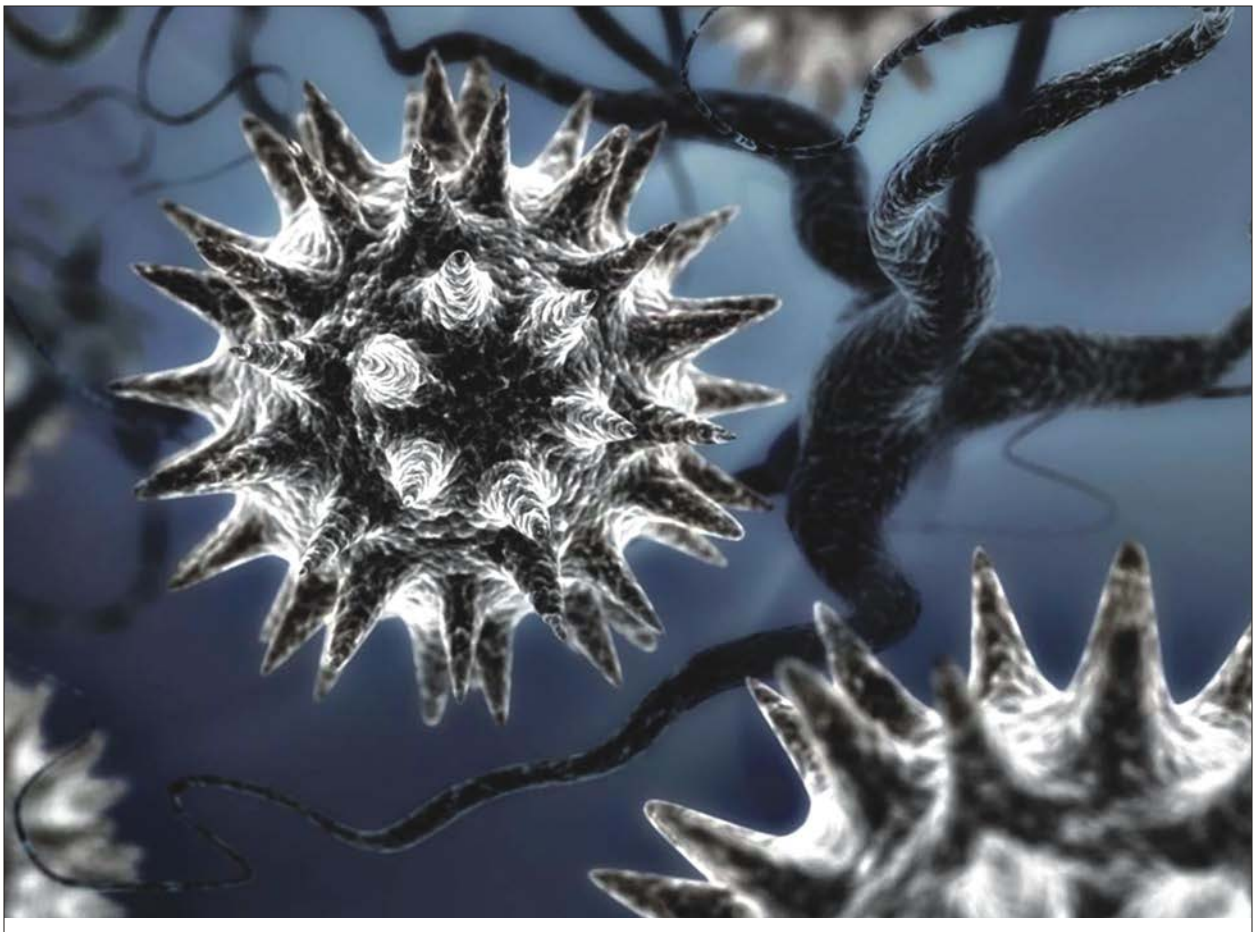

International Journal of Virology

ISSN 1816-4900

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS International Journal of Virology

ISSN 1816-4900
DOI: 10.3923/ijv.2016.10.17

Research Article

First Report of Recombination Analysis of Betasatellite and Aplhasatellite Sequence Isolated from an Ornamental Plant Marigold in India: An *in silico* Approach

