BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, March 2017. Vol. **14**(1), 315-318

Molecular Detection of Mungbean Yellow Mosaic India Virus (MYMIV) infecting soybean in Madhya Pradesh

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http://dx.doi.org/10.13005/bbra/2448

(Received: 27 December 2016; accepted: 18 January 2017)

Detection of MYMIV infecting soybean was carried out during *Kharif* 2016 using DNA-A (cp) and DNA-B specific molecular markers. Among ten leaf samples collected from different villages, nine were found to possess virus irrespective of the density of whiteflies in concerned region. One sample from Tigan village was found to be virus free even though it was having yellow mosaic disease (YMD) symptoms and whitefly density in an area was moderate. Deficiency of micronutrients could be a reason for YMD like symptoms in virus free plant. There was no correlation between the density of whiteflies and presence of virus in host plant. Molecular markers can be successfully used for detection of MYMIV infecting soybean.

Keywords: YMD, MYMIV, Molecular markers, Soybean, Whitefly.

Soybean is the main *Kharif* crop of the Madhya Pradesh state of India. The present area under soybean in the state is 6.31 million ha with production of 5.24 million tonnes and productivity 831 kg/ha (Anonymous 2016). Productivity of soybean is less than the potential yield of recommended varieties. Among different biotic factors yellow mosaic diseases (YMD) are major constraints on the productivity of legume crops in India. In Central India, YMD of soybean and blackgram is caused by Mungbean Yellow Mosaic India Virus (MYMIV) (Usharani *et al*., 2004; Malathi *et al*., 2005; Girish *et al*., 2005; Ramesh *et al*., 2013). MYMIV possesses bipartite single-stranded circular DNA genomes *viz*., DNA-A and DNA-B (Qazi *et al*., 2006 and Brown *et al*., 2015). MYMIV is transmitted by the whitefly, *Bemisia tabaci* (Govindan *et al*., 2014) and infect the legumes such as soybean, blackgram, greengram etc.

Recent advancement of molecular techniques is useful for clarifying epidemiology of MYMIV. In particular, detections of MYMIV by polymerase chain reaction (PCR) from the plants and whiteflies can be a key technology. Molecular markers are developed for DNA-A and DNA-B genome of MYMIV and are being used to detect viruliferous whitefly using PCR in recent past (Accotto and Sardo, 2010). Very recently molecular differentiation and rapid detection of MYMV and MYMIV from soybean leaf has been reported by Ramesh *et al*, (2016). Therefore the current study was carried out to detect MYMIV infection in soybean using molecular markers.

MATERIALS AND METHODS

Plant materials

Soybean leaf samples were collected from different villages of Jabalpur district of Madhya

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Pradesh state, having variable density of whiteflies (Table 1) during *Kharif* 2016.

DNA isolation and PCR amplification

DNA from soybean leaf samples was isolated using DNeasy Plant Mini Kit (Qiagen) from the plants showing YMD symptoms. Molecular markers were designed for DNA-A (cp) and DNA-B genomes of MYMIV (Table 2). PCR was carried out with genomic DNA using molecular markers in Bio-Rad Thermal cycler. The reaction was carried out in 25 µl volumes, which contains 1.0µl (25ng) of soybean genomic DNA, 1.0µl (2.5pmole) of forward and reverse primers each, 1.0µl (2.0mM) of dNTPs, 1.0µl of Taq buffer (10X), 1.0µl of $MgCl_2$ (25mM) and 1 units of Taq polymerase. All the chemicals and plasticwares used were obtained

Table 1. Soybean samplescollected from different villages and whitefly density

Village	Date of Collection	Crop age	Density of Whitefly
Kuwakheda	20.08.2016	40-45 days	High***
Kalapatha	20.08.2016	after sowing	High***
Malakheda	20.08.2016	(Vegetative	Moderate**
Kevlari	20.08.2016	stage)	High***
Karhaiya	22.08.2016		Moderate**
Mukanwara	22.08.2016		Moderate**
Katrabelkheda	22.08.2016		Moderate**
Tigan	24.08.2016		Low*
Bargi	24.08.2016		Moderate**
Sihora	24.08.2016		High***

*<5 whiteflies/plant; **6-10 whiteflies/plant; ***>11 whiteflies/plant

Table 2. Molecular markers and their sequences

Molecular Marker	Sequence	Amplicon size (bp)
DNA-A (cp)	Forward: ACACGGATCCGTTGCATACACAGGATTTG	750
	Reverse: ACACGAGCTCCTCTACCCCGATATCGAATG	
DNA-B	Forward: AGCCTATGACACCGTCAAGAGGA	541
	Reverse: CGCCGGGACAACGGCATAT	

