Pepper yellow leaf curl Indonesia virus, a new bipartite begomovirus species that belongs to distinct clade of Old World geminiviruses

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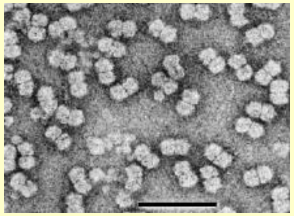
Pepper yellow leaf curl Indonesia virus exhibiting bright yellowing symptoms



Particles of Gemini virus as shown in E.M (Bar=100nm)

Geminiviruses are a large and diverse group of plant viruses characterized by bisegmented (geminate 30 X 20 nm) particles, by their circular ssDNA genome that replicate in the host cell nucleus and by being transmitted in a persistent manner by insect vectors. Emergence of pepper yellow leaf curl disease in Indonesia was first reported in 1999 in West Java (Rusli *et al*.,1999) and in 2003 the disease had been widely spread in Java with the highest incidence and severity occurred particularly in Central Java (Sulandari *et al*., 2006). The emergence of the disease was thought to be associated with upsurge of whitefly population on the crops. The whitefly vector spreaded the virus very quickly throughout the chilli pepper growing area. Later on, an epidemic of this disease was reported in southern and West Sumatra beginning 2005 , imposing a great problem for chili pepper cultivation.

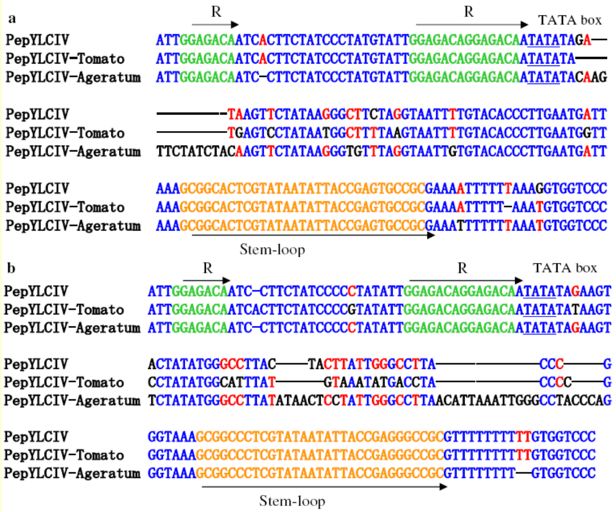
METHODOLOGY

PCR was carried out as described by Sharma et al. (2005) with specific primers designed (Sakata et al. 2009). We constructed infectious clones of pPepVA1.4 and pPeVB3.0 in binary vector pBI121& agro-inoculated to Pepper, *N. benthamiana and* confirmed through southern blot analysis. .

RESULTS AND DISCUSSION

We report here the complete nucleotide sequences of PepYLCIV bipartite begomoviruses from pepper, tomato and ageratum PeYLCIV (Acc No. AB267834-39). DNA-A alone was infectious in pepper and *N. benthamiana* and association with DNA B increases symptom severity, were confirmed through southern blot analysis (Fig.1). Phylogenetic analysis of DNA-A and DNA-B components showed that PepYLCIV is distinct from other geminiviruses and clustered separately from them (Fig.2 a&b). Similarly, PepYLCIV-Tomato, PepYLCIV and ageratum also clustered among PepYLCIV clade. Comparison of CR sequences of the DNA A and DNA B components shows that divergence is due to a 10 ntd. Insertion in the DNA A CR and 20 ntd in DNA B CR, though they shared identical iterons and stem loop sequences, suggesting that they represented two components of the one virus (Fig.3). Tsai et al. (2006) have recently identified the yellow virus on pepper having monopartite genome only .

Pepper yellow leaf curl Indonesia virus-B[Indonesia:2005] (PepLCIV-B[ID:05]) is a distinct begomovirus species isolated from pepper exhibiting leaf curling and bright yellowing symptoms. In addition, we have isolated two new strains of this virus were also reported to infect ageratum and tomato hence designated as *Pepper yellow leaf curl Indonesia virus*-A[Indonesia:Ageratum:2005] (PepLCIV-B[ID:Age:05]) and *Pepper yellow leaf curl Indonesia virus*-A[Indonesia:Tomato:2005] (PepLCIV-B[ID:Tom:05]) respectively. This virus has bipartite genomes organization. All these viruses are transmissible by whitefly *Bemisia tabaci* (Genn.). Pepper virus DNAs from PepLCIV-B[ID:05], PepLCIV-B[ID:Tom:05] and PepLCIV-B-A[ID:Age:05] DNA-As) were noticeably distinct, forming a separate branch from the other viruses infecting pepper. A considerable divergence is observed in the common region (CR) of the genomic components of PepLCIV-B[ID:05] (77%), PepLCIV-B[ID:Tom:05] (82%) and PepLCIV-B-A[ID:Age:05] (75%). PepLCIV-B[ID:05] DNA-A alone is infectious in pepper and *N. benthamiana* plants and association with DNA-B increases symptom severity. Ageratum is a common weed that often grows near tomato and pepper fields in Southern Asia; hence, it could be a reservoir for PepLCIV-B[ID:05].