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Abstract

The aim of this study were to present and highlight the *in silico* recombination analysis approach for in depth study about the nature of the virus. To detect the possibility of recombination in betasatellite and alphasatellite, Recombination Detection Program (RDP) was utilized, which is based on a pair wise scanning approach. The betasatellite and alphasatellite genomes were isolated from an ornamental plant Marigold and is identified as a new recombinant species, sharing nucleotide identity with other isolates reported from China and Pakistan. One factor favoring the spread of viruses among these plants is that many dicotyledonous species in India are hosts of whiteflies of the *B. tabaci* complex, which are the known or likely vectors of all the viruses. The present study remarkably suggests that the exchange of DNA with other viruses would create a new disease complex posing a serious threat to agriculture crops and horticulture ornamental plants production.

Key words: Geminivirus, Begomovirus, horticulture crops, infection

Received: August 22, 2015 Accepted: November 22, 2015 Published: June 15, 2016

Citation: Avinash Marwal, Rajneesh Prajapat and R.K. Gaur, 2016. First Report of Recombination Analysis of Betasatellite and Aplhasatellite Sequence Isolated from an Ornamental Plant Marigold in India: An *in silico* Approach. Int. J. Virol., 12: 10-17.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Ornamentals can retain the virus that can be transmitted by the insect vector back to crop plants (Assuncao *et al*., 2006) causing yields loss of the crops (Morales and Anderson, 2001). Additionally, because they act like virus reservoirs, recombination and generation of new viral genomes is facilitated (Frischmuth *et al*., 1997). Once present in the new host, these indigenous viruses would have rapidly evolved via recombination and pseudo recombination (Jovel *et al*., 2007), giving rise to the species currently detected in the field (Castillo-Urquiza *et al*., 2008).

Recombination has played and continues to play, a pivotal role in geminiviral evolution and may be contributing to the emergence of new forms of geminiviruses because the high frequency of mixed infections of Begomoviruses provides an opportunity for the emergence of new viruses arising from recombination among strains and/or species (Harrison and Robinson, 1999). In some cases, the recombinants exhibited a new pathogenic phenotype which is often more virulent than the parents (Zhou *et al*., 1997).

With the development of reliable computational recombination detection tools and an increasing number of available genome sequences, many studies have reported evidence of recombination in a wide range of virus genera. The computational analysis suggested that interspecific recombination has resulted in remarkable diversity among geminiviruses and could be a major cause of the emergence of new geminivirus diseases (Padidam *et al*., 1999).

It was earlier reported the molecular characterization of complete genome of a betasatellite and alphasatellite isolated from an ornamental plant Marigold (Marwal *et al*., 2013) i.e., Ageratum leaf curl betasatellite (ALCB: KC589700) and Marigold leaf curl alphasatellite (MLCuA: KC206078). The article presents and highlights the *in silico* recombination analysis approach for in depth study about the nature of the virus. Thus, in order to take a step forward to find a cure against such viruses that causes major crop loss worldwide.

MATERIALS AND METHODS

Recombination between divergent genomes is believed to be a major mechanism by which diversity amongst viruses is generated (Robertson *et al*., 1995). To detect the possibility of recombination in betasatellite and alphasatellite, Recombination Detection Program (RDP) was utilized, which is based on a pair wise scanning approach. It usually 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 (Posada and Crandall, 2001). Using various recombination detection methods the conclusion of recombination studies were evaluated (Posada, 2002). The recombination breakpoint could be identified by using RDP, GENECONV, Maximum-Chi, BOOTSCAN, CHIMAERA and 3SEQ methods. All these methods were implemented in RDP v.3.44 (Martin *et al*., 2005). Recombination positions were recognized by only RDP method and other methods such as GENECONV, Bootscan, Maxchi and Chimera were not found suitable for recombination analysis because of lowest recombination breakpoint detection accuracy.

The Ageratum leaf curl betasatellite (ALCB: KC589700) and Marigold leaf curl alphasatellite (MLCuA: KC206078) sequence were subjected to recombination analyses in the year 2014 using RDP method used to drive automated recombination scan and the manual checking of automated analysis results. Analysis was allowed by employing Bonferroni correction with confidence greater than 95% (p-value 0.05). In RDP analysis, the length of the window was set to 10 variable sites and the step size was set to one nucleotide. p-values were estimated by randomizing the alignment 1,000 times.