Table 3. PCR programme

Steps followed in Thermal cycler	Temperature in ºC for one cycle	Timefor one cycle
Marker	DNA-A(cp)/DNA-B Specific	
Step 1	94ºC	1 min.
Step 2	94ºC	20 sec.
Step 3	56ºC	20 sec.
Step 4	72ºC	1 min.
	Step 2 – Step 4 are repeated for 30 cycles	
Step 5	72ºC	3 min.
Step 6	Hold at 15ºC until ready to load onto gel	

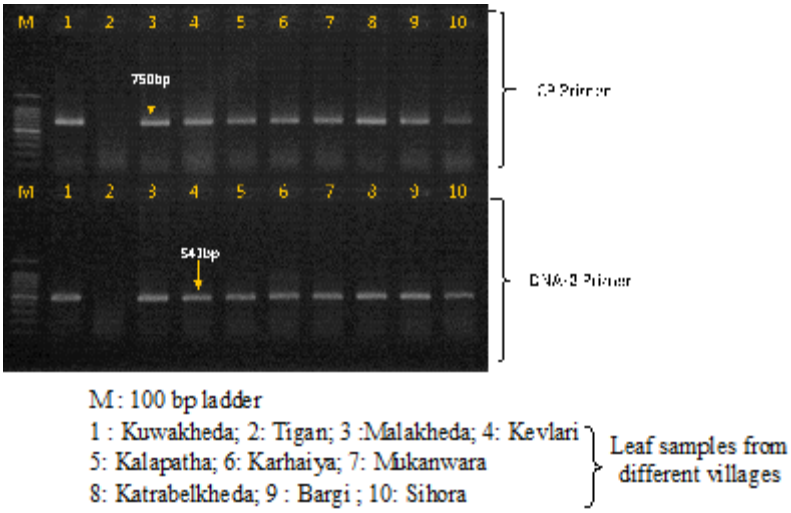

Fig. 1. Molecular detection of MYMIV from soybean leaf samples

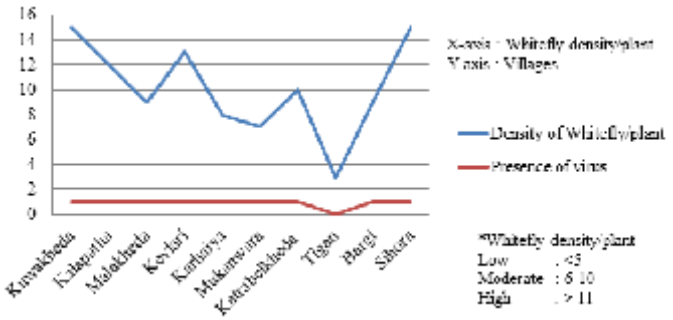

Fig. 2. Correlation between density of whiteflies and MYMIV in soybean

from Genei and Tarsons respectively. PCR Programme (Table 3) was standardized to carry out amplification with DNA-A and DNA-B genome specific primers. The amplified products were resolved on 1.0% agarose gel and visualized under Syngene gel documentation system.

RESULTS AND DISCUSSION

Soybean leaf samples collected from ten different villages, possessing YMD symptoms were subjected to PCR amplification using DNA-A (cp) and DNA-B specific primers. Among ten samples, nine were found to possess both DNA-A (cp) and DNA-B genome of MYMIV (Figure 1). In these nine villages there was variability in whitefly density. Villages *viz*., Malakheda, Karhaiya, Mukanwara, Katrabelkheda, Tigan, Bargi, where whitefly density was low/moderate; the leaf samples of these villages were found to possess virus *i.e.* DNA-A (cp) and DNA-B +ve. This indicates that most of the whiteflies present in those areas were viruliferous and sufficient to transmit MYMIV in soybean.

One leaf sample collected from Tigan village was found to be virus free (Figure 1) even though YMD symptoms were present on the leaves and whitefly density was moderate in the area. This indicates that whiteflies present were non-viruliferous hence there was no presence of virus in the soybean leaf, morever the YMD like symptoms which appeared on leaves might be due to other reasons such as deficiency of micronutrients. Further no correlation was

observed between the whiteflies density and presence of virus in the soybean host plant (Figure 2).

The present study supports the previous studies carried out by Accotto and Sardo (2010); Naimuddin *et al* (2011) and Ramesh *et al* (2016) where molecular markers were used for rapid detection of Begomoviruses, MYMIV and MYMV/ MYMIV, respectively.

CONCLUSION

Molecular markers can be successfully used for detection of MYMIV infecting soybean. Deficiencies of micronutrients could be a reason for YMD symptoms in virus free plant. There is no correlation between the density of whiteflies and MYMIV in host plant.

ACKNOWLEDGEMENT

Authors are thankful to Director of Research, Jawaharlal Nehru Agricultural University, Jabalpur and Japan International Cooperation Agency (JICA), Japan for providing funds to carry out research under project for maximization of soybean production in Madhya Pradesh.

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