Virology & Mycology

Akhtar J et al, Virol-mycol 2016, 5:1
http://dx.doi.org/10.4172/2161-0517.1000153

Research Article Open Access

Identification of Resistant Sources of *Vigna spp.* against Yellow Mosaic Disease

Jameel Akhtar[1*], Hem Chandra Lal[2], PK Singh[3], S Karmakar[4] Narinder Kumar Gautam[5], and Atul Kumar[6]

[1]*Division of Plant Quarantine, ICAR-National Bureau of Plant Genetic Resources, New Delhi-110012, India*

[2]*Department of Plant Pathology, Birsa Agricultural University, Ranchi-834006, India*

[3]*Department of Entomology, Birsa Agricultural University, Ranchi-834006, India*

[4]*Department of Agronomy, Birsa Agricultural University, Ranchi-834006, India*

[5]*Division of Germplasm Evaluation, ICAR-National Bureau of Plant Genetic Resources, New Delhi-110012, India*

[6]Division of Seed Science & Technology, ICAR-Indian Agricultural Research Institute, New Delhi-110012, India

[*]**Corresponding authors:** Jameel Akhtar et al, Division of Plant Quarantine, ICAR-National Bureau of Plant Genetic Resources, New Delhi, India, E-mail: jameelbau@rediffmail.com

Received date: November 27, 2015; **Accepted date:** January 21, 2016; **Published date:** February 22, 2016

Abstract

The cultivation of green gram (*Vigna radiata* (L.) and black gram (*Vigna mungo* (L.) Hepper) in Jharkhand, India, is adversely affected by most destructive yellow mosaic disease (YMD), but the etiological agent in this region is not identified so far. Disease incidence and severity as high as 100 per cent in farmers' fields is common, often resulting in considerable yield losses in both the crops. Therefore, an attempt was made during crop seasons 2009 and 2010 at three locations *viz*., Ranchi, Dumka and Chianki of Jharkhand state with aim to confirm the identity of etiological agent of YMD as well as to identify the resistant sources of green gram and black gram against YMD. The identity of the etiological agent causing YMD in *Vigna* spp. in Jharkhand, India was confirmed as *Munbean yellow mosaic India virus* (MYMIV) in polymerase chain reaction using species specific primers. Out of twelve genotypes of green gram, only two genotypes, Meha and ML 1477 and two of eight genotypes of black gram, KU 323 and BS 23-13 were recorded either as resistant or highly resistant at all the three locations of Jharkhand which could be used as resistant donors against MYMIV in crop improvement programmes to develop high-yielding lines/ varieties and increased acreage of green gram and black gram crops.

Keywords: Black gram; Green gram; Resistance; Field screening; Yellow mosaic disease

Introduction

Legumes, which are major source of dietary proteins, minerals and vitamins, are grown world over. In India and Southeast Asia, among *Vigna* spp. grown as grain legumes during July to October - *Kharif* season [1], black gram (Vigna mungo L. Hepper) and green gram (*Vigna radiata* L.) Wilczek) are more important ones. The production of these two major *kharif* pulses is adversely affected by most destructive YMD, which was first reported by Nariani (1960) [2]. Whitefly-transmitted bipartite begomoviruses named as *Mungbean yellow mosaic India virus* (MYMIV), *Mungbean yellow mosaic virus* (MYMV), *Horsegram yellow mosaic virus* (HgYMV) and *Dolichus yellow mosaic virus* (DoYMV) are four distinct etiological agents of this disease in legume crops in India and other South Asian countries [3-8]. Disease incidence and severity as high as 100 per cent in farmers' fields is common, often resulting in considerable yield losses in green gram and black gram in India [7,10] and in Pakistan [11]. The disease has been recorded since long back on green gram and black gram in Jharkhand, India too, but the etiological agent is not identified so far. There is enough literature that refers resistant genotypes of green gram and black gram against YMD [12,13] but without identifying the etiological agent. Since three distinct viruses i.e. MYMIV, MYMV and HgYMV are reported to cause YMD in *Vigna* spp., accurate identification of the virus is important. Therefore, the present study was conducted with the aim to confirm the identity of etiological agent of YMD and make available genetic stock of *Vigna* spp. resistant to YMD, which is a prerequisite for breeding programme for varietal improvement to achieve the effective and practical management of this disease at farmers' field.