RESULTS AND DISCUSSION

The specific recombination events including the recombination breaks and hot spots have not been reported so far in Marigold infecting betasatellite and alphasatellite. It is also currently unknown as to whether the sequences in particular parts of the Begomovirus genomes are exchangeable between different species and/or members of the same genus from different geographical locations. In addition, Northern India seems to be unusually rich in virus biodiversity; which were investigated for the extent of recombination events and examined their role in the evolution of virus in India and its neighboring countries.

***In silico* recombination analysis of Ageratum leaf curl betasatellite (ALCB: KC589700):** Besides the apparent importance of recombination in begomovirus evolution the marks that it has left on currently sampled Begomovirus genome sequences also have major implications when it was attempted to use these sequences to infer the evolutionary histories of begomoviruses (Garcia-Andres *et al*., 2006). Consequently, the detailed characterization of recombination amongst marigold infecting Begomovirus is a prerequisite for understanding how these important pathogens are evolving. While analyzing the betasatellite molecule associated with leaf curl disease of ornamental Marigold plants, a total of four

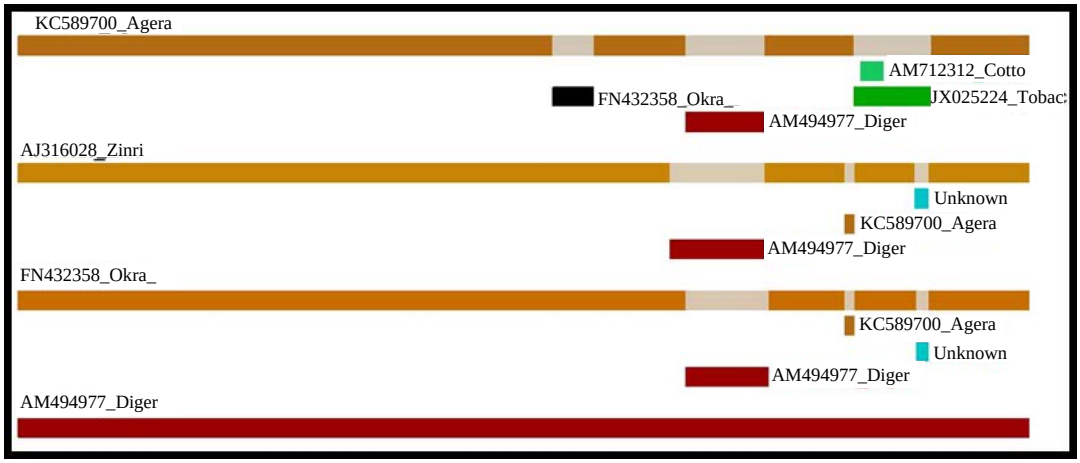

Fig. 1: Diagram of the schematic sequence display representing the RDP recombination map of the recombinant fragments for the Ageratum leaf curl betasatellite (ALCB: KC589700) sequence. Each color/pattern represents a sequence specific of a virus. The virus genome organization is represented under the diagram, positioning the different viral genes named according to the Begomovirus convention

