RESEARCH ARTICLE

GENOME SEQUENCE ANALYSIS OF DNA-A FROM *OKRA YELLOW VEIN MOSAIC VIRUS* ISOLATES

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ARTICLE INFO

ABSTRACT

Entire DNA-A of four *okra yellow vein mosaic virus* (OYVMV) Indian biotypes were isolated from virus infected leaf tissue of okra using PCR amplification. Isolated complete DNA-A from experimental biotypes were cloned and sequenced. DNA-A sequences from all these biotypes were compared and further analyzed using the bioinformatics tools. ClustalW analysis of these sequences confirmed variation into the DNA-A amongst OYVMV isolates. It is observed that out of the AV1 gene which encodes coat protein (CP) is the most conserved region, and AC4 protein the most variable. Results also concluded that there is variability in DNA-A of all four experimental biotypes. There are recombinational host spots within DNA-A of these four biotypes. Among all four experimental biotypes of OYVMV, Madurai biotype was found to be more recombinant and formed by major genetic changes into its DNA-A.

INTRODUCTION

Okra (*Abelmoschus esculentus* L) is commonly known as bhindi or lady's finger belonging to family *Malvaceae*. It is an important fruit vegetable crop cultivated in various states of India. Several species of the genus *Abelmoschus* are grown in many parts of the world among them *Abelmoschus esculentus* is most commonly cultivated in Asia and has a great commercial demand due to its nutritional values. The genus *Abelmoschus* was established by Medikus in 1787. However most authors followed De-Candolle (1824) and treated it as a section of *Hibiscus*. One of the major problems with this crop is infection and yield loss due to the fast growing, widely spread *okra (Bhindi) yellow vein mosaic virus*. The *okra yellow vein mosaic virus* (OYVMV) disease is characterized by a homogenous interwoven network of yellow vein enclosing islands of green tissue within its leaf. In extreme cases, infected leaves become yellowish or creamy color. If plants are infected within 20 days after germination, their growth is retarded, few leaves and fruits are formed and the loss may about 94%. The extent damage declines with delay in infection of the plants. Plants infected 50 to 65 days after germination suffer a loss of 49-84% respectively (Sastry and Singh, 1974). The vector transmitting the *okra yellow vein mosaic virus* is *Bemisia tabaci* Genn.

Several attempts have been made to manage the whitefly (Pun *et al.,* 1999). But the recombinations occurring within the genome of geminiviruses created difficulties to develop the resistance or effective tolerance against the *okra yellow vein mosaic virus.* Geminiviruses belongs to the family Geminiviradae and have circular single stranded (ss) DNA genome which is responsible for major crop losses worldwide (Moffat, 1999). Most of the Begomoviruses have bipartite genome, termed DNA A and DNA B. DNA A and DNA B share the region of ~ 200 nucleotides (nt) within the intergenic region called the common region. The DNA A component encodes the proteins required for viral DNA replication (Sunter *et al.,* 1987), while the DNA B encodes two proteins that are essential for systemic movement and symptom expression (Brown and Nelson, 1988, Nouiry *et al.,* 1994).

Recently certain monopartite Begomoviruses indicating *Agerantum yellow vein mosaic virus* (AYVMV), *Cotton leaf curl virus* (CLCuV), some Begomoviruses infecting tomato (TYLCV) and tobacco (TLCV) in China and *Okra (Bhindi) yellow vein mosaic virus* (OYVMV) in India have been found to require a satellite molecule called β (beta) for introduction of disease symptoms in the same host plants (Briddon *et al.,* 2001, Jose and Usha, 2003, Zhou *et al.,* 2003). Begomovirus genomes have either one (monopartite) or two (bipartite) DNA components ranging from 2.5 to 2.8 Kb in size. These viruses replicate in the host cell nucleus

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via a double-stranded (ds) DNA intermediate, termed replicative form (RF). The RF is used as a template for transcription as well as replication. Both strands code for viral proteins (Hanley-Bowdoin *et al.,* 1999). The DNA A of bipartite begomoviruses and monopartite begomoviruses have a very similar genome organization and encode 5-6 overlapping open reading frames (ORFs). The virion-sense strand (V) of DNA A encodes the coat protein (CP, *AV1/V1*) that encapsidates the viral ssDNA. The DNA A of Old World begomoviruses encodes an additional ORF AV2/V2 that has been implicated in virus movement (Padidam *et al.,* 1996, Rigden *et al.,* 1993). The DNA A complementary-sense (C) strand encodes the replication-associated protein (Rep, *AC1/C1*), a transcriptional activator protein (TrAP, AC2/C2), and a replication enhancer protein (REn, *AC3/C3*). TrAP is involved in the control of both viral and host gene expression. Some DNA A of bipartite viruses and all monopartite viruses encodes *AC4/C4* that participates in cell-cycle control (Briddon & Stanley, 2006).

Natural recombination between TYLCV-Israel and TYLCV has been reported by Navas-Castillo *et al.,* (2000). Evidence has been provided by Kirthi *et al.,* (2002) that AV1, AV2, AC1 and intergenic regions of the viral genome contain potential sites of recombinations among TYLCV strains / species. In China, several Begomoviruses infecting squash, tobaco and tomato have been reported (Zhou *et al.,* 2001; Xie *et al.,* 2002). Jovel *et al.,* (2004) cloned and sequenced two molecules of DNA A (A1, A2) and three of DNA B (B1, B2, B3) of *Abutilon mosaic virus.* Their results demonstrated that the intergenic regions of DNA B2 appears to be the product of the recombination between DNA B1 and DNA A2. These results showed that a co infection of begomoviruses could persist over decades, producing a reservoir of partially recombined but distinct geminiviruses.

MATERIALS AND METHODS

Genomic DNA isolation from virus infected leaf

Whole genomic DNA extraction was carried out from the young tender leaf samples of Yellow Vein Mosaic Virus infected Okra. Four virulent biotypes were selected from India. These were from 1) Madurai, 2) Aurangabad, 3) Delhi and 4) Abohar. These different biotypes covered all different parts of India i.e. South, Central and North India. Okra yellow vein mosaic virus DNA A sequences were collected from various accessions from the NCBI (National Center for Biotechnology Information) site. The DNA A sequences were further analyzed using Bioinformatics tools. The genome specific primers were designed to get the DNA A genomes amplified through the PCR amplification.

Genomic DNA of okra was isolated by using protocol of Lodhi *et al.,* (1994) devised for genomic DNA isolation from Grape vine. Since okra plant contains latex type substance which may degrade the DNA, PVP was added to the extraction buffer during grinding of leaf samples. Quality of the isolated DNA of the entire four samples was checked on 0.8% agarose gel. 20 µl of DNA loaded into the gel with the 5 µl of gel loading dye.

PCR amplification of DNA-A using genome specific primers

Master mix for PCR was prepared by adding 2 µl of Taq Buffer (10X), 2 µl dNTP's (10 mM), 0.4 µl of Taq Polymerase (3U/µl), 7 µl of BSA (1mg/ml), 0.2 µl of Tween 20, 2 µl of DNA-A genome specific forward primer reverse primer, 3 µl of template DNA from each biotype and 3.4 µl of SMQ to make final volume to 20 µl. All the above components were mixed in a 0.2 ml PCR tube and PCR machine was set by giving the programs for first denaturation at 94°C for 3 minutes, second denaturation at 94°C for 15 seconds, annealing for 30 seconds and extension at 72°C for 5 minutes. Each PCR reaction was performed with 38 cycles.

