New Disease Reports

First report of *Mungbean yellow mosaic India virus* on kidney bean in Nepal

M.S. Shahid [1,2]*, B.J. Pudashini [1], G.B. Khatri-Chhetri [3], M. Ikegami [4] and K.T. Natsuaki [1]

[1] *Department of International Agricultural Development, Tokyo University of Agriculture, 1-1-1, Sakuragaoka Setagaya-ku, Tokyo 156-8502, Japan*; [2] *Department of Biosciences, COMSATS Institute of Information Technology, Sahiwal 57000, Pakistan*; [3] *Department of Plant Pathology, Institute of Agriculture and Animal Science Tribhuvan University, Chitwan 44209, Nepal*; [4] *NODAI Research Institute, Tokyo University of Agriculture, 1-1-1, Sakuragaoka, Setagaya-ku, Tokyo 156-8502, Japan*

*E-mail: shafiqinayat@yahoo.com

Received: *18 Jan 2012.* ***Published:*** *30 Jun 2012.* ***Keywords:*** *geminivirus, begomovirus, bipartite, Phaseolus vulgaris*

Kidney bean (*Phaseolus vulgaris*) is an important legume crop that is grown widely in Nepal. In December 2010, *P. vulgaris* plants showing severe mosaic, yellowing and leaf curling symptoms were observed in different fields in the vicinity of Chitwan, Nepal (Fig. 1). Disease incidence was 70-80%. To identify the suspected causative viral agent, total DNA was extracted from eleven plants with symptoms and three plants without symptoms, collected from five locations ("Darai village", "Pakaudi", "Pokhara", "Rampure" and "Malepatan") and resolved in agarose gels and blotted onto nylon membranes. A full-length *Mungbean yellow mosaic India virus* (MYMIV) DNA-A clone isolated from mungbean (unpublished results) was used as a probe in Southern hybridisation that revealed bands typical of begomoviruses in all samples with symptoms, but not for symptomless ones, indicating the association of a begomovirus with the disease.

To further confirm the virus identity, begomovirus-specific degenerate primers were used (Briddon *et al*., 1994) in polymerase chain reactions (PCR), using the total DNA preparations as templates. With DNA from plants with symptoms, an amplified product of the expected size was obtained, cloned and sequenced. To sequence the remaining DNA region, a specific primer pair HOG1/ HOG2 (5'-ATGAATTCCTTGGATGTTTC-3/5'-AGGAATTCATTGGGGCCCAA-3') was designed to amplify the full-length begomovirus DNA-A component by PCR. Rolling circle amplification (RCA) was used to clone a DNA-B of MYMIV from samples showing typical mungbean yellow mosaic disease symptoms. Multiple clones harbouring the same restriction fragment length polymorphism (RFLP) pattern were produced, and one from each amplification (designated clones 13P and S31 for DNA-A and DNA-B, respectively) was selected and sequenced completely. No evidence for the association of DNA satellites was found by using DNA101/DNA102 and Bet01/ Bet02 specific primers for DNA satellites (Briddon *et al*., 2002; Bull *et al*., 2003). The DNA-A component isolated from *P. vulgaris* (GenBank Accession No. JN543395) showed the highest sequence identity (SI) of 97% with the DNA-A component of MYMIV-[PK:VrA4:07] (FM208836), followed by 95.7% nucleotide SI with an unpublished DNA-A sequence of MYMIV (AY271895) originating from Nepal and isolated from an unknown host in 2003, for which no cognate DNA-B was identified. The DNA-B component (JN543396) showed the highest level of SI of 99% with the DNA-B of MYMIV-[IN:ND:Cp:04 (AY939925). This indicates that the leaf curl disease of *P. vulgaris* was associated with the bipartite begomovirus MYMIV. Based on the nucleotide SI with MYMIV-[legumes: Pakistan] and the demarcation threshold in species identification (Fauquet *et al*., 2008), the virus isolated from the diseased *P. vulgaris* in Chitwan is considered as a variant of MYMIV and was named as MYMIV-PK[NP:Ch:10]. To our knowledge, this is the first report of natural infection of *P. vulgaris* by MYMIV in Nepal.

References

Briddon RW, Markham P, 1994. Universal primers for the PCR amplification of dicot-infecting geminiviruses. Molecular Biotechnology , 202-205. [http://dx.doi.org/10.1007/BF02921559]

Briddon RW, Bull S, Mansoor S, Amin I, Markham P, 2002. Universal primers for the PCR-mediated amplification of DNA β. A molecule associated with some monopartite begomoviruses. *Molecular Biotechnology* **20**, 315-318. [http://dx.doi.org/10.1385/MB:20:3:315]

Bull S, Briddon RW, Markham P, 2003. Universal primers for the PCR-mediated amplification of DNA 1: A satellite-like molecule associated with begomovirus-DNA β complexes. *Molecular Biotechnology* **23**, 83-86. [http://dx.doi.org/10.1385/MB:23:1:83]

Fauquet CM, Briddon R, Brown JK, Moriones E, Stanley J, Zerbini M, Zhou X, 2008. Geminivirus strain demarcation and nomenclature. *Archives of Virology* **153**, 783-821. [http://dx.doi.org/10.1007/s00705-008-0037-6]

Figure 1

To cite this report: Shahid MS, Pudashini BJ, Khatri-Chhetri GB, Ikegami M, Natsuaki KT, 2012. First report of *Mungbean yellow mosaic India virus* on kidney bean in Nepal. *New Disease Reports* **25**, 30. [http://dx.doi.org/10.5197/j.2044-0588.2012.025.030]

This report was published on-line at www.ndrs.org.uk where high quality versions of the figures can be found.