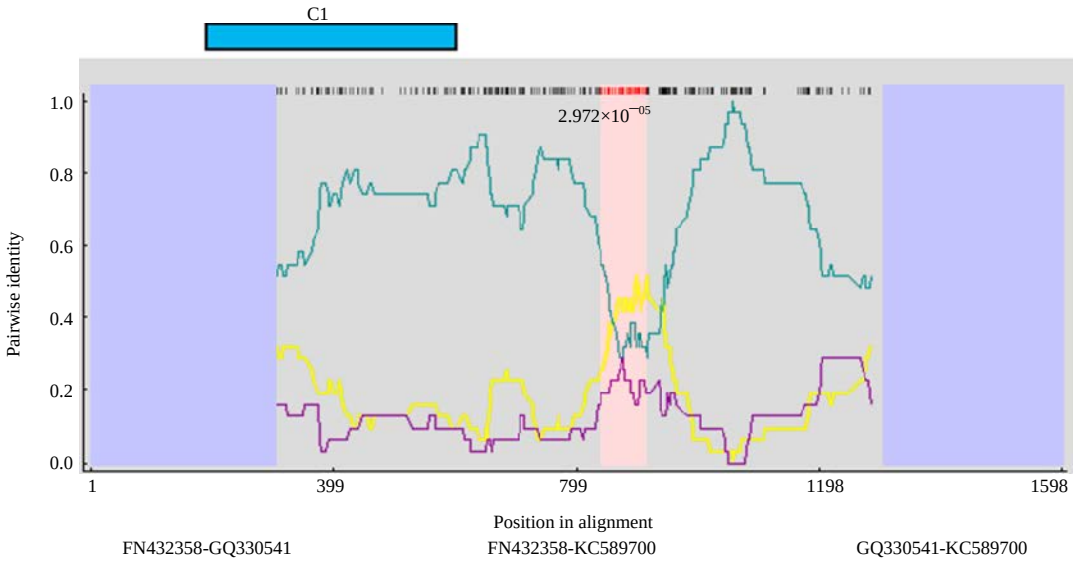

Fig. 2: An RDP pairwise identity plot for the piece of sequence from the major parent (FN432358_Okra). Uppermost bares indicating positions of informative sites; pink region indicates breakpoint positions suggested by the RDP software method. The pairwise identity plot have major parent: minor parent plot (FN432358_ Okra: GQ330541_Peppe; yellow), major parent: recombinant plot (FN432358_ Okra: KC589700_ Agera; dark blue) and minor parent: recombinant plot (GQ330541_ Peppe: KC589700_ Agera; purple)

recombination events were detected in the genome as exhibited by schematic sequence display (Fig. 1).

The first evidence is given in Fig. 2 where breakpoint begin from 717th [position 842 in alignments] position and ending breakpoint ends at 783th [position 910 in alignments] position. Approximate p-value for this region was 2.947×10^{-04}. The beginning breakpoint probability was found as 0.048 and the ending breakpoint probability was recorded to be 821.2. The region probability (MC uncorrected) was 2.607 E-08 and region probability (MC corrected) was 2.972 E-05. The ORF beta C1 is devoid of any recombination but recombination was observed in A rich region of the

betasatellite sequence. The major parent was identified as Okra leaf curl virus satellite DNA beta (FN432358) identified in Pakistan and were found infecting *Sonchus arvensis*. Whereas the minor parent was Pepper leaf curl virus satellite beta DNA (GQ330541) found infecting pumpkin in India.

To date, many natural begomoviruses recombinants have been reported (Sanz *et al*., 1999). Although the biological significance of begomovirus recombination is not clearly understood, in many parts of the world epidemics associated with the emergence of recombinant begomoviruses have been reported. The second recombination was again detected in the A rich region downstream of the first recombinant sequence where breakpoint begin from 90th [position 1053 in alignments] position and ending breakpoint ends at 965th [position 1180 in alignments] position (Fig. 3).

Approximate p-value for this region was 6.005×10^{-03}. Here the contribution of major parent in the RDP plot was by Digera arvensis yellow vein disease-associated DNA beta (AM494977) infecting *Digera arvensis* in Pakistan. The minor parent was identified as Cowpea severe leaf curl-associated *DNA beta* (AY728263) infecting cowpea plant in India. The beginning breakpoint probability was found as 4002.5 and the ending breakpoint probability was recorded to be 0.682. The region probability (MC uncorrected) was 5.268 E-06 and region probability (MC corrected) was 6.005 E-03. The recombination sites are distributed non-randomly along the genome. The third recombination breakpoints were detected further downstream of the second recombinant fragment of the betasatellite sequence. Beginning breakpoint position was 1085th [position 1328 in the alignment]and ending breakpoint position was 1122th [position 1367 in the alignment]. The region probability (MC uncorrected) was 6.216 E-14 and region probability (MC corrected) was 7.086 E-11.Approximate p-value for this region was 7.086×10^{-11}. In this case the major parent was identified as Okra leaf curl virus satellite beta DNA (FN432358) identified in Pakistan and were found infecting *Sonchus arvensis* (Fig. 4). The minor parent in the RDP plot was found to be Cotton leaf curl betasatellite (AM712312) causing disease symptoms in *G. annumalum* in Pakistan.