All PCR amplicons were analyzed on 0.8% agarose gel in 1X TAE, agarose gel was prepared by dissolving the 0.8 gm of agarose. Agarose gel was stained with Ethidium bromide (EtBr). Ethidium bromide stain was prepared by adding the 5 µl EtBr stock solution (10 mg/ml) to 100 ml TBE running buffer.

Cloning of PCR amplicons

The ligation of the amplicon was carried out as per the user's manual provided with the pGEM-T Easy Vector Kit (Promega, USA). *Escherichia coli* JM109 and DH5α was used as the host cell. The ligation mixture was prepared by adding 5 µL of 2X rapid ligation buffer, 2 µL of vector (100-150 ng), 6 µL insert (300-400 ng) and 1 µL T4 DNA ligase (3 U/µL). Final volume was made to 15 µL with SMQ water and a ligation reaction was set up. For getting the maximum number of transformants, the reaction was performed overnight at 4°C. Plasmid DNA was isolated by using alkaline lysis method (Sambrook *et al.* 1989).

Preparation of Competent Cell Lines

To get the pure culture for carrying the insert DNA, a single cell culture of *E. coli* JM 109 was developed. From these cultures, a single colony was inoculated in 2 mL of LB medium and grown overnight at 37°C. Then, 50 mL of LB medium was inoculated with a 5 mL overnight culture of the *E. coli* and grown at 37°C at 200 rpm. When an optimal density (OD_{600}) of 0.5-0.8 was reached, cells were chilled on ice for 15-20 min and centrifuged at 4000 rpm at 4°C for 15 min. The cells were then washed with one volume (50 mL) of ice cold sterile water and centrifuged again. The pellet was resuspended in 1 mL of 100 m*M* $CaCl_2$. This was then dispensed in 200 µL aliquots to eppendorf tubes and kept at 4°C overnight. The cells were finally suspended in 0.004 volumes (2 mL) of ice cold glycerol. Aliquots of 25-50 µL were placed into ice cold eppendorf tubes, snap frozen in liquid nitrogen and stored at -80°C.

Transformation of E. coli

The competent *E. coli* JM109 and DH5α cells were transformed as described by Sambrook *et al* (1989). DNA (~50 ng) was added to the competent *E. coli* cells, mixed and kept on ice for 30 min. The cells were then incubated at 42°C for 2 min. To each tube 800 µL of LB broth was added and further incubated at 37°C for 1h. The cells were pelleted by centrifugation and resuspended in 200 µL of LB broth and spread on LB medium plates containing appropriate antibiotic, IPTG (40 µg/mL) and 40 µg/mL X-

gal. In total, 13 transformed colonies were grown on the LB agar selective medium.

Isolation and Identification of Insert

Recombinant plasmids were isolated using alkaline lysis method as described by Sambrook *et al* (1989) and characterized by restriction digestion. The plasmid was digested with *ApaI and Sac*II restriction endonucleases to isolate the insert from the vector plasmid. The digested fragment was electrophoresed on 0.8% agarose gel for 45 min at 60 V and further the insert DNA was used for sequencing

Sequencing of DNA A genome

The insert DNA was bidirectionally sequenced using Beckman Coulter CEQ™ 8000 Genetic Analysis System. DNA sequencing reactions were set up using CEQ™ DTCS Quick Start Kit Dye Terminator Cycle sequencing kit. Sequences of viral genomes were confirmed by comparisons and alignments performed with the Basic Local Alignment Search Tool (BLAST; National Center for Biotechnology Information).

Bioinformatics for sequence analysis

Clustal analysis of all the DNA A and β DNA fragments was carried out by using ebi ClustalW analysis tool (www.ebi.ac.uk/clustalw*)*. ClustalW is a tool for aligning multiple protein or nucleotide sequences. The alignment is achieved via three steps: pairwise alignment, guide-tree generation and progressive alignment. Conserved regions within the DNA-A sequences were analyzed.

Recombination analysis by Recombination Detection Program (RDP3)

RDP3 is a Windows 95/98/NT/XP/VISTA program for detecting and analysing recombination signals in a set of aligned DNA sequences (Martin *et al.,* 2005). Once RDP3 has scanned an alignment and enumerated all detectable recombination signals, it begins the task of trying to distill all the detectable recombination signals down to a minimal set of unique recombination events that could account for the signals. This program helps to indentify the site of recombinations within the sequences. This is available on the web *http://darwin.uvigo.es/rdp/rdp.html*. DNA-A and β-DNA sequences of all four biotypes were analyzed using the RPD3 tool (Martin and Rybicki, 2000).

RESULTS AND DISCUSSION

Genomic DNA isolation from okra leaf tissue

Isolation of genomic DNA from Okra leaf tissue is tedious due to presence of the high mucilage into the leaf. Genomic DNA was isolated by using Lodhi *et al.,* (1994) protocol for genomic DNA isolation. Genomic DNA isolation of all four *okra yellow vein mosaic virus* infected leaf samples collected from all the biotypes was carried out using the same method. Quality of isolated DNA was checked on 0.8% agarose gel. A good quality of DNA was seen under a UV transilluminator.

PCR amplification of DNA

The forward primer 5'AGTGGTGGGTCCAGAAC3' of 17 nucleotides with 58% GC content and melting temperature (Tm) 59°C and a reverse primer 5'TATTATACGGATGGCCGC3' of 18 nucleotides with 50% GC content and melting temperature (Tm) 57°C was used for amplification of DNA-A from all the four OYVMV biotypes. DNA-A of OYVMV was amplified using the above mentioned forward and reverse primers with temperature gradient from 55°C to 60°C to study the exact annealing temperature. Finally primers produced a desired amplicon at annealing temperature of 58°C. Figure 1 reveals the PCR amplification products checked on 0.8% Agarose gel. After PCR amplification, out of OYVMV samples collected from the various regions of India produced a DNA-A genome amplicon of ~2700 bp.

More recently, Jose and Usha (2002) reported PCR amplification of DNA-A using begomovirus component equivalent to DNA-A in diseased bhendi plants. Again Jose and Usha (2003) have isolated a DNA-A and β-DNA component from BYVMV infected bhendi tissue, which together with BYVMV, causes typical yellow vein disease symptoms in bhendi. BYVMV was initially amplified using begomovirus specific primers. PCR amplicon were cloned, sequenced and the 2.7 kbp amplified product was compared with various other BYVMV isolates. Besides this, they have also reported the amplification, cloning and characterization of β-DNA component from Madurai isolate. An approximately 1.35 kbp β-DNA fragment was amplified from diseased bhendi plants using non overlapping primers located in the highly conserved region found in all β-DNA sequences and was clones into pGEM-T.

Ha *et.al.* (2008) described molecular characterization of begomoviruses and DNA satellites from Vietnam. They collected samples from a range of crop and weed plants exhibiting characteristic geminivirus symptoms (vein yellowing, leaf curling, chlorosis and stunting). Total DNA was extracted and PCR amplifications were carried out using degenerate primers for amplification of DNA-A, DNA-B, β-DNA and Nanoviruses like DNA-1.

