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In silico Recombination Analysis: A Study for Geminivirus Host Mobility

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ABSTRACT

Recombination plays a key role in the evolution of geminiviruses and may be contributing to the emergence of new strains/species. The high frequency of mixed infections of begomoviruses in different host allows the emergence of new viruses arising from recombination among species. With the development of computational recombination detection tools and an increasing number of available genome sequences, many studies have reported evidence of recombination in a wide range of Geminiviridae genera. The *in silico* analysis suggested that interspecific recombination has resulted significant diversity among geminiviruses and emergence of new geminivirus diseases.

Key words: Recombination, Geminiviridae, begomoviruses, mixed infections, *in silico*

INTRODUCTION

Geminiviridae is the largest family of plant DNA viruses that infect a broad range of plants causing devastating diseases for important crops (i.e., fruits, vegetables, ornamental plants and fiber crops (Morales and Anderson, 2001; Mansoor *et al*., 2003) and medicinal weeds (Prajapat *et al*., 2011a, b). Geminiviruses are characterized by circular single stranded DNA (ssDNA) genomes that encapsidated in twinned quasi isometric particles. The Geminiviridae family classified into four genera based on the genome organization and host range: *Mastrevirus, Curtovirus, Topocuvirus* and *Begomovirus* (Fauquet and Stanley, 2003; Van Regenmortel *et al*., 1999).

Begomovirus is the largest genus of the plant virus family Geminiviridae which members infect only dicotyledonous plants and transmitted by whitefly (*Bemisia tabaci*). *Begomovirus* those have bipartite genomes, comprising a DNA-A components required for replication and encapsidation and a DNA-B components required for virus movement and each of about 2.6-2.8 kb (Hanley-Bowdoin *et al*., 1999) or monopartite with all genes resident on one (DNA A-like) ssDNA of about 2.8 kb (Stanley *et al*., 2005).

The International Committee on Taxonomy of Viruses (ICTV) recommend taxonomic criterion for species of begomoviruses based on the applicability of these criteria to the large number of characterized begomoviruses. The comparative analyses of full-length DNA-A sequences were considered, based on recombination events that readily occur among begomoviruses (Pita *et al*., 2001; Fauquet *et al*., 2003). The nucleotide sequence identity of the A component up to 89% was established to distinguish different species from strains (Fauquet *et al*., 2003). Genomic organizations of begomoviruses are presented in Fig. 1.

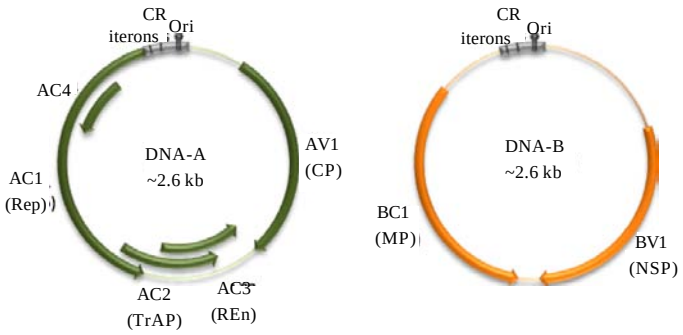

Fig. 1: Genomic organization of begomoviruses: ORF positions and directions of translation are indicated by arrows in DNA-A and DNA-B (Wyant, 2011)

Recombination defines "as the exchange of DNA between similar DNA components" and pseudorecombination is "the exchange of DNA components" (Polston and Anderson, 1997; Belete *et al*., 2011). The diversity of begomovirus species is associated with mixed infections, in which recombination and pseudorecombination events may explain the frequent emergence of new begomoviruses. Both events have been demonstrated in the laboratory (Garrido-Ramirez *et al*., 2000) and under natural conditions (Pita *et al*., 2001). Some isolates of Tomato Yellow Leaf Curl Virus (TYLCV), Potato Yellow Mosaic Virus (PYMV) and Mimosa Yellow Vein Virus (MYVV) are good examples of recombination and pseudorecombination (Pita *et al*., 2001; Monci *et al*., 2002; Prajapat *et al*., 2011b).