Recombination sites have been reported in both the DNA and RNA viruses (Prasanna and Rai, 2007). Presumably, the different pathotypes could simultaneously infect a host cell and exchange genetic materials through recombination. The recombination observed between geographically separated isolates probably represents older events, which may have occurred before their present separation (Gagarinova *et al*., 2008). Movement of vectors and/or infected plant materials could be another factor for the gene flow between the widely separated locations (Rojas *et al*., 2005).

The final and the fourth recombination were detected. In this case the breakpoint begins from 1076th [position 1319 in

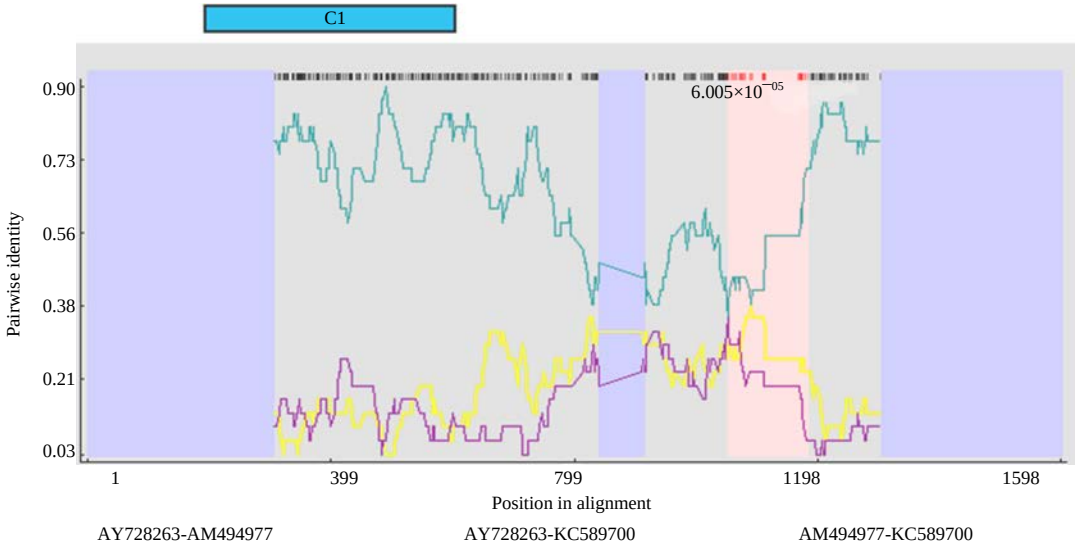

Fig. 3: An RDP pairwise identity plot for the piece of sequence from the major parent (AM494977_Diger). Uppermost bares indicating positions of informative sites; pink region indicates breakpoint positions suggested by the RDP software method. The pairwise identity plot have major parent: minor parent plot (AM494977_Diger: AY728263_Cowpe; yellow), major parent: recombinant plot AY728263_Cowpe: KC589700_ Agera; dark blue) and minor parent: recombinant plot (AY728263_Cowpe: KC589700_ Agera; purple)