Materials and Methods

In order to identify the resistant sources against YMD, initial screening during the year 2008 against YMD consisted of 110 accessions each of black gram and green gram at Ranchi resulted in highly resistant/ resistant response of nine and seven accessions of black gram and green gram, respectively. To verify the identified resistant sources against YMD, the field trial consisting of 9 genotypes of *V. radiata* and 6 genotypes of *V. mungo* was laid in randomized block design with plot size of 2.5 × 2.4 m^2 and three replications at three locations of Jharkhand, India, namely Ranchi (23° 17' N latitude and 85° 19' E longitude), Dumka (24° 27' N latitude and 87° 25' E longitude) and Chianki (23° 50' N latitude and 83° 55' E longitude) with an altitude of 625, 137 and 237 meters above mean sea level, respectively. The sowing was done on 24th June during both the cropping seasons i.e. 2009 and 2010. Disease was scored after 4 and 8 weeks of sowing using 0 to 9 scale [14] and disease index was calculated using formula given under [15] and data were statistically analyzed using ANOVA.

$$Disease\ index = \frac{n_1X_1 + n_2X_2 + n_3X_3 + n_4X_4 + n_5X_5}{\text{Total number of leaves observed} \times \text{Maximum Grade}} \times 100$$

To identify the causal virus associated with YMD, the total DNA was extracted by using Nucleopore plant mini kit from 3 randomly selected leaves of yellow mosaic infected plants individually of each location according to the prescribed protocols. Species specific three primers i.e. MYMIV-AV1F/ MYMIV-AV1R (5'GTA TTT GCA KCA WGT TCA AGA3'/ 5'AGG DGT CAT TAG CTT AGC3') specific for MYMIV, MYMV-CPF/MYMV-CPR (5'ATG GG(GT) TCC GTT GTA TGC TTG3'/5'GGC GTC ATT AGC ATA GGC AAT3') specific for MYMV and HgYMV-CPF/HgYMV-CPR (5'ATG CTT GCA ATT AAG TAC TTG CA3'/5'TAG GCG TCA TTA GCA TAG GCA3') specific for HgYMV, respectively, were used for PCR, which was performed according to the prescribed protocols in a T1 Thermocycler, programmed for 35 cycles with one step of initial denaturation for 3 min., and denaturation for 30 seconds at 940 C, 1 minute annealing temperature at 54°C for the primers pair specific for MYMIV, MYMV and at 57°C and for HgYMV and 1 minute extension at 72°C followed by a one-step final extension for 10 min at 72°C. PCR assays were conducted with DreamTaq Green PCR Master Mix (Fermetas) in total reaction mixture volume of 25 µL that contained DNA template (@50 ng/µL) -2 µL, primer (@25 pmole/ µL)-1 µL each and dH2O-21 µL. PCR amplicons were analyzed by 1% agarose gel electrophoresis in Tris-acetate EDTA (TAE) containing 0.1% ethidium bromide. The gel was examined under UV trans-illuminator and photographed using a digital camera (Sony DSCW270).

Results and Discussion

Field observations on disease index of YMD in Vigna spp. at all the 3 locations during both the crop seasons 2009 and 2010, presented in Table 1 revealed that the amount of disease as well as symptoms produced on genotypes differed significantly (Figure 1). The symptoms observed in the present investigations have also been recorded and described by several workers [2,11,16,17]. The average disease index of YMD at Ranchi ranged from 0.00 to 63.34 per cent, at Dumka from 0.00 to 47.67 per cent and at Chianki from 0.00 to 70.50 per cent (Table 1). Out of three locations, maximum YMD disease index on V. radiata was recorded at Ranchi, whereas, Chianki had maximum disease index on V. mungo genotypes during both the crop seasons followed by Dumka.