Mol. Marker : DNA ladder (7.5, 6.0, 5.5, 3.6, 2.0 kp).
Sample No. 1: PCR amplified DNA-A from Abohar Biotype
Sample No. 2: PCR amplified DNA-A from Delhi Biotype
Sample No. 3: PCR amplified DNA-A from Aurangabad Biotype
Sample No. 4: PCR amplified DNA-A from Madurai Biotype
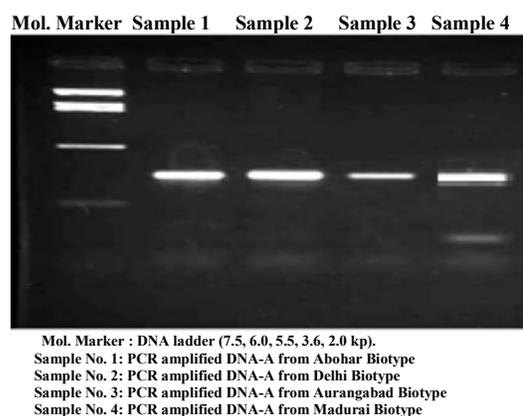
Fig. 1. PCR amplified DNA-A of OYVMV biotypes.

Sequence Analysis of *OYVMV DNA-A*

The complete nucleotide sequences of DNA-A of four virus isolates were determined. Plasmid DNA from the pGEM-T vector was used for the DNA sequencing. The insert DNA was bi-directionally sequenced using Beckman Coulter CEQ™ 8000 Genetic Analysis System.

Fig. 2. Graphical representation of DNA-A genome presenting the variability into genome size of OYVMV DNA-A genomes of each biotypes.

DNA sequencing reactions were set up using CEQ™ DTCS Quick Start Kit Dye Terminator Cycle sequencing kit. Variability in DNA-A genome of all experimental biotypes is briefed in figure 2. The lengths of four DNA-A molecules from yellow vein mosaic virus infected okra were 2743 nt of Abohar isolate, 2729 nt of Delhi isolate, 2743 nt of Aurangabad isolate and 2727 nt of Madurai isolate. Jose and Usha (2008) reported a DNA-A of 2741 nt from Madurai isolate. Similarly Zhou *et. al.* (1998) also noted a DNA-A of 2744 nt from cotton leaf curl virus (CLCuV) from Pakistan isolate which belongs to Geminiviradeae family.

Sequence comparisons and alignments were performed with the Basic Local Alignment Search Tool (BLAST; National Center for Biotechnology Information). The NCBI Blast tools confirmed that the sequences belong DNA-A of *okra yellow vein mosaic virus*. The sequenced DNA-A genome was showing above 95% sequence similarity with the other published DNA-A from OYVMV. It confirmed the isolation of OYVMV DNA-A genome. These confirmed sequences were used for further DNA-A genome analysis.

ClustalW analysis of DNA-A

After ClustalW analysis and comparison within the DNA-A of all four biotypes it is observed that all four biotypes have sequence divergence for DNA-A genome of OYVMV. ClustalW has been used for finding similarity in various studies like recombination analysis of replication initiator protein of geminiviruses (Vadivukarasi *et.al.* 2006) pseudo-recombinations in tomato yellow spot virus (Andrade *et.al.* 2006). Results of sequence similarity comparison between DNA-A of all four biotypes is mentioned in figure 3. After ClustalW analysis of DNA-A from all four biotypes, it has been noted that within Delhi and Abohar biotypes there is maximum sequence similarity of 98%. It represents a variance of 2% in DNA-A genome. While after comparison of DNA-A between Madurai and Aurangabad biotypes, it found that there is minimum sequence similarity of 86%. It reveals a difference of 14% into the DNA-A genome of these two biotypes.

Sequence similarity comparison between DNA-A of all four OYVMV biotypes using ClustalW tool

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OYVMV/DNAA/   Delhi       ACCGGATGGCCGCGCGATTTTTTTTAGTGGTGGGTCCAGAACGCACGACG 50
OYVMV/DNAA/Abohar         ACCGGATGGCCGCGCGATTTTAGTTAGTGGTGGGTCCAGAACGCACGACG 50
OYVMV/DNAA/Madurai        GCCGGATGGCCGCGCGATTTTT-TAAGTGGTGGGTCCAGAACGCACGACG 49
OYVMV/DNAA/Aurangabad     ACGGGATGGCCGCGCGATTTTT-TAAGTGGTGGTTCCAGAACGCACGACT 49
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OYVMV/DNAA/   Delhi       ATGCAGACTCAAAGCTTAGATAACGCTTCTTTGGCTATAAGTA-GTTGCG 99
OYVMV/DNAA/Abohar         ATGCAGACTCAAAGCTTAGATAACGCTTCTTTGGCTATAAGTA-CTTGCG 99
OYVMV/DNAA/Madurai        ATGCAGACTCAAAGCTTAGATAACGCTCCTTCGGCTATAAGTA-CGTGCG 98
OYVMV/DNAA/Aurangabad     ATGCAGACTCAAAGCTTAGATAACGCTCCTTCGGCTATAAGTAACGTGCG 99
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OYVMV/DNAA/   Delhi       CACTAAGTTTAAATTCAAAACATCTGGGATCCACTAGTAAACGAGTTCCC 149
OYVMV/DNAA/Abohar         CACTAAGTTTAAATTCAAAACATGTGGGATCCACTAGTAAACGAGTTCCC 149
OYVMV/DNAA/Madurai        CACTAAGTTTCAATTCAAAAAATGTGGGATCCACTATTAAACGAATTTCC 148
OYVMV/DNAA/Aurangabad     CACTAAGTTTCAAATTCAAAAATGTGGGATCCACTATTAAACGAGTTCCT 149
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OYVMV/DNAA/   Delhi       GGATACGGTTCACGGGTTTCGTTGTATGCTATCTGTAAAATATTTGCAAC 199
OYVMV/DNAA/Abohar         GGATACGGATCACGGGTTTCGTTGTATGCTATCTGTAAAATATTTGCAAC 199
OYVMV/DNAA/Madurai        GGATACAATTCACGGGTTTCGTTGTATGCTATCTCTAAAATATTTGCAAC 198
OYVMV/DNAA/Aurangabad     GGATACGGTTCACGGTTTTCGTTGTATGCTTTCTGTGAAATATTTGCAAC 199
                          ******   ****** ************** *** * ************

OYVMV/DNAA/   Delhi       TTTTGTCGCAGGATTATTCTCCAGATACTCTTGGGTACGAGTTAATACGG 249
OYVMV/DNAA/Abohar         TTTTGTCGCAGGATTATTCTCCAGATACTCTTGGGTACGAGTTAATACGG 249
OYVMV/DNAA/Madurai        TTTTGTCGCAGGATTATTCTCCAGATACGCTTGGGTACGAGTTAATACGG 248
OYVMV/DNAA/Aurangabad     TTTTGTCGCAGGAGTATTCACCAGATACGCTTGGTTACGATTTAATACGG 249
                          ************* ****** ******** ***** ***** ***** *********