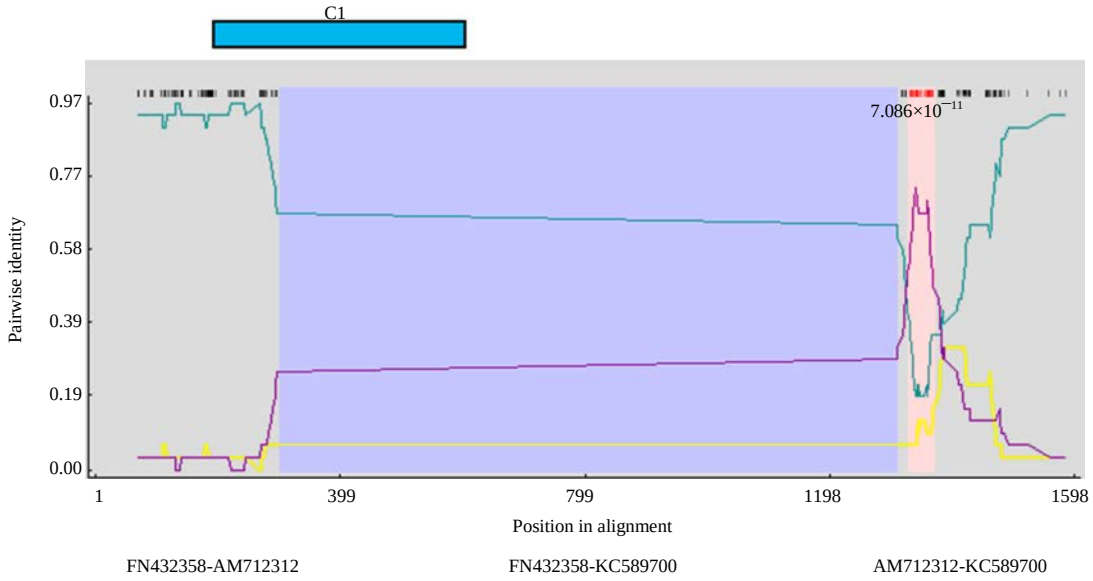

Fig. 4: An RDP pairwise identity plot for the piece of sequence from the major parent (FN432358_Okra). Uppermost bares indicating positions of informative sites; pink region indicates breakpoint positions suggested by the RDP software method. The pairwise identity plot have major parent: minor parent plot (FN432358_ Okra: AM712312_Cotto; yellow), major parent: recombinant plot (FN432358_ Okra: KC589700_ Agera; dark blue) and minor parent: recombinant plot (AM712312_Cotto: KC589700_ Agera; purple)

alignments] position and ending breakpoint ends at 1195th [position 1442 in alignments] position. Approximate p-value for this region was 1.130×10^{-12}. The major parent was encountered to be Okra leaf curl virus satellite beta DNA (FN432358) identified in Pakistan and were found infecting *Sonchus arvensis* (Fig. 5) and the minor parent was discovered as Tobacco leaf chlorosis betasatellite (JX025224) responsible for begomovirus symptoms in tobacco plants accounted in India. The KA p-value was recorded as 9.929 E-16 and the Global KA p-value was recorded as 1.130 E-12.

It may be wiser to develop virus derived resistance strategies using genome regions that are less recombinogenic as this will make it more difficult for viruses to overcome resistance by simply replacing targeted genome regions with variants that are not targeted. The major parent was found to be the same in first and third and fourth recombination event. The interesting findings were the evolution of Ageratum leaf curl betasatellite (ALCB: KC589700) sequence from betasatellites prevailing in India, as well as from other isolates existing in Pakistan. Therefore this is the first report of recombination in betasatellite infecting Marigold.

***In silico* recombination analysis of Marigold leaf curl alphasatellite (MLCuA: KC206078):** Diversity among viruses is generated by mutation, recombination, reassortment and Denovo gene acquisition. Viruses are believed to evolve much faster than their hosts due to lack of fidelity in replication as well as short duration of multiplication. As per the result of sequence display (Fig. 6) only one recombination event was detected in Marigold leaf curl alphasatellite (MLCuA: KC206078). Sequences used for the comparison were obtained from the Gen Bank database. The detection of potential sequences, identification of likely parent sequences and localization of possible recombination break points were carried out using RDP method.

In begomoviruses the recombination hot-spots map to complementary-sense gene transcription initiation and termination sites and virion strand origins of replication. The reason complementary gene transcription initiation and termination sites may be more predisposed to recombination than other sites is possibly that these are the regions where the most frequent clashes between transcription and replication complexes occur (Lefeuvre *et al*., 2007).

