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**Thesis Title** - Molecular Characterization of Begomovirus Causing Leaf curl Disease of Tobacco in  
India

### **ABSTRACT-**

The tobacco crop in Pusa, Bihar, India was severely affected by leaf curl disease (TbLCD) and yellow leaf curl disease (TbYLCD). In 2007, 30% incidence of TbLCD was found in many popular varieties of tobacco. Two different begomoviruses were isolated and characterized from TbLCD and TbYLCD affected tobacco plants collected from Pusa, Bihar. The full-length sequence of DNA-A [HQ180391] of the *Begomovirus* associated with TbLCD, showed that it is different from all the previously sequenced begomoviruses as it had <89% sequence homology with all the known begomoviruses. The isolate was, therefore, tentatively named as Tobacco leaf curl Pusa virus [India: Pusa: 2010]. A betasatellite was also associated with TbLCD. Based on its sequence (HQ180395) homology, it was identified as a variant of Tomato leaf curl Bangladesh betasatellite ToLCBDB [IN: Raj: 03]. An alphasatellite (HQ180392) was also associated with the disease, it had highest (87%) nucleotide sequence identity with Tomato leaf curl alphasatellite. The TbYLCD has been described for the first time. The affected plants developed characteristic mild leaf curl and yellowing of the leaves in commercial tobacco fields in Pusa, Bihar. The associated *Begomovirus* was identified as a strain of *Radish leaf curl virus* (RaLCV) based on nucleotide sequence of the viral genome (2761 nucleotides; EU194914). The betasatellite (HQ180397) associated with TbYLCD was identified as a variant of Chilli leaf curl betasatellite (ChLCB). Phylogenetically, the two begomoviruses characterized in this study formed distinct branch in the Asian clade. Phylogenetic analysis of the nucleotide sequences of the betasatellites showed distinct clustering of the betasatellites associated with the diseases of malvaceous and solanaceous crops. Five betasatellites isolated from different tobacco growing villages of Pusa, Bihar varied from 1310 to 1377 nt in the length of their molecules; these molecules had an ORF  $\beta$ C1, an adenine-rich region, and satellite conserved region.

For developing serodiagnostic, the coat protein of TbLCPuV was expressed as recombinant protein in pET expression vector and polyclonal antiserum was produced. The infection was efficiently detected by ELISA and PCR indicating that the polyclonal antiserum raised using recombinant CP protein can be effectively used in diagnosis of TbLCD. To demonstrate Koch's Postulates, an infectious clone of TbLCPuV was prepared and agroinoculated to *Nicotiana benthamiana* and tomato. TbLCPuV alone could induce severe leaf curl in tobacco plants. Co-inoculation of TbLCPuV with the associated betasatellite molecule induced more severe symptom in tobacco plants, and co- inoculation of TbLCPuV with the associated betasatellite and alphasatellite induced symptom similar to plant agroinfected with TbLCPuV and the associated betasatellite tobacco plants.

## FINDINGS

- New species of *Begomovirus* (TbLCPuV) associated with TbLCD was found in northern India.
- A new disease of tobacco with characteristic mild leaf curl and yellowing symptoms on the leaves was observed in 2007 in commercial tobacco fields in Pusa, Bihar, India. The associated begomovirus was identified as a strain of *Radish leaf curl virus* (RaLCV).
- First time Tomato leaf curl Bangladesh betasatellite and Chilli leaf curl betasatellite were found associated with TbLCPuV and RaLCV.
- Alphasatellite associated with TbLCPuV identical to Tomato leaf curl alphasatellite was also found.
- Considerable diversity was found in the begomoviruses causing TbLCD. Five betasatellite isolated from different location in Pusa, Bihar, India varied from 1310 to 1377nt. Their nucleotide sequence identity varied from 88 to 92%.
- The polyclonal antisera was raised against TbLCPuV. The specificity of the antiserum was tested by ELISA and PCR. The infection was efficiently detected by ELISA indicating that the polyclonal antiserum raised against the recombinant CP protein can be effectively used in diagnosis of TbLCD.
- To investigate the infectivity of TbLCPuV, it was alone able to induce severe leaf curl in tobacco plants. TbLCPuV with associated betasatellite molecule induced more severe symptom only tobacco plants and in another combination of TbLCPuV with associated betasatellite and alphasatellite induced similar symptom only in tobacco plants.