OYVMV/DNAA/   Delhi       GATTTAATTTGTATTTTACGCTCACGTAATTATGTCGAAGCGAGCTGCCG 299
OYVMV/DNAA/Abohar         GATTTAATTTGTATGCTACGCTCCCGTAATTATGTCGAAGCGAGCTGCCG 299
OYVMV/DNAA/Madurai        GATTTAATTTGTATTTTACGTTCCCGTAATTATGTCGAAGCGAGCTGCCG 298
OYVMV/DNAA/Aurangabad     GATTGAATTTGTATTGTCCGTTCTCGTAATTATGTCGAAGCGAGCTGCCG 299
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OYVMV/DNAA/   Delhi       ATATCGTCATTTCTACGCCCGCGTCGAAAGTACGCCGGCGTCTGAACTTC 349
OYVMV/DNAA/Abohar         ATGTCGTCATTTCTACGCCCGCGTCGAAAGTACGCCGGCGTCTGAACTTC 349
OYVMV/DNAA/Madurai        ATATCGTCATTTCTACGCCCGCGTCGTAAGTACGCCGGCGTCTGAACTTC 348
OYVMV/DNAA/Aurangabad     ATATCGTCATTTCTACGCCCGCGTCGAAAGTACGCCGGCGTCTGAACTTC 349
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OYVMV/DNAA/   Delhi       GGCAGCCCATACACCAGCCGTGCTGCTGCCCCCATTGTCCGCGTCACAAA 399
OYVMV/DNAA/Abohar         GGCAGCCCATACACCAGCCGTGCTGCTGCCCCCATTGTCCGCGTCACAAA 399
OYVMV/DNAA/Madurai        GGCAGCCCATACACCAGCCGTGCTGCTGCCCCCATTGTCCGCGTCACAAA 398
OYVMV/DNAA/Aurangabad     GGCAGCCCATACACCAGCCGTGCTGCTGCTCCCATTGTCCGCGTCACAAA 399
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OYVMV/DNAA/   Delhi       ACAACAGGCATGGACAAACAGGCCTATGAACAGGAAACCCACAATGTACC 449
OYVMV/DNAA/Abohar         ACAACAGGCATGGACAATCAGGCCTATGAACAGGAAACCCAGAATGTACC 449
OYVMV/DNAA/Madurai        ACAACAGGCATGGACAAACAGGCCTATGAACAGGAAACCCAGAATGTACC 448
OYVMV/DNAA/Aurangabad     ACAACAGGCATGGACAAACAGGCCTATGAACAGGAAACCCAGAATGTACC 449
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OYVMV/DNAA/   Delhi       GGATGTACAGAAGTCCGGATGTTCCAAGGGGATGTGAGGGTCCCTGTAAG 499
OYVMV/DNAA/Abohar         GGATGTACAGAAGTCCGGATGTTCCAAGGGGATGTGAGGGTCCCTGTAAG 499
OYVMV/DNAA/Madurai        GGATGTACAGAAGTCCGGATGTTCCAAGGGGATGTGAGGGTCCCTGTAAG 498
OYVMV/DNAA/Aurangabad     GGATGTACAGAAGTCCGGATGTTCCTAGGGGATGTGAGGGTCCCTGTAAG 499
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OYVMV/DNAA/   Delhi       GTACAGTCGTTTGAATCTCGACACGATGTCGTTCATATTGGTAAGGTAAT 549
OYVMV/DNAA/Abohar         GTACAGTCGTTTGAATCTCGACACGATGTCGTTCATATTGGTAAGGTAAT 549
OYVMV/DNAA/Madurai        GTACAGTCGTTTGAGTCTCGACACGATGTCGTTCATATTGGTAAGGTAAT 548
OYVMV/DNAA/Aurangabad     GTACAGTCGTTTGAATCTCGACACGATGTTGTCCATATTGGTAAGGTAAT 549
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OYVMV/DNAA/   Delhi       GTGTATTTCGGATGTTACGCGTGGAGTCGGTTTGACCCATCGTATAGGTA 599
OYVMV/DNAA/Abohar         GTGTATTTCGGATGTTACGCGTGGAGTCGGTTTGACCCATCGTATAGGTA 599
OYVMV/DNAA/Madurai        GTGTATTTCGGATGTTACGCGTGGAGTCGGTTTGACCCATCGTATAGGTA 598
OYVMV/DNAA/Aurangabad     GTGTATTTCTGATGTTACGCGTGGAGTCGGTTTGACCCATCGTATAGGTA 599
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OYVMV/DNAA/   Delhi       AGCGTTTTTGTGTCAAGTCAGTTTATGTTTTAGGTAAGATATGGATGGAC 649
OYVMV/DNAA/Abohar         AGCGTTACTGTGTCAAGTCAGTTTATGTGTTAGGTAAGATATGGATGGAC 649
OYVMV/DNAA/Madurai        AGCGTTTTTGTGTCAAGTCAGTTTATGTTTTAGGTAAGATATGGATGGAC 648
OYVMV/DNAA/Aurangabad     AGCGTTTTTGTGTCAAGTCAGTTTATGTTTTAGGTAAGATATGGATGGAC 649
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OYVMV/DNAA/   Delhi       GAGAACATCAAGACCAAGAACCATACGAATTCGGTGATGTTTTTCCTTGT 699
OYVMV/DNAA/Abohar         GAGAACATCAAGACCAAGAACCATACGAATTCGGTGATGTTTATCCTAGT 699
OYVMV/DNAA/Madurai        GAGAACATCAAGACGAAGAACCATACGAATTCGGTGATGATTTTCCAAGT 698
OYVMV/DNAA/Aurangabad     GAGAACATCAAGAGCAAGAACCATACGAATTCGGTGATGTTTTTCCTTGT 699
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OYVMV/DNAA/   Delhi       TCGTGATCGACGACCGGTAGATAAACCACAAGATTTTGGTGAAGTATTTA 749
OYVMV/DNAA/Abohar         TCGTGATCGACGACCGGTAGATAAACCACAAGATTTTGGTGAAGTATTTA 749
OYVMV/DNAA/Madurai        TCGTGATCGACGACCGGTAGATAAACCACAAGATTTTGGTGAAGTATTTA 748
OYVMV/DNAA/Aurangabad     TCGTGATCGACGACCGACAGATAAACCACGAGATTTTGGTGAAGTATTTA 749
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OYVMV/DNAA/   Delhi       ATATGTTTGATAACGAGCCTAGTACGGCGACCGTGAAGAACATGCATAGG 799
OYVMV/DNAA/Abohar         ATATGTTTGATAACGAGCCTAGTACGGCGACGGTGAAGAACATGCATAGG 799
OYVMV/DNAA/Madurai        ATATGTTTGATAATGAGCCCAGTACGGCGACCGTGAAGAACATGCATAGG 798
OYVMV/DNAA/Aurangabad     ATATGTTTGATAACGAGCCCAGTACGGCCACCGTTAAGAACATGCATAGG 799
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OYVMV/DNAA/   Delhi       GATCGATACCAGCTGTTGAGGAAATGGCATGCAACCGTTACAGGTGGACA 849
OYVMV/DNAA/Abohar         GATCGATACCAGGTGTTGAGGAAATGGCATGCAACCGTTACTGGTGGACA 849
OYVMV/DNAA/Madurai        GATCGTTACCAGGTGTTGAGGAAATGGCATGCAACCGTTACTGGTGGTCA 848
OYVMV/DNAA/Aurangabad     GATCGGTACCAGGTGTTGAGGAAATGGCATGCAACCGTTACTGGTGGACA 849
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OYVMV/DNAA/   Delhi       ATATGCGAGTAAGGAGCAGGCTTTGGTCAAGAAGTTTGTTAGGGTTAACA 899
OYVMV/DNAA/Abohar         ATATGCGAGTAAGGAGCAGGCTTTGGTCAAGAAGTTTGTTAGGGTTAACA 899
OYVMV/DNAA/Madurai        ATATGCAGCGAGGGAACAGGCGTTGGTTAAGAAATTTGTCAGGGTTAACA 898
OYVMV/DNAA/Aurangabad     ATATGCAGCTAGGGAACAGGCGTTGGTTAAGAAGTTTGTCAGGGTTAACA 899
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OYVMV/DNAA/   Delhi       ACTACGTTGTTTACAACCAGCAGGAAGCAGGAAAATACGAGAATCACACC 949
OYVMV/DNAA/Abohar         ACTACGTTGTTTACAACCAGCAGGAAGCAGGAAAATACGAGAATCACACC 949
OYVMV/DNAA/Madurai        ATTATGTTGTTTACAACCAGCAGGAGGCAGGAAAATACGAGAATCACACC 948
OYVMV/DNAA/Aurangabad     ATTACGTTGTTTACAACCAGCAGGAGGCAGGAAAATACGAGAATCACACT 949
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OYVMV/DNAA/   Delhi       GAGAATGCATTGATGCTTTATATGGCTTGTACCCATGCTAGCAATCCTGT 999
OYVMV/DNAA/Abohar         GAGAATGCATTGATGCTTTATATGGCTTGTACCCATGCTAGCAATCCTGT 999
OYVMV/DNAA/Madurai        GAGAATGCATTGATGCTTTATATGGCTTGTACCCATGCTAGTAACCCAGT 998
OYVMV/DNAA/Aurangabad     GAGAATGCATTGATGCTTTATATGGCTTGTACCCATGCTAGTAACCCAGT 999
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OYVMV/DNAA/   Delhi        TTATGCTACTCTTAAGATTAGAATATATTTTTATGACTCTGTAACGAACT 1049
OYVMV/DNAA/Abohar          TTATGCTACTCTTAAGATTAGAATATATTTTTATGACTCTGTAACGAACT 1049
OYVMV/DNAA/Madurai         GTATGCTACGCTTAAGATTCGGATTTATTTTTATGACTCTGTAACGAATT 1048
OYVMV/DNAA/Aurangabad      TTATGCTACGCTTAAGATTCGGATATATTTTTATGACTCTGTAACGAACC 1049
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OYVMV/DNAA/   Delhi        AATATTAATAAAGTTTAAATTGTATATCTGAATATTGGTCTACATACATT 1099
OYVMV/DNAA/Abohar          AATATTAATAAAGTTTAAATTGTATATCTGAATATTGGTCTACATACATT 1099
OYVMV/DNAA/Madurai         GA-ATTAATAAAGTTTGAATTTTATATCTGAATATTGGTCTACATACATT 1097
OYVMV/DNAA/Aurangabad      ATATGATCTAGAGTTTTAATTTTATATCTGAATATTGGTCTACATACATT 1099
                          ** ***** **** **************************