Variability of begomovirus species: Since recombination events showed in *Begomovirus* genus, partial sequences were not sufficient to distinguish new species (Fauquet *et al*., 2003). Studies to obtain complete sequences of the genomes of begomoviruses from appropriate samples to determine if they are indeed new species of begomoviruses. In addition recombination and pseudorecombination events can generate severe hybrids, as in the case of African Cassava Mosaic Virus (ACMV) (Pita *et al*., 2001). In less than 15 years ACMV isolates and species are no longer geographically distinct, the viruses are spread throughout the continent (Legg and Thresh, 2000; Pita *et al*., 2001).

Based on the phylogenetic analysis of viral sequences, it was proposed that, analogously to curtoviruses, Tomato pseudo-curly top virus (TPCV) resulted from recombination between mastreviruses and begomoviruses (Briddon *et al*., 1996; Palmer and Rybicki, 1998). A key factor in the genesis and spread of the pandemic was the recombination of two distinct cassava mosaic begomoviruses to produce a novel and more virulent hybrid (Pita *et al*., 2001).

In southern Spain, a natural recombinant between Tomato yellow leaf curl Sardinia virus (TYLCSV) and Tomato yellow leaf curl (TYLCV) was detected and an infectious clone of a recombinant isolate (ES421/99) was obtained and characterized. Field studies revealed that the recombinant strain is becoming the predominant strain in the region in which it was detected (Monci *et al*., 2002).

***In silico* recombination analysis:** Recombination between divergent genomes is a major mechanism by which diversity amongst viruses is generated (Robertson *et al*., 1995). Recombination

Detection Program (RDP) utilized to detect the possibility of recombination in geminivirus isolates by using their sequence information which based on pair wise scanning approach. It runs under Windows 95/98/NT/XP/VISTA/7 and couples a high degree of analysis automation with an interactive and detailed graphical user interface.

The conclusions of recombination studies based on the evaluation of different methods of recombination detection (Posada and Crandall, 2001; Posada, 2002). The recombination breakpoint could be identified by using Recombination detection program [RDP] (Fig. 2), Geneconv (Fig. 3), Maximum-Chi (Fig. 4), Bootscan, Chimaera and 3SEQ methods (Table 1). All these methods were implemented in RDP v.3.44 (Martin *et al*., 2005). Default RDP v.3.44 settings were used throughout (Bonferroni correction and P-value), other than that sequences were considered as circular, consensus daughters were found and breakpoints were polished.

Natural virus recombinants: Newly emerging geminiviruses are causing severe disease epidemics in cotton, grain, legumes, tomato and other staple food and cash crops in tropical and subtropical regions of the world (Boulton, 2003; Khan, 2000). These viruses cause a variety of

Table 1: Different recombination detection methods available in RDP3

Method	Estimates regions	Estimates breakpoints	Identifies recombinants	p-value calculation	References
RDP method	+	+	+	Binomial distribution	Martin and Rybicki (2000)
GENECONV	+	+	+	Blast-Like Karlin-Altschul and Permutation	Padidam *et al*. (1999)
BOOTSCAN	+	+	+	Bootstrapping, binomial distribution and χ^2	Salminen *et al*. (1995)
Maximum χ^2	+/-	+	+	χ^2 and Permutation	Smith (1992)
CHIMAERA	+/-	+	+	χ^2 and Permutation	Posada and Crandall (2001)
3SEQ	+	+	+	Exact test	Boni *et al*. (2007)

(RDP3: Instruction manual at http://darwin.uvigo.es/rdp/rdp.html)