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a
PepYLCIV           ATTGGAGACAATCACTTCTATCCCTATGTATTGGAGACAGGAGACAATATATAGA--
PepYLCIV-Tomato    ATTGGAGACAATCACTTCTATCCCTATGTATTGGAGACAGGAGACAATATATA----
PepYLCIV-Ageratum  ATTGGAGACAATC-CTTCTATCCCTATGTATTGGAGACAGGAGACAATATATACAAG

PepYLCIV           ----------TAAGTTCTATAAGGGCTTCTAGGTAATTTTGTACACCCTTGAATGATT
PepYLCIV-Tomato    ----------TGAGTCCTATAATGGCTTTTAAGTAATTTTGTACACCCTTGAATGGTT
PepYLCIV-Ageratum  TTCTATCTACAAGTTCTATAAGGGTGTTTAGGTAATTGTGTACACCCTTGAATGATT

PepYLCIV           AAAGCGGCACTCGTATAATATTACCGAGTGCCGCGAAAATTTTTTAAAGGTGGTCCC
PepYLCIV-Tomato    AAAGCGGCACTCGTATAATATTACCGAGTGCCGCGAAAATTTTT-AAATGTGGTCCC
PepYLCIV-Ageratum  AAAGCGGCACTCGTATAATATTACCGAGTGCCGCGAAATTTTTTTAAATGTGGTCCC

b
PepYLCIV           ATTGGAGACAATC-CTTCTATCCCCCTATATTGGAGACAGGAGACAATATATAGAAGT
PepYLCIV-Tomato    ATTGGAGACAATCACTTCTATCCCCGTATATTGGAGACAGGAGACAATATATATAAGT
PepYLCIV-Ageratum  ATTGGAGACAATC-CTTCTATCCCCCTATATTGGAGACAGGAGACAATATATAGAAGT

PepYLCIV           ACTATATGGGCCTTAC----TACTTATTGGGCCTTA-------------CCC----G
PepYLCIV-Tomato    CCTATATGGCATTTAT------GTAAATATGACCTA-------------CCCC---G
PepYLCIV-Ageratum  TCTATATGGGCCTTATATAACTCCTATTGGGCCTTAACATTAAATTGGGCCTACCCAG

PepYLCIV           GGTAAAGCGGCCCTCGTATAATATTACCGAGGGCCGCGTTTTTTTTTGTGGTCCC
PepYLCIV-Tomato    GGTAAAGCGGCCCTCGTATAATATTACCGAGGGCCGCGTTTTTTTTTGTGGTCCC
PepYLCIV-Ageratum  GGTAAAGCGGCCCTCGTATAATATTACCGAGGGCCGCGTTTTTTT--GTGGTCCC
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Fig 3. Pairwise alignment of CR sequences of DNA A and DNA B of Pepper associated viruses

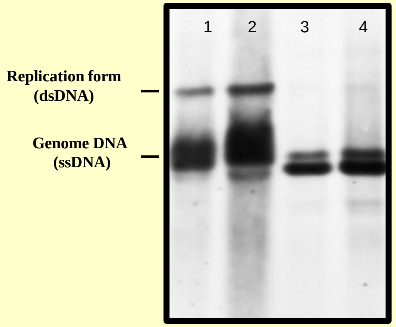

Fig 1. Southern blot analysis of pepper plants infected with PepLCIV-B[ID:05] and viral DNA presence in pepper samples collected from fields (lane 1,2) and agro-inoculation with pPepVA1.4 + pPeVB3.0 clone (lane 3,4) were detected.

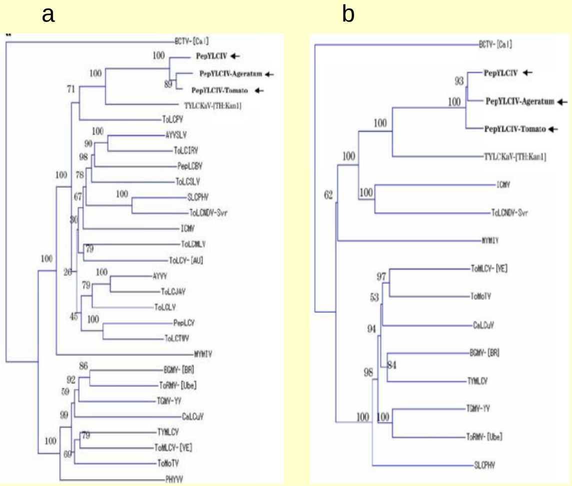

Fig 2. Phylogenetic relationships among begomovirus DNA A (a) and DNA B (b).

References:
Rusli et al. 1999. Bull HPT 11:26; Sulandri et al. 2006, Proc 3rd Asian Conf. Pl. Pathol 140.; sharma et al. 2005 virus genes' Sakata et al. 2009 Arch Virol 153:2307; Tsai et al. 2006. Plant Dis. 90:2247.