OYVMV/DNAA/   Delhi        GTTTGATTAATTACATTGTACAATACATGTTCAACGGCTTTAATAACTAA 1149
OYVMV/DNAA/Abohar          GTTTGATTAATTACATTGTACAATACATGTTCAACGGCTTTAATAACTAA 1149
OYVMV/DNAA/Madurai         GTCTGATTAACTACATTGTACAATACATGTTCGACGGCTTTAATAACTAA 1147
OYVMV/DNAA/Aurangabad      GTTTGATTAATTACATTGTACAATACATGTTCAACGGCTTTAATAACTAA 1149
                          ** ******* ******************** *****************

OYVMV/DNAA/   Delhi        ATTAATTGAGATTACACCTAGATTGTTGAGATGTTTGAGGACTTGGGTTT 1199
OYVMV/DNAA/Abohar          ATTAATTGAGATTACACCTAGATTGTTGAGATGTTTGAGGACTTGGGTTT 1199
OYVMV/DNAA/Madurai         ATTAAGTGAGATTACACCTAGATTATTGAGATATTTGAGGACTTGGGTTT 1197
OYVMV/DNAA/Aurangabad      ATTAAGTGAGATTACACTTAGATTGTTGAGATACTTGAGTACTTGGGTTT 1199
                          ***** *********** ****** ******* ***** **********

OYVMV/DNAA/   Delhi        TGAATACCCTTAAGAAAAGACCAGTCGGAGGGTGTAAGGTCGTCCAGATT 1249
OYVMV/DNAA/Abohar          TGAATACCCTTAAGAAAAGACCAGTCGGAGGGTGTAAGGTCGTCCAGATT 1249
OYVMV/DNAA/Madurai         TGAATACCCTTAAGAAAAGACCAGTCGGAGGGTGTAAGGTCGTCCAGATT 1247
OYVMV/DNAA/Aurangabad      TGAATACCCTTAAGAAAAGACCAGTCGGAGGGTGTAAGGTCGTCCAGATT 1249
                          **************************************************

OYVMV/DNAA/   Delhi        CGGAAGGTTAGACAACACTTGTGTATTTCCAGAGCTTTCCGTAGGTTGTA 1299
OYVMV/DNAA/Abohar          CGGAAGGTTAGAAAACACTTGTGTATTTCCAGAGCTTTCCGTAGGTTGTA 1299
OYVMV/DNAA/Madurai         CGGAAGGTTAGAAAACACTTGTGTATTTCCAGAGCTTTCCGTAGGTTGTA 1297
OYVMV/DNAA/Aurangabad      CGGAAGGTTAGAAAACACTTGTGCACTCCCAGAGCTTTCCGAAGGTTGTA 1299
                          *********** ********** * * ************* ********

OYVMV/DNAA/   Delhi        GTTGAAATGGATCCTGAGTGTTATTATGTCCATGTTCGTCGTGAATGGAC 1349
OYVMV/DNAA/Abohar          GTTGAAATGGATCCTGAGTGTTATTATGTCCATGTTCGTCGTGAATGGAC 1349
OYVMV/DNAA/Madurai         GTTGAAATGGATCCTGAGTGTTATTATGTCCATGTTCGTCGTGAATGGAC 1347
OYVMV/DNAA/Aurangabad      GTTGAATTGGATCCTCATTGTTATGATGTCCATGTTCGTCGTGAATGGAC 1349
                          ****** ******** * ****** ************************

OYVMV/DNAA/   Delhi        GGTTGTCGTGGTTGAGGATCTTGAAATAGAGGCGATTTGGAACCTTCCAG 1399
OYVMV/DNAA/Abohar          GGTTGTCGTGGTTGAGGATCTTGAAATAGAGGGGATTTGGAACCTTCCAG 1399
OYVMV/DNAA/Madurai         GGTTGTCGTGGTTGAGGATCTTGAAATAGAGGCGATTTGGAACCTTCCAG 1397
OYVMV/DNAA/Aurangabad      GGTTGTCGTGGCTGAGGATTTTGAAATAAAGCGGATTTGGAACCTCCCAG 1399
                          *********** ******* ******** ** ************ ****

OYVMV/DNAA/   Delhi        ATATAGACGCCATTCTTTGCTTGAGCTGCAGTGATGCGTTCCCCTGTGCG 1449
OYVMV/DNAA/Abohar          ATATAGACGCCATTCTTTGCTTGAGCTGCAGTGATGCGTTCCCCTGTGCG 1449
OYVMV/DNAA/Madurai         ATATAGACGCCATTCTTTGCTTGAGCTGCAGTGATGCGTTCCCCTGTGCG 1447
OYVMV/DNAA/Aurangabad      ATAAAGACGCCATTCGTTGAATGAGCTGCAGTGATGCGTTCCCCTGTGCG 1449
                          *** *********** *** **************************