Crop species	Genotype	Infection coefficient (%) and reaction of yellow mosaic disease at different locations											
		Ranchi				Dumka				Chianki			
		2009	2010	Average	Reaction	2009	2010	Average	Reaction	2009	2010	Average	Reaction
Vigna radiata	ML 1477	2.33	4	3.17	R	0.33	2.7	1.52	R	0	0	0	HR
		(8.13)*	-11			-1.91	-9.1			0	0		
V. radiata	SML 829	11.67	13.3	12.49	MR	13.33	13.3	13.32	MR	8.33	9.3	8.82	MR
		-19.88	-21			-21.33	-21.4			-16.6	-17.8		
V. radiata	SML 668	23	22	22.5	MS	6	6.3	6.15	MR	1.67	3.7	2.69	R
		-28.63	-28			-14.09	-14.5			-4.31	-10.9		
V. radiata	Meha	1.33	3	2.17	R	1	2.7	1.85	R	0	0	0	HR
		-6.53	-9.3			-4.62	-9.1			0	0		
V. radiata	SML 610	11.67	11.3	11.49	MR	13.33	13.7	13.52	MR	3.33	5.3	4.32	R
		-19.88	-20			-19.68	-21.6			-8.61	-13.1		
V. radiata	PS 16	33.33	35.7	34.52	S	18.33	18.3	18.32	MS	5	5	5	R
		-35.2	-35			-23.52	-25.3			-10.5	-10.5		
V. radiata	Pusa Vishal	11.67	12	11.84	MR	13.33	13.3	13.32	MR	3.33	4	3.67	R
		-19.49	-20			-17.59	-21.4			-8.61	-10.7		
V. radiata	SML 771	61.67	65	63.34	S	45.33	48.7	47.02	S	33.33	34.7	34.02	S
		-51.82	-54			-38.74	-44.2			-35.2	-36.1		
V. radiata	SML 836	43.33	42.3	42.82	S	31.67	32	31.84	S	0	0	0	HR
		-41.14	-41			-32.37	-34.4			0	0		
V. radiata	PDM 11	3.67	4	3.84	R	3.67	5.33	5.5	R	10	9.7	9.85	MR
		-10.52	-12			-10.52	-12.36			-18.4	-17.9		
V. radiata	ML 1333	6.67	6.7	6.69	MR	3.33	4.3	3.82	R	0	0	0	HR

		-12.29	-15			-6.14	-11.9			0	0		
V. radiata	Sunaina	30	31.7	30.85	S	30	33.3	31.65	S	11.67	12.3	11.99	MR
		-33.14	-34			-32.75	-35.2			-19.5	-20.5		
V. mungo	KU 323	3.67	4.3	3.99	R	0	0	0	HR	1.7	1.7	1.7	R
		(10.52)*	-12			0	0			-4.31	-7.2		
V. mungo	Pant U 19	7.67	8	7.84	MR	3.67	4	3.84	R	14.33	16	15.17	MS
		-15.92	-16			-10.95	-11.4			-22.1	-23.6		
V. mungo	KU 99-20	46.67	47	46.84	S	53.33	54.3	53.82	S	70	71	70.5	S
		-43.07	-43			-46.9	-47.5			-56.9	-57.4		
V. mungo	BS 23-13	0	0	0	HR	0	0	0	HR	0	0	0	HR
		0	0			0	0			0	0		
V. mungo	IPL 01-52	11	12.7	11.85	MR	8.33	8.7	8.52	MR	0	0	0	HR
		-19.21	-19			-16.59	-16.59			0	0		
V. mungo	WBU 108	35	37	36	S	31.67	31.67	31.67	S	6	7.7	6.85	MR
		-36.22	-37			-34.13	-34.2			-14.8	-15.9		
V. mungo	Uttra	3	4.3	3.65	R	3.33	4.7	4.02	R	10	9.3	9.65	MR
		-3.72	-12			-8.61	-12.3			-18	-17.7		
V. mungo	Birsa Urd	36.67	37.7	37.19	S	48.33	47	47.67	S	26.7	28.3	27.5	MS
		-37.19	-38			-43.96	-43.3			-29.5	-32.1		
	CD (p=0.05)	7.22	8.63			9.38	9.45			11.33	13.67		
	CV	19.02	20.2			22.33	23.9			29.09	21.7		

Table 1: Multilocational evaluation of *Vigna spp.* against YMD during crop seasons 2009 and 2010. *Figures in parenthesis are transformed values.

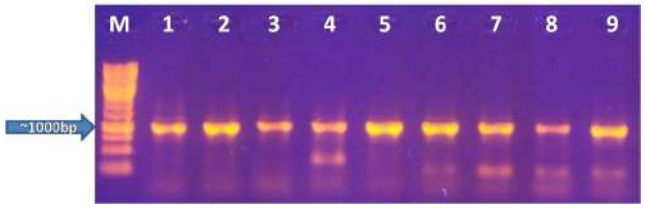

Figure 1: Gel electrophoresis of PCR amplified products of MYMIV using specific primers pair (NM1/NM2) of the greengram and blackgram samples randomly selected and collected from three locations. Lane M=1 kb DNA ladder, Lane 1-3=Ranchi, Lane 4-6=Dumka and Lane 7-9=Chianki.

