New Disease Reports

First report of natural occurence of *Papaya leaf crumple virus* on soyabean in India

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Soybean (*Glycine max*, family *Fabaceae*) is a most important nutrient crop. It is native to East Asia and is grown widely as a pulse rich in protein, and also has numerous other uses. Symptoms of leaf crumple disease at Lalitpur (24.6° N, 78.4° E), India and yellow mosaic disease at Lucknow (26.8° N, 80.9° E), India, were noticed on a number of soybean plants in 2013. The naturally infected soybean plants exhibited yellow mosaic on leaves (Fig. 1a) and leaf crumpling and distortion symptoms (Fig. 1b). Severely infected plants were stunted and bore less number of flowers and pods as compared to the healthy ones. Based on leaf crumple and yellow mosaic symptoms, begomovirus infection was suspected. Twenty-two leaf samples of infected soybean plants were collected from each location and total DNA was isolated using a plant genomic DNA isolation kit (Sigma, USA). The PCRs were performed with begomovirus degenerate primers (Rojas *et al*., 1993) which resulted in expected size amplicon of ~1.2 kb from 16/22 samples of yellow mosaic diseased plants collected from Lucknow and 12/22 samples of leaf crumple diseased plants collected from Lalitpur, but not from the healthy samples (Fig. 2). This indicated the presence of begomovirus in the soybean plants that had been sampled.

To identify the complete genome sequence of these begomoviruses, total DNAs isolated from a representative leaf sample exhibiting yellow mosaic and leaf crumple symptoms were subjected separately to rolling circle amplification (RCA, TempliPhi kit, GE Healthcare, USA) followed by digestion with *Eco*RI, *Bam*HI, *Hind*III, *Sal*1, *Xho*I, *Pst*I and *Xba*I enzymes. Electrophoresis of products digested only with *Bam*HI resulted in a DNA fragment of ~2.7 kb in both the samples. The products obtained ~2.7 kb were cloned separately into the pCAMBIA1300 vector, sequenced and deposited in GenBank with Accession Nos. KR052025 (MJS-1 isolate from yellow mosaic sample of Lucknow) and KR071789 (MJS-2 isolate from crumpled leaf sample of Lalitpur). The sequence analyses of MJS-1 and MJS-2 isolates showed the typical genome of begomovirus containing six open reading frames (V2 and V1 in sense strand and C1-C4 in antisense strand). BLASTn analysis of nucleotide sequence of begomovirus isolate MJS-1 under study showed 98-99% nucleotide sequence identity with *Mungbean yellow mosaic India virus* (MYMIV) isolates of *Vigna unguiculata* (DQ389153) and of *G. max* (EU523045, KC852204) reported from India. However, begomovirus isolate MJS-2 shared 94-97% identity with isolates of *Papaya leaf crumple virus* (PaLCrV) from *Carica papaya* (HM140367, HM140368, HM140369) and *Solanum nigrum* (KJ028210) both from India, and *C. papaya* (HE580236) from Pakistan. During phylogenetic analysis by MEGA v6.0 program (Tamura *et al.*, 2013) using nucleotide sequences of MYMIV and PaLCrV and some other begomoviruses available in GenBank database, MJS-1 and MJS-2 isolates showed close relationships with MYMIV and PaLCrV isolates, respectively (Fig. 3), hence the begomovirus isolated from soybean were identified as MYMIV and PaLCrV. A literature survey revealed the natural occurrence of MYMIV (Girish and Usha 2005), *Tomato leaf curl Karnatka virus* (Raj *et al*., 2006a) and *Cotton leaf curl Kokhran virus* (Raj *et al*., 2006b) on soybean in India. The existence of PaLCrV is reported on *C. papaya* (Singh-Pant *et al*., 2012), *S. nigrum* and *Andrographis paniculata* from India. However, this is the first report of natural occurrence of PaLCrV in soybean from India.

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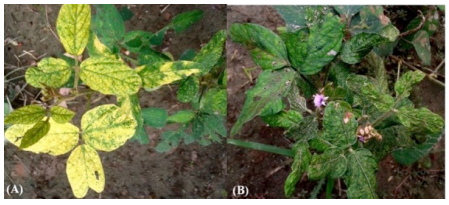

Figure 1

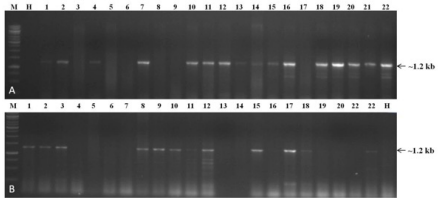

Figure 2

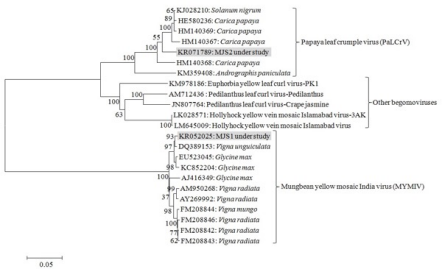

Figure 3

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