KK and Baisakh B. 2013. Reaction of local and mutant lines of blackgram (*Vigna mungo* [L] Hepper) to yellow mosaic virus and powdery

mildew. Journal of Plant Protection and Environment **10**: 57-60.

Pavishna M, Kannan R, Arumugam Pillai M and Rajinimala N. 2019. Screening of blackgram genotypes against mungbean yellow mosaic virus disease. Journal of Pharmacognosy and Phytochemistry **8**(3): 4313-4318.

Peeta Gopi, Satyanarayana A, Rama Krishna A and Sambasiva Rao KRS. 2016. Evaluation of blackgram germplasm for resistance against YMV. Journal of Plant Pathology & Microbiology **7**(7):2157-7417

Singh RA and De RK. 2006. Major diseases and their management. *In*: Advances in Mungbean and Urdbean (Eds. Ali M, Kumar S), Indian Institute of Pulses Research, Kanpur. Pp. 283-334

Williams FJ, Grewal JS and Amin KS. 1968. Serious and new diseases of pulse crops in India in 1966. Plant Disease Reporter **52**:300-304.