New Disease Reports

First report of natural infection of *Mungbean yellow mosaic India virus* in two wild species of *Vigna*

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A number of wild relatives of the two cultivated species of *Vigna* (*V. mungo* and *V. radiata*) are known to occur in different parts of India and are exploited for their useful genes, including resistance to yellow mosaic disease (YMD). There are publications on the characterisation and use of wild accessions of *Vigna* spp. that refer to resistance to YMD in some (Singh, 2004; Bisht *et al*., 2005) without identifying the causal virus involved. Since three distinct viruses (*Mungbean yellow mosaic India virus*, *Mungbean yellow mosaic virus* and *Horsegram yellow mosaic virus*) are reported to cause YMD in cultivated species of *Vigna* (Malathi & John, 2008), proper identification of the yellow mosaic-causing virus is important. We report here natural infection with *Mungbean yellow mosaic India virus*, a begomovirus, as the causal agent of YMD of two wild *Vigna* species, *V. hainiana* and *V. trilobata*.

During the rainy season of 2010, accessions of wild species of *Vigna hainiana* (IC-331615, National Bureau of Plant Genetic Ressources, New Delhi, India) and *V. trilobata* (IC-331436), grown at Indian Institute of Pulses Research, Kanpur, India, showed symptoms like yellowing of inter-veinal tissue and bright yellow spots in the leaves (Fig. 1a, b). All plants of *V. hainiana* (8 plants) and *V. trilobata* (15 plants) were affected. The disease severity measured in terms of percentage foliage yellowing was 80% in the former and 30% in the latter. Whiteflies (*Bemisia tabaci*) were also noticed feeding on plants of these accessions. Type of symptoms and presence of whitefly led us to suspect the involvement of a begomovirus. The association of a begomovirus was confirmed by PCR using primer pairs specific to MYMIV and MYMV (see Fig. 4 for details) that commonly infect cultivated species of *Vigna* in different parts of India (Karthikeyan *et al*., 2004; Usharani *et al.,* 2004). Rolling circle amplification (RCA) was performed using REPLI-g® Mini Kit (Cat. No. 150023, Qiagen, USA) as per manufacturer's instructions and restriction digestion was also carried out to further confirm the presence of DNA A and DNA B of MYMIV.

Total DNA extracted from infected samples using a Qiagen DNeasy Plant Mini Kit was used as template in PCR and for RCA. In PCR, all the four samples drawn from each of the two accessions showing yellow mosaic symptoms gave positive results with MYMIV specific primer pairs NM1/NM2 and MYMIV-MPF/ MYMIV- MPR and yielded amplicons of ~1000bp and ~900bp respectively (Fig. 2a, b), indicating presence of both DNA A and DNA B components. Results with MYMV specific primers were negative. RCA products were subjected to restriction digestion using unique restriction enzymes *Eco*RV (specific for DNA A) and *Bgl* I (specific for DNA B) of MYMIV. Digested products were subjected to agarose gel electrophoresis which revealed the presence of ~2.7 kb DNA bands, further confirming the presence of MYMIV with both DNA A and DNA B components (Fig. 3). To the best of our knowledge, this is the first report of MYMIV infecting *V. hainiana* and *V. trilobata*.

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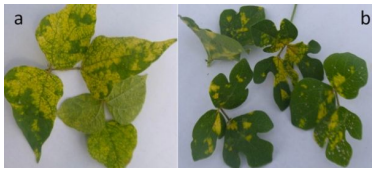

Figure 1

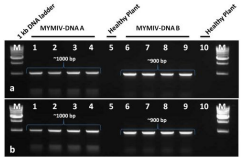

Figure 2

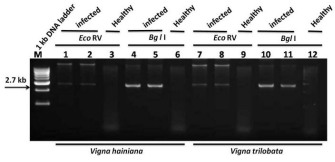

Figure 3

Target virus	Target DNA	Primer	Sequence
MYMIV	DNA A	NM1	5'GTA TTT GCA KCA WGT TCA AGA 3'
		NM2	5' AGG DGT CAT TAG CTT AGC 3'
	DNA B	MYMIV-MPF	5'ATG GAA AAT TAT TCA GGT GCA 3'
		MYMIV-MPR	5'CTA CAA CGC TTT GTT CAC ATT 3'
MYMV	DNA A	MYMV-CP-F	5' ATG GG (T/G) TCC GTT GTA TGC TTG 3'
		MYMV-CP-R	5' GGC GTC ATT AGC ATA GGC AAT 3'
	DNA B	MYMV-MPF	5' ATG GAG AAT TAT TCA GGC GCA 3'
		MYMV-MPR	5' TTA CAA CGC TTT GTT CAC ATT 3'

Figure 4

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