Remarkably the recombination was detected in a small portion of the rep ORF of the aplhasatellite. The beginning breakpoint position was 936th [position 1101 in the alignment] and ending breakpoint position was 974th [position 1147 in the alignment]. The region probability (MC uncorrected) was 9.393 E-09 and region probability (MC corrected) was 2.686 E-06. Approximate p-value for this

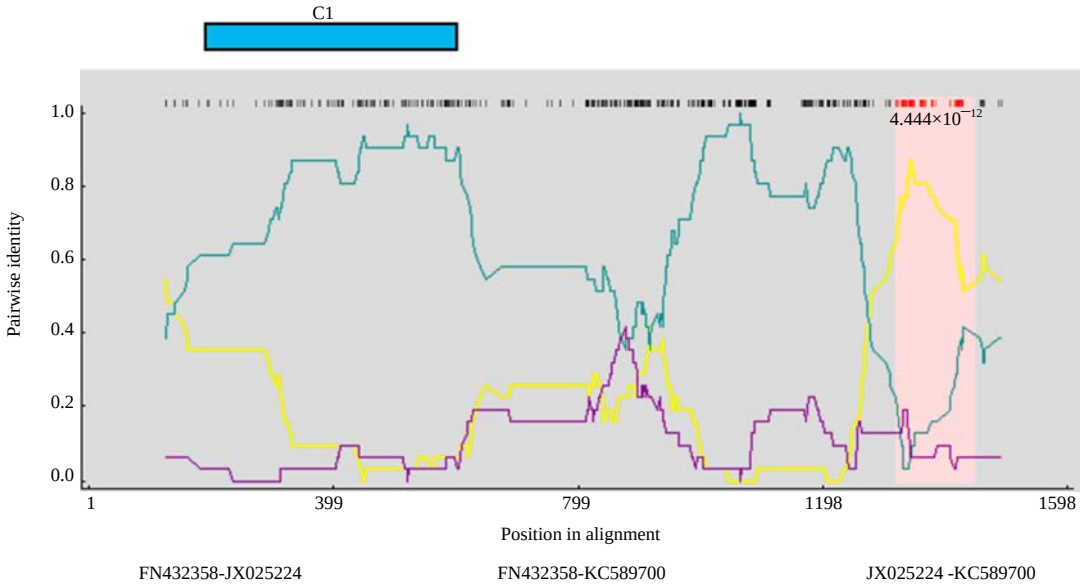

Fig. 5: An RDP pairwise identity plot for the piece of sequence from the major parent (FN432358_Okra). Uppermost bares indicating positions of informative sites; pink region indicates breakpoint positions suggested by the RDP software method. The pairwise identity plot have major parent: minor parent plot (FN432358_ Okra: JX025224_Tobac; yellow), major parent: recombinant plot (FN432358_ Okra: KC589700_ Agera; dark blue) and minor parent: recombinant plot (JX025224_Tobac: KC589700_ Agera; purple)

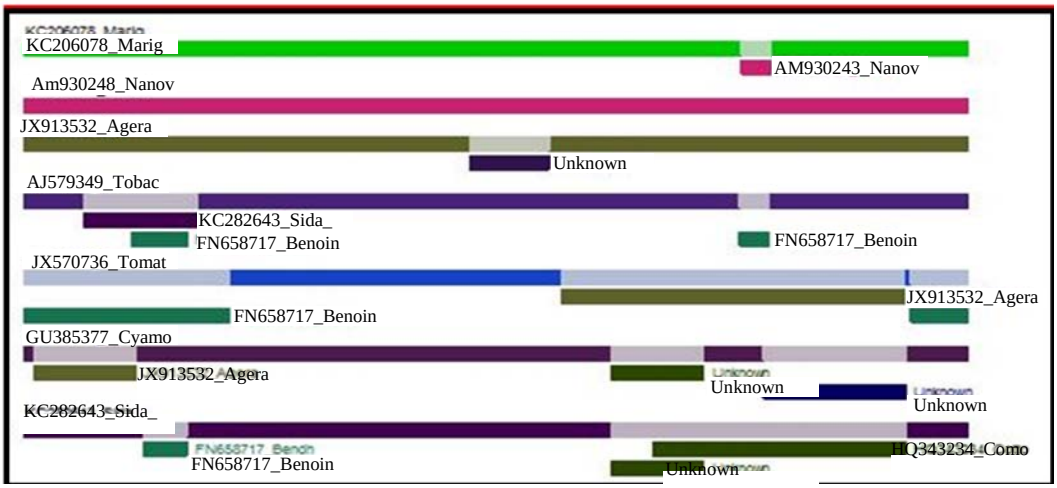

Fig. 6: Diagram of the schematic sequence display representing the RDP recombination map of the recombinant fragments for the Marigold leaf curl alphasatellite (MLCuA: KC206078) sequence. Each color/pattern represents a sequence specific of a virus. The virus genome organization is represented under the diagram, positioning the different viral genes named according to the begomovirus convention