Further, results revealed that out of 9 genotypes of V. radiata showed some degree of variation in disease index at different locations and taking data of Ranchi and Dumka into consideration, finally 3 genotypes, ML 1477, Meha and PDM 11 were recorded as resistant, whereas, same two genotypes, ML 1477 and Meha showed highly resistant reaction at Chinaki but, genotype PDM 11 was recorded as moderately resistant. Seven genotypes of V. mungo showed some degree of variation in disease index at different locations and all the genotypes showed different disease response at different locations except 2 genotypes, BS 23-13 and KU 99-20 which were uniformly, recorded as highly resistant and susceptible at all the three locations, respectively. In both the Vinga species, none of the resistant genotypes at one location could be noticed as susceptible at other two locations. Among V. radiata genotypes, SML 771 were recorded with maximum disease index of 65.00 per cent at Ranchi, whereas, among V. mungo genotypes, KU 99-20 was recorded with maximum disease index of 70.50 per cent at Chianki. But, it was interesting to note that V. mungo genotypes had relatively higher disease index of YMD across the locations.

In V. radiata, out of 9, only two genotypes, SML 829 and SML 771 were recorded with uniform disease reactions at all the three locations as moderately resistant (MR) and susceptible (S) reactions, respectively and two of V. mungo, BS 23-13 and KU 99-20 were recorded with uniform disease reactions at all the three locations as highly resistant (HR) and susceptible (S) reactions, respectively.

Further, when the host differential reactions of was correlated with disease index at different locations, accordingly disease reactions, based on location wise disease index, were recorded on all the genotypes except SML 836 of V. radiata and Pant U 19 of V. mungo. This variation in disease reactions under field conditions on the genotypes of Vigna spp. shows host response to the pathogen as well as environment. Similar results have also been recorded and described by earlier workers [5,13,17,18,19,20,21].

PCR test yielded amplified DNA fragments of the expected sizes, ~1000 bp (Figure 2) with only the primer pair's specific for MYMIV in all the samples collected from all the three locations. No amplification was observed with other primers pairs specific to MYMV and HgYMV, indicated that the virus causing YMD in black gram and green gram genotypes at all the three locations was MYMIV only. However, the differences observed in the disease index/ reaction might be due to the other factor like weather parameters, load of initial inoculum of the virus or different genetically divergent isolates of MYMIV prevailing at the three locations. High disease incidence and severity at farmers' fields has been observed resulting in considerable yield losses in green gram and black gram in northern part of India and elsewhere is mainly due to MYMIV [6,11].

Experimental findings suggest that the genotypes showing resistant or highly resistant reaction under natural epiphytotics may prove as resistant donors against MYMIV if incorporated in crop improvement programmes to develop high-yielding varieties and increased acreage of Vigna spp. Moreover, it will help in reducing the use of pesticide for vector control and improving soil health thereby environment friendly [22,23].

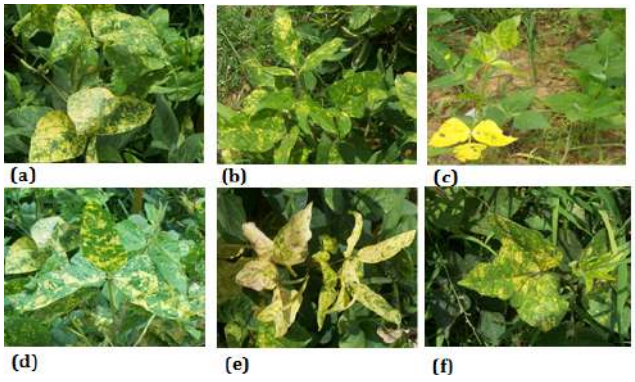

Figure 2: Characteristic YMD sympt¬oms observed on greengram cv. Sunaina at a) Ranchi; b) Dumka; and c) Chianki and on blackgram cv. Birsa Urd 1 at d) Ranchi; e) Dumka; and f) Chianki.

Acknowledgement

The authors are grateful to the Director of Research, Birsa Agricultural University, Kanke, Ranchi, Jharkhand, India and Dr. Md. Akram, Sr. Scientist, Division of Crop Protection, Indian Institute of Pulses Research, Kanpur, UP, India for providing necessary facilities to conduct the research experiments. The help and co-operation received from Project Coordinator, AICRIP-MULLaRP, Kanpur is also acknowledged.

