

In : 5ème colloque du Réseau Evolution Virale - REV, 2010/09/30-2010/10/01, Montpellier, France.

Frequency and nature of recombinant genomes isolated in plants co-infected with two begomoviruses.

C.Urbino, G.Thébaud, A. Antolik, M.Granier, R.Froissart, S. Blanc, M. Peterschmitt.

Begomoviruses (Family Geminiviridae) are circular ssDNA viruses transmitted by the whitefly *Bemisia tabaci*. They replicate in the nucleus of infected cells by recombination prone mechanisms, rolling circle replication and recombination dependant replication. Recombinant begomoviruses were frequently reported, some of them being implicated in emerging diseases. Consistently, Garcia Andres et al. (2007) showed that recombinant viral genomes occurred frequently in tomato plants 120 days after their inoculation with two tomato yellow leaf curl (TYLC) associated begomoviruses. About 50% of the isolated viral genomes were recombinants and nearly 100% after vector transmission (whitefly *Bemisia tabaci*) to a new tomato plant. This study brought up several questions regarding the delay until recombinants are detected, the distribution of recombination breakpoint and the influence of the vector which is expected to stimulate recombination after transmission in host plant. We addressed these questions by studying the frequency and nature of recombinant genomes in tomato plants coinfecting with two TYLC associated begomoviruses, either by artificial inoculation with *Agrobacterium tumefaciens*, or naturally with *B. tabaci*. The coinfecting viruses were Tomato yellow leaf curl virus (TYLCV) and Tomato leaf curl Mayotte virus (ToLCYTV) which were previously used for generating artificial recombinants using DNA-shuffling (see F. Vuillaume presentation). These two begomoviruses exhibit 18% nucleotide differences spread throughout the genome. Viral genomes isolated from co-infected plants using the commercial TempliPhi kit were cloned and analysed with RFLP assay and sequencing. Several of our results contrast or complete those of Garcia Andres (2007).

- (i) Recombinant genomes were detected as early as 30 days post-inoculation (dpi) but with a relatively low frequency (0-2%). Thereafter, the frequency reached 6% at 60 dpi and 50% at 150 dpi.
- (ii) Recombination breakpoints were not concentrated to a specific region of the genome suggesting that recombination patterns are specific of each pair of begomoviruses.
- (iii) Selected recombinants, i.e. those detected more than one time in a plant at 150 dpi or more, exhibited contrasted patterns between plants co-infected under identical experimental conditions revealing the stochastic determinants of the evolutionary pathways.
- (iv) The supposed vector stimulation of recombination frequency was not detected in our experiment.