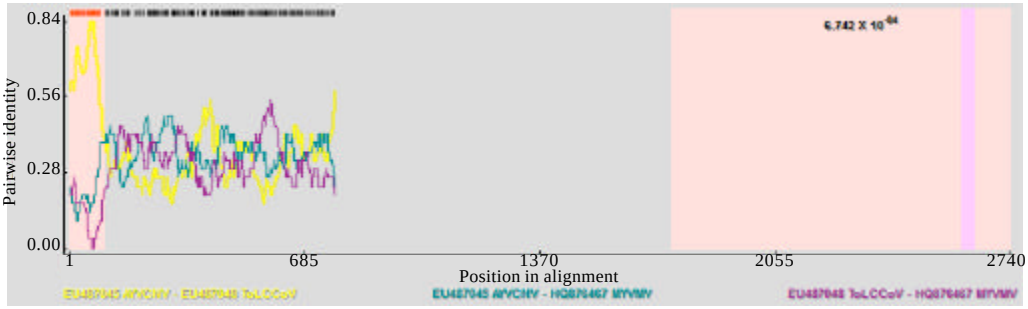

Fig. 2: An RDP pairwise identity plot for the piece of sequence from major parent (EU487045 AYVCNC) that breakpoint begin from 774th [position 1758 in alignment] position in alignment and ending breakpoint at position 94th in alignment of Mimosa Yellow vein virus (HQ876467). Approximate p-value for this region was 6.742×10^{-04}.Uppermost bares indicating positions of informative sites, pink region indicates breakpoint positions suggested by the GENECONV method. The pairwise identity plot have major parent: minor parent plot (EU487045 AYVCNV: EU487048 ToLCCoV; yellow), recombinant: major parent plot (HQ876467 MYVMV: EU487045 AYVCNV; dark blue) and recombinant: minor parent plot (HQ876467 MYVMV: EU487048 ToLCCoV; purple)

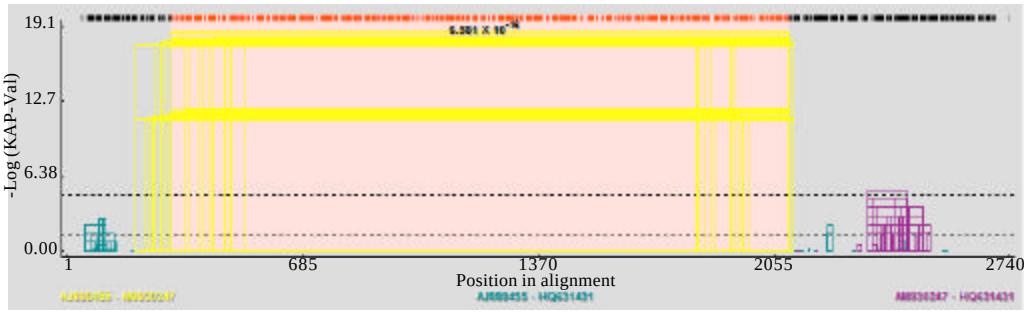

Fig. 3: The GENECONV plot of high scoring fragment (AJ888455) in recombinant *Verbesina encelioides* leaf curls alphasatellite (HQ631431). The approximate p-value was 6.581×10^{-16}. In this case the left and right bounds of the pink region indicate breakpoint positions suggested by the GENECONV method

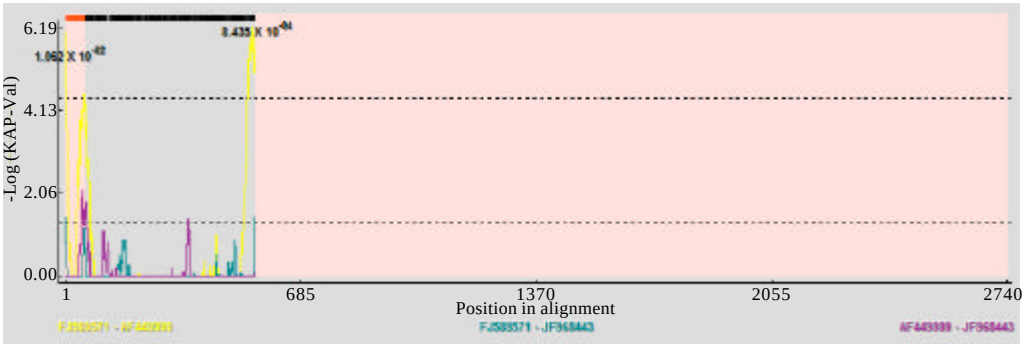

Fig. 4: An example MaxChi plot of recombinant *Ocimum* leaf curl virus (JF968443) for beginning breakpoint position was 557th and ending breakpoint position was 54th in alignment. In this case the left and right bounds of the pink region indicate breakpoint positions suggested by the GENECONV method. Approximate p-values of two peaks were 1.062×10^{-02} and 8.435×10^{-04}. Graph shows the schematic linearized map of putative recombinant fragments within the CP gene of the *Ocimum sanctum* begomovirus and related begomovirus isolates. Each horizontal line represents the genotype of one virus isolate and the color-coded boxes represent the tentative origins of the putative recombinant fragments

symptoms in host plant species and are spreading at an alarming pace due to a high rate of recombination (Briddon *et al*., 2003; Mansoor *et al*., 2003). The rapidly evolve of begomoviruses, due to recombination and pseudorecombination (Hou and Gilbertson, 1996; Padidam *et al*., 1999). The emergence of new begomvirus strains / species in nature by recombination between previously existing species has been demonstrated (Zhou *et al*., 1997; Saunders *et al*., 2001).