OYVMV/DNAA/   Delhi        AGAATCCATGGTTGTGGCAGTTGATGCTAAGATAATAAAAACACCCGCAT 1499
OYVMV/DNAA/Abohar          AGAATCCATGGTTGTGGCAGTTGATGCTAAGATAATAAAAACACCCGCAT 1499
OYVMV/DNAA/Madurai         AGAATCCATGGTTGTGGCAGTTGATGCTAAGATAATAACTGCATCCGCAT 1497
OYVMV/DNAA/Aurangabad      GGAATCCATGGTTGTGGCAGTTGATACTTAGGTAATAGCTGCATCCACAC 1499
                          *********************** ** ** *****   ** ** **

OYVMV/DNAA/   Delhi        TCAAGATCTACTCTCCTCCTCCTGTTGCGTCTCTTCGCTTCCCTGTGCTG 1549
OYVMV/DNAA/Abohar          TCAAGATCTACTCTCCTCCTCCTGTTGCGTCTCTTCGCTTCCCTGTGCTG 1549
OYVMV/DNAA/Madurai         TCAAGGTCCACTCGTCTCCTCCTGTGCACTCGCTTCGCTTCCTTGTGTTG 1547
OYVMV/DNAA/Aurangabad      TCAAGGTCCACTCGTTTCCTCCTGTGCGCTCTCTTCGCTTCCCTGTGTTG 1549
                          ***** ** ****   ********    ** ********** **** **

OYVMV/DNAA/   Delhi        TACTTTGATTGGTACCTGAGTACAACGGTTGGGTGAGAAAGACGAATGCT 1599
OYVMV/DNAA/Abohar          TACTTTGATTGGTACCTGAGTACAACGGTTGGGTGAGAAAGACGAATGCT 1599
OYVMV/DNAA/Madurai         AACTTTGATTGGTACCCGAGTACAACGGTTGGGTGAGAAAGACGAATGCT 1597
OYVMV/DNAA/Aurangabad      AACTTTGATTGGTACCGGAGTAGAGCGGTTGGGTGAGAAAGACGAATGTT 1599
                          ************** ***** * *********************** *

OYVMV/DNAA/   Delhi        GCATTTTTTAAAGCCCACGCTTTCAAAGCTGAGTTCTTTTCCTCGTCCAG 1649
OYVMV/DNAA/Abohar          GCATTTTTTAAAGCCCACGCTTTCAAAGCTGAGTTCTTTTCCTCGTCCAG 1649
OYVMV/DNAA/Madurai         GCATTTTTTAAAGCCCACGCTTTCAAAGCTGAGTTCTTTTCCTCGTCTAA 1647
OYVMV/DNAA/Aurangabad      GCATTCTTTAAAGCCCAGGATTTTAATGCTGAGTTCTTATCCTCGTCTAA 1649
                          ***** *********** * *** ** ********** ******** *

OYVMV/DNAA/   Delhi        ATACTCTTTATAGCTAGCGTTGGGCCCAGGATTGCAGAGGAAGATTGTTG 1699
OYVMV/DNAA/Abohar          ATACTCTTTATAGCTAGCGTTGGGCCCAGGATTGCAGAGGAAGATTGTTG 1699
OYVMV/DNAA/Madurai         AAACTCTTTATAGCTTGCGTTGGGGCCTGGATTGCAGAGAAAGATTGTTG 1697
OYVMV/DNAA/Aurangabad      GAACTCTTTATAGCTTGCATTGGGCCCTGGGTTGCAGAGGAAGATTGTTG 1699
                          ************* ** ***** ** ** ******** **********
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OYVMV/DNAA/   Delhi       GTATTCCACCTTTAATTTGAACTGGCTTCCCGTACTTTGTGTTCGATTGC 1749
OYVMV/DNAA/Abohar         GTATTCCACCTTTAATTTGAACTGGCTTCCCGTACTTTGTGTTGGATTGC 1749
OYVMV/DNAA/Madurai        GAATGCCTCCTTTAATTTGAACTGGCTTCCCGTACTTTGTGTTTGATTGC 1747
OYVMV/DNAA/Aurangabad     GTATGCCGCCTTTAATTTGAACTGGCTTCCCGTACTTTGTGTTTGATTGC 1749
                          *  **  **  *********************************  ******


OYVMV/DNAA/   Delhi       CAGTCCCTTTGGGCCCCCATGAACTCTTTAAAGTGCTTGAGGAAGTGCGG 1799
OYVMV/DNAA/Abohar         CAGTCCCTTTGGGCCCCCATGAACTCTTTAAAGTGCTTGAGGAAGTGCGG 1799
OYVMV/DNAA/Madurai        CAGTCCCTTTGGGCCCCCATGAATTCTTTAAAGTGTTTCAGATAATGCGG 1797
OYVMV/DNAA/Aurangabad     CAGTCCCTTTGGGCCCCCATGAATTCTTTAAAGTGTTTTAGGAAGTGTGG 1799
                          *********************** *********** **  **  *  **  **

OYVMV/DNAA/   Delhi       ATCTACGCCATCAATGACGTTATACCAAGCGTCGTTACTGTACACCTTTG 1849
OYVMV/DNAA/Abohar         ATCTACGCCATCAATGACGTTATACCAAGCGTCGTTACTGTACACCTTTG 1849
OYVMV/DNAA/Madurai        GTCAACATCATCTATAATGTTGAACCACGCATCGTTTGAATACACTTTAG 1847
OYVMV/DNAA/Aurangabad     ATCGACGTCATCAATGACGTTATACCAAGCGTCGTTACTGTACACCTTTG 1849
                          ** **  **** ** * ***   **** ** *****    ***** ** *

OYVMV/DNAA/   Delhi       GGCTTAGATCTAGATGCCCACATAAATAATTATGTGGGCCTAAAGACCTA 1899
OYVMV/DNAA/Abohar         GGCTTAGATCTAGATGCCCACATAAATAATTATGTGGGCCTAAAGACCTA 1899
OYVMV/DNAA/Madurai        GGCTTAGATCCAAGTGCGCACATAAATAATTATGTGGCCCCAAGGAACGG 1897
OYVMV/DNAA/Aurangabad     GGCTTAACTCCAGATGCCCGCATAAATAGTTATGTGGGCCTAAAGACCTA 1899
                          ******  **  *   *** * ********* ********** ** ** ** *

OYVMV/DNAA/   Delhi       GCCCACATTGTCTTCCCAGTACGACTATCACCCTCAATTACTATACTTTG 1949
OYVMV/DNAA/Abohar         GCCCACATTGTCTTCCCAGTACGACTATCACCCTCAATTACTATACTATG 1949
OYVMV/DNAA/Madurai        GCCCATTGTGTGTTTTCCCAGTTCTAGACTCCCCCTCTAGAACTAAACTCAA 1947
OYVMV/DNAA/Aurangabad     GCCCACATTGTTTTTTCCAGTACGACTATCTCCCTCAATTACTATACTTTG 1949
                          *****    *** ** ***** * *   ** ***** *   **** ***

OYVMV/DNAA/   Delhi       AGGTCTCAGGGGCCGCGCAGCGGCGTCGACAACATTCTCAGACACCCATT 1999
OYVMV/DNAA/Abohar         AGGTCTCAGGGGCCGCGCAGCGGCGTCGACAACATTCTCAGACACCCATT 1999
OYVMV/DNAA/Madurai        AGGTCTCTGAGGCCGCGCAGCGGCGTCCATGACGTTCTCCCGACGCCCACT 1997
OYVMV/DNAA/Aurangabad     AGGTCTCAGGGGCCGCGCAGCGGCATCGACAACGTTCTCGCACGCCCACT 1999
                          *******  * ************** ** *   ** *****   ** **** *

OYVMV/DNAA/   Delhi       CTTCAAGTTCTTCTGGAACTTGATCGAAAGAAGAAGAGGAAAAAGGAGAA 2049
OYVMV/DNAA/Abohar         CTTCAAGTACTTCTGGAACTTGATCGAAAGAAGAAGAGGAAAAAGGAGAA 2049
OYVMV/DNAA/Madurai        CTTCAAGTTCTTCTGGAACTTGATCAAAAGAAGAAGATAAAAATGGACAA 2047
OYVMV/DNAA/Aurangabad     CTTCAAGTTCTTCTGGAACTTGATCAAATGAAGAAGAAGAAAAAGGAGAA 2049
                          ********  **************** **  ********   **** *** **

OYVMV/DNAA/   Delhi       ACATAAGGAGCTGGTGGCTCCTGAAAGATTCTGTCTAGATTTGCATTTAA 2099
OYVMV/DNAA/Abohar         ACATAAGGAGCTGGTGGCTCCTGTAAGATTCTGTCTAGATTTGCATTTAA 2099
OYVMV/DNAA/Madurai        ACATAAACCTCCTGAGGAGGAGTAAAAATCCTATCTAAATTTGAATTTAA 2097
OYVMV/DNAA/Aurangabad     ACATAAGGAGCTGGAGGCTCCTGAAAATATCTATCTAAATTAGAATTTAA 2099
                          ******    *  *  * **    **    **  ** **** *** * ******

OYVMV/DNAA/   Delhi       ATTATGAAATTGTAGTACAAAATCTTTAGGAGCTAGCTCCTTGATGACTC 2149
OYVMV/DNAA/Abohar         ATTATGAAATTGTAGTACAAAATCTTTAGGAGCTAGCTCCTTGATTACTC 2149
OYVMV/DNAA/Madurai        ATTATGATATTGTAAGACATAATCTTTGGGGGCTAATTCTTTAAGAACTC 2147
OYVMV/DNAA/Aurangabad     ATTATGAAATTGAAGTAGAAAGTCTCTTGGGGCTTTCTCCTTCAGTATAT 2149
                          *******  ****  *   * *  *** *  ** ***    ** **  *   *

OYVMV/DNAA/   Delhi       TAAGAGCCTCTGACTTACTTCCCGCGTTAAGTGCTGCGGCGTAAGCGTCG 2199
OYVMV/DNAA/Abohar         TAAGAGCCTCTGACTTACTTCCCGCGTTAAGTGCTGCGGCGTAAGCGTCG 2199
OYVMV/DNAA/Madurai        TAAGAGCCTCTGCCTTACTTCCTGTGTTAAGTGCTGCCGCGTAAGCGTCA 2197
OYVMV/DNAA/Aurangabad     TGAGGGCCTGAGCTTTGGACCCTGCGTTGATTGCCTCGGCATATGCGTCG 2199
                          *  ** ****  *   **     ** *  *** *  ***   *  **  ** *****

OYVMV/DNAA/   Delhi       TTGGCTGTTTGTTGCCCTCCTCTTGCTGATCTTCCGTCGATCTGAAATTC 2249
OYVMV/DNAA/Abohar         TTGGCTGTTTGTTGCCCTCCTCTTGCTGATCTTCCGTCGATCTGAAATTC 2249
OYVMV/DNAA/Madurai        TTGGCTGATTGTTGTCCTCCCCTTGCAGATCTGGCGTCGATCTGGAATTC 2247
OYVMV/DNAA/Aurangabad     TTGGCAGTTTGGCAACCTCCTCTAGCTGATCTTGCATCGACTTGGAAAAC 2249
                          ***** * ***      ***** ** ** *****   * ****   ** **   *

OYVMV/DNAA/   Delhi       CCCCCAGTCGAGAATGTCCCCGTCCTTGGCGATGTAGGACTTGACGTCGG 2299
OYVMV/DNAA/Abohar         CCCCCAGTCGAGAATGTCCCCGTCCTTGGCGATGTAGGACTTGACGTCGG 2299
OYVMV/DNAA/Madurai        CCCCCATTCAAGGGTATCTCCGTCCTTGTCGATATAGGACTTGACGTCGG 2297
OYVMV/DNAA/Aurangabad     TCCATGATCAAGGATGTCTCCGTCTTTCTCGATGTAGGTTTTGACATCGC 2299
                          **     ** **  * ** ***** **   **** ****   ***** ***

OYVMV/DNAA/   Delhi       AGCTGGATTTAACTCCCTGAATGTTCGGATGGAAATGTGCTGACCTGGTT 2349
OYVMV/DNAA/Abohar         AGCTGGATTTAACTCCCTGAATGTTCGGATGGAAATGTGCTAACCTGGTT 2349
OYVMV/DNAA/Madurai        AGCTGGATTTAGCTCCCTGAATGTTTGGATGGAAATGTGCTGACCTGTTT 2347
OYVMV/DNAA/Aurangabad     TTGAGCTTTTAGCTCCCTGAATGTTCGGATGGAAATGTGCTGACCTAGTT 2349
                          *    **** ************* ***************** ****  **

OYVMV/DNAA/   Delhi       GGGGATACGAGGTCGAAGAATCTGTTATTTTTGCACTTGTATTTCCCTTC 2399
OYVMV/DNAA/Abohar         GGGGATACGAGGTCGAAGAATCTGTTATTTTTGCACTTGTATTTCCCTTC 2399
OYVMV/DNAA/Madurai        GTGGCGACCAAGTCGGAGAATCTCTGATTCTGGCACTTGTATTTTCCTTC 2397
OYVMV/DNAA/Aurangabad     GGGGAGGTGAGGTCGAAGAATCTATTGTTCCTGCACTGGAACTTTCCTTC 2399
                          *  **       * * **** ******* *   **    ***** * * ** *****
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OYVMV/DNAA/   Delhi        GAACTGGATGAGCACGTGAAGATGAGGTTCCCCATTTTCATGAAACTCTC 2449
OYVMV/DNAA/Abohar          GAACTGGATGAGCACGTGAAGATGAGGTTCCCCATTGCCATGAAACTCTC 2449
OYVMV/DNAA/Madurai         GAACTGGATGAGCACGTGAAGATGAGGTTCCCCATTTTCATGAAGCTCAC 2447
OYVMV/DNAA/Aurangabad      GAACTGGATGAGAACATGCAATTGAGGATTCCCATCTTCATGAAGTTCTC 2449
                           ************ ** ** *  ***** * *****   ****** ** *