References

1. Pandya BP, Singh DP, and Sharma BL (1977) Screening of mungbean (Vigna radiata L.Wilczek) germplasm for field resistance to yellow mosaic virus. Tropical Grain Legume Bulletin 7: 13-14.
2. Nariani TK (1960) Yellow mosaic of mung (Phaseolus aureus L.). Indian Phytopathology. 13: 24-29.
3. Green SK (1999) In project 3, legumes for crop diversification. AVRDC Report-1999, pp.35-43.
4. Karthikeyan AS, Vanitharani R, Balaji V, Anuradha S, Thillaichidambaram P, et al. (2004) Analysis of an isolate of Mungbean yellow mosaic virus (MYMV) with a highly variable DNA B component. Arch Virol 149: 1643-1652.
5. Kundagrami S, Basak J, Maiti S, Kundu A, Das B, et al. (2009) Agronomic, genetic and molecular characterization of MYMIV-tolerant mutant lines of Vigna mungo. International Journal of Plant Breeding and Genetics 3: 1-10.
6. Naimuddin, Akram M and Pratap A (2011) First report of natural infection of Mungbean yellow mosaic India virus in two wild species of Vigna. New Disease Reports 23: 21.
7. Pant V1, Gupta D, Choudhury NR, Malathi VG, Varma A, et al. (2001) Molecular characterization of the Rep protein of the black gram isolate of Indian mungbean yellow mosaic virus. See comment in PubMed Commons below J Gen Virol 82: 2559-2567.
8. Akhtar J, Lal HC, Kumar Y, Singh PK, Ghosh J, et al. (2013) Multiple disease resistance in green gram and black gram germplasm and management through chemicals under rain-fed conditions. Legume Research 37: 101-109.
9. Varma A, Dhar AK and Mandal B (1992) MYMV transmission and control in India. In: Mungbean Yellow Mosaic Disease (S. K. Green and D. Kim, eds.) Asian Vegetable Research and Development Centre, Bangkok, Thailand. pp. 8-27.
10. Hussain M, Qazi J, Mansoor S, Iram S, Bashir M, et al. (2004) First report of Mungbean yellow mosaic India virus on mungbean in Pakistan. New Disease Reports 9: 13.
11. Dey SK, Singh DR (1973) Resistance test of mung (Phaseolus atoms L.) against yellow mosaic in Uttar Pradesh. Indian J Farm Sci 1: 113.
12. Honda Y, Ikegami M (1986) Mungbean yellow mosaic virus. AAB/CMI Descriptions of Plant Viruses No. 323. Commonwealth Mycological Institute, Kew, England.
13. Nene YL, Srivastava SK, Naresh JS (1972) Evaluation of urdbean (Phaseolus mungo L.) and mungbean (Phaseolus aureus L.) germplasm and cultivars against mungbean yellow mosaic virus. Indian Journal of Agricultural Sciences 42: 251-254.
14. Vidhyasekaran P Arjunan G and Ranganathan K (1976). Field tolerance of some green gram types to yellow mosaic. Madras Agri J 63: 54-55.
15. Ahmad M (1975) Screening of mungbean (Vigna radiata) and urdbean (V. mungo) germplasm for resistance to yellow mosaic virus. J Agri Res 13: 349-354.
16. Bashir M, Iqbal S, Ghafoor A, Ahmad Z and Qureshi AS (2002) Variability in cowpea germplasm for reaction to virus infection under field conditions. Pakistan Journal of Botany 34: 47-48.
17. Mayee CD, Datar VV (1986) Phytopathometry, Marathawada Agricultural University, Parbhani. Technical Bulletin No. 1, pp.145-146.
18. Wheeler BEJ (1969) An Introduction to Plant Diseases, John Wiley and Sons Limited, London.
19. Chhabra KS, Kooner BS, Sharma AK, Saxena AK (1988) Sources of resistance in mungbean (Vigna radiata) to insect pests and mungbean yellow mosaic virus. In: Mungbean; Proceedings of the Second International Symposium, Nov. 16-20 1987, Bangkok, Thailand. AVRDC, Shanhua, Tainan, Taiwan. pp. 308-314.
20. Jalaluddin M and Shaikh MAQ (1981) Evaluation of mungbean (Vigna radiata L. Wilczek) germplasm for resistance to mungbean yellow mosaic virus. Sabrao Journal 13: 61-68.
21. Pandya BP, Singh DP, Sharma BL (1977) Screening of mungbean (Vigna radiata L.Wilczek) germplasm for field resistance to yellow mosaic virus. Tropical Grain Legume Bulletin 7: 13-14.
22. Malathi VG, John P (2008) Geminiviruses infecting legumes. In: Characterization diagnosis and management of plant viruses. Volume 3: vegetables and pulses crops (G.P. Rao, P.L. Kumar, R.J. Holguin-Pena, (ed.), Houston, TX, USA: Studium Press LLC, pp. 97-123.
23. Chiemsombat P (1992) Mumgbean yellow mosaic disease in Thailand: A review. In: Proceedings of an International Workshop. (eds.) S.K. Green and D. Kim, July 2-3 1991, Bangkok, Thailand. AVRDC, Shanhua, Tainan, Taiwan. pp. 54-58.