It was observed that the different species of geminiviruses seem to recombine easily if infectious pseudorecombinant clones used (Unseld *et al*., 2000). In addition, examples of natural recombination have also been reported recently in cotton (Zhou *et al*., 1998; Sanz *et al*., 1999) and cassava (Zhou *et al*., 1997; Pita *et al*., 2001). In some cases, the new geminiviruses species those arising as a consequence of recombination, exhibited a new pathogenic phenotype which is often

more virulent than the parents (Fauquet *et al*., 2005; Girish and Usha, 2005; Rojas *et al*., 2005; Garcia-Andres *et al*., 2006; Kon *et al*., 2006; Rothenstein *et al*., 2006).

Begomoviruses are considered to be an emergent group of plant viruses, due to the high incidence and severity of diseases caused by them over the last three decades, in tropical and subtropical regions of the world (Polston and Anderson, 1997; Legg and Thresh, 2000; Morales and Anderson, 2001; Briddon *et al*., 2003). *Ageratum conyzoides* plants exhibiting yellow vein symptoms, collected from China, contained begomoviral DNA-A like molecules. Sequence alignment shows that Ageratum Yellow Vein China Virus (AYVCNV) has arisen by recombination among viruses related to Ageratum yellow vein virus, Papaya leaf curl China virus and an unidentified begomovirus (Xiong *et al*., 2007).

Weeds can retain the virus that can be transmitted by the insect vector back to crop plants (Assuncao *et al*., 2006) causing yield loss of the crops. Additionally, because they act like virus reservoirs, recombination and generation of new viral genomes is facilitated (Frischmuth *et al*., 1997; Jovel *et al*., 2007; Morales and Anderson, 2001). Once present in the new host, these indigenous viruses would have rapidly evolved via recombination and pseudo recombination, giving rise to the species currently detected in the field (Castillo-Urquiza *et al*., 2008). The new geminiviruses originating from molecular recombination or pseudorecombination, as has been exemplified by *Sida micrantha* mosaic-associated viruses (SimMV). One of such viruses has developed recently and naturally by recombination between a DNA-A and a DNA-B components of different ancestors (Jovel *et al*., 2007).

The presence of multiple and recombinant betasatellites in *Digera arvensis* indicates that weeds could be important sources of multiple begomovirus components that affect crop plants. The presence of a recombinant betasatellite suggested that weeds are likely vessels for recombination and evolution of components of begomovirus complexes (Mubin *et al*., 2009). *Rhynchosia minima* (family Fabaceae) weed species exhibiting bright golden mosaic symptoms were previously associated with begomovirus infection in Yucatan, Mexico. Recombination analysis of the Rhynchosia yellow mosaic Yucatan virus (RhYMYuV) genome indicated that the DNA-A component has arisen through intermolecular recombination (Hernandez-Zepeda *et al*., 2010). Therefore, the high frequency of mixed infections of begomoviruses facilitates the emergence of new viruses arising from recombination among strains/species (Harrison and Robinson, 1999; Power, 2000).

CONCLUSIONS

Viruses cause a variety of symptoms in host plant species and are spreading easily due to a high rate recombination and pseudorecombination events that contribute in the evolution of new virus strains/species. Inter-specific recombination has resulted in remarkable diversity among geminiviruses and that is the major cause of the emergence of new geminivirus diseases in tropical and subtropical regions. In some cases, the recombinants exhibited a new pathogenic phenotype which is often more virulent than the parents. With the development of reliable computational recombination detection tools and an increasing number of available genome sequences, many research reports have demonstrated evidence of recombination in a wide range of Geminiviridae genera.

This study could be used to understand the role of recombination and pseudorecombination in evolution of new Geminiviridae species and genetic diversity information could be considered for the planning of disease management strategies.

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