OYVMV/DNAA/   Delhi        TGCAGATTCTAATGTATTTTTTGTTTACTGGGGTTTGTAGGTTTTGTAAT 2499
OYVMV/DNAA/Abohar          TGCAGAGTCTAATGTATTTTTTGTTTACTGGGGTTTGTAGGTGCTGTAAT 2499
OYVMV/DNAA/Madurai         TGCAGATCTTTATGTATTTTTAAATGTTGGGGTTTGTATATTTTGTAGT 2497
OYVMV/DNAA/Aurangabad      TGCAGATTCTAATGGATTTTTTGTTTACTGGGGTTTGGAGATTTAAGAAT 2499
                           ******   * *** *******   *   ********* * *    * *

OYVMV/DNAA/   Delhi        TGGGAAAGTGCCTCCTTTTTAGTAAGAGAGCATGTGGGATAAGTGATGAA 2549
OYVMV/DNAA/Abohar          TGGGAAAGTGCCTCCTTTTTAGTAAGAGAGCATGTGGGATAAGTGATGAA 2549
OYVMV/DNAA/Madurai         TGGGATAATGCCTCTTCTTTATTAAGAGAGCATTGTGGATAAGTAAGGAA 2547
OYVMV/DNAA/Aurangabad      TGGGAAAGTGCTTTCTCTTTTCTTAGGGAGCACTTGGGATAAGTGAGAAA 2549
                           *****  *  *** *  *  *  ***   *  ** *****   ******** *   **

OYVMV/DNAA/   Delhi        ATAATTTTTGGCATATATCTGAAATTGTTTGGGAGGA--GCCAAATGACT 2597
OYVMV/DNAA/Abohar          ATAATTTTTGGCATATATCTGAAATTGTTTGGGAGGA--GCCAT-TGACT 2596
OYVMV/DNAA/Madurai         ATAATTTTTGGAATTGATGACAAAACGCCTTGGAGGCATGTTGACTATTT 2597
OYVMV/DNAA/Aurangabad      ATAAATTTTGGCATTTATTTTAAACCGA-TGGGGGGCTGCCATGTTGACT 2598
                           ****  ****** **  **   ***   *  * ** **         *   *

OYVMV/DNAA/   Delhi        TG-TCAATCGGTACCCAGATCTAATCCTATGT---CAATTGGTGAACGGT 2643
OYVMV/DNAA/Abohar          TGGTCAATCGGTACCCAGATCTAATCCTATGT---CAATTGGTGAACGGT 2643
OYVMV/DNAA/Madurai         TTGAGACCCGATTGACCGCTCTTACAAC-TCT---CCCAGTATATCGGG 2643
OYVMV/DNAA/Aurangabad      AAGTCAATCCGGTGTGCTCTTTAACTCTCTGCATGTATCGGTGTTTTGGA 2648
                           *  *        * *  *   *          *             **

OYVMV/DNAA/   Delhi        GCCCTATATATAGTGGGTACTGAATGGCATTATTTGTAATTACAAAAGGA 2693
OYVMV/DNAA/Abohar          GCCCTATATATAGTGGGTACTGAATGGCATTATTTGTAATTACAAAAGGA 2693
OYVMV/DNAA/Madurai         TCCCTATATATAGTGAGACCCAAATGGCATAATT-GTAATAAAACAACTT 2692
OYVMV/DNAA/Aurangabad      GTCCTATATATATGGAGACTCTAATGGCATTAA--AATGTAAAATAAAAG 2696
                           **********  *  *   ******** *    * *  *  **

OYVMV/DNAA/   Delhi        -AATTCAAAATCTACACGCTCCAA--AAAGCGGCCATCG----------- 2729
OYVMV/DNAA/Abohar          -AATTCAAGATCTACACGCTCCAA--AAAGCGGCCATCCGTATAATATTA 2740
OYVMV/DNAA/Madurai         TAATTTGAAAATCAAACGAAAAGGCTAAAGCGGCC--------------- 2727
OYVMV/DNAA/Aurangabad      AAAACACCTATTTGAATTCCAAGG--AAAGCGGCCATCCGTCTAATATC- 2743
                            **     *      *         *********

OYVMV/DNAA/   Delhi        ---
OYVMV/DNAA/Abohar          GTC 2743
OYVMV/DNAA/Madurai         ---
OYVMV/DNAA/Aurangabad      ---
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Fig. 3. Graphical comparison of sequence similarity between DNA-A genome of all four OYVMV biotypes.

DNA-A genome sequences comparison between Delhi and Madurai as well as Delhi and Aurangabad biotypes showed 88% similarity and 12% distinctness in the DNA-A genome. Similarly the sequences similarity of 87% was observed between Madurai and Abohar as well as Abohar

and Aurangabad biotypes with 13% genome variability into DNA-A. Above results reflect genome variability within all four biotypes which was considered for the molecular studies. This also highlights that as we compare the DNA-A genome of Abohar biotypes from northern part of India with the DNA-A of Madurai biotype from southern part of India or Aurangabad biotypes from central part of India, the variation within the genome goes on increasing. Similarly comparison between Aurangabad and Madurai biotypes showed 14% variability into DNA-A sequence. This result clearly proves that these viruses recombine for their adoptability into the particular area.

Conserved regions within the DNA-A of OYVMV genome

During ClustalW analysis, it is also observed that that there are several conserved regions within the nucleotide sequences of OYVMV DNA-A. These regions were identified and marked. After a full length complete genome sequence analysis of DNA-A, all conserved regions within the genome is noted. Table No. 1 showed the conserved region within various genes from DNA-A of all experimental biotypes. The nucleotides representing the coat protein AV1 gene showed maximum conserved

regions. In-depth analysis, it is also noticed that there is maximum conserved region between the DNA-A genome sequence of Aurangabad and Madurai biotypes. However, it has also been observed that Aurangabad isolate has an AV1 nonfunctional Pre-coat protein gene might have resulted through mutations.

Table 1. Conserved regions between the DNA-A genome of four OYVMV biotypes.

Complement		Total Nucleotides	Conserved region representing gene
Start Nucleotide	End Nucleotide		
34	49	43 nt	AV1 gene Coat Protein
51	76	26 nt	AV1 gene Coat Protein
274	301	28 nt	AV1 gene Coat Protein
327	377	51 nt	AV1 gene Coat Protein
560	605	46 nt	AV1 gene Coat Protein
813	840	28 nt	AV1 gene Coat Protein
926	990	65 nt	AV1 gene Coat Protein
1012	1101	90 nt	AC3 gene of Replication Enhancer protein
1190	1261	72 nt	AC3 gene of Replication Enhancer protein
1325	1360	36 nt	AC3 gene of Replication Enhancer protein
1421	1449	29 nt	AC2 gene
1451	1474	24 nt	AC2 gene
1574	1597	24 nt	AC1 gene of Rep protein
1708	1742	35 nt	AC1 gene of Rep protein
1744	1772	29 nt	AC1 gene of Rep protein

family (Keese and Gibbs 1993; Morse 1994; Gibbs *et. al.* 1995; Holland 1998). It has now been accepted that recombination contributed to the diversity of geminiviruses and therefore, to the emergence of new variants and species\ reported worldwide. For instance, cotton leaf curl disease in Pakistan became severe during the past decade, causing extensive damage to cotton production. In Trinidad Tobago, a geminivirus disease on tomato observed in 1989 has reemerged throughout the country. A new cassava mosaic virus has devastated cassava production in Uganda. Tomato production in Spain