region was 2.686×10^{-06}. In this case the major parent was identified as nanovirus-like particle rep gene for truncated replication associated protein, clone UK8 (AM930248) identified in Pakistan and were found infecting *Sonchus arvensis* (Fig. 7). The minor parent in the RDP plot was found to be Tobacco curly shoot virus associated DNA 1 rep gene for replication-associated protein (AJ579349) found in association with tobacco plants in China.

Both the major and minor parents are from different countries and are responsible for the alphasatellite evolution.

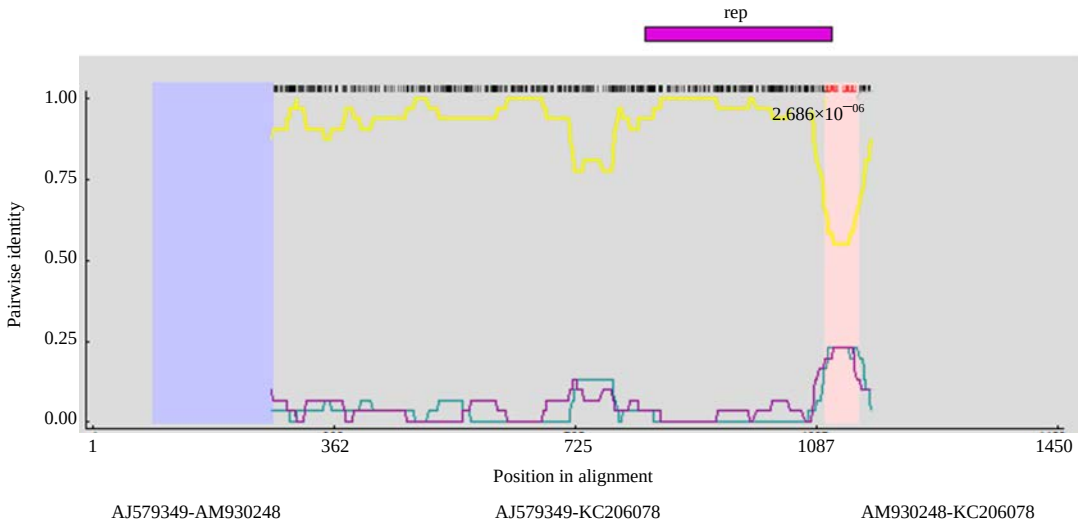

Fig. 7: An RDP pairwise identity plot for the piece of sequence from the major parent (AM930248_Nanov) Uppermost bares indicating positions of informative sites; pink region indicates breakpoint positions suggested by the RDP software method. The pairwise identity plot have major parent: minor parent plot (AM930248_Nanov: AJ579349_Tobac; yellow), major parent: recombinant plot (AM930248_Nanov: KC206078_ Marig; dark blue) and minor parent: recombinant plot (AJ579349_Tobac: KC206078_ Marig; purple)

These data provide evidence of the significant contribution of recombination to the genetic diversification of emerging begomovirus population structures in India from neighboring countries. Therefore this is the first report of recombination in alphasatellite infecting Marigold.

CONCLUSION

A recombination may result in significant changes in the biological properties of virus isolates with the ability to adopt and sustain in different environmental conditions. The interesting findings were the evolution of Ageratum leaf curl betasatellite (ALCB: KC589700) and Marigold leaf curl alphasatellite (MLCuA: KC206078) sequence from isolates prevailing in India, as well as from other isolates existing in Pakistan and China. It is also possible that exchange of genome could extend the virus host range thereby emergence of new diseases in cultivated crop plants and other ornamental plants. One factor favoring the spread of viruses among these plants is that many dicotyledonous species in India are hosts of whiteflies of the *B. tabaci* complex, which are the known or likely vectors of all the viruses. Perhaps this is the first report of recombination in betasatellite and alphasatellite infecting Marigold, which would provide significant information for understanding the diversity and evolution of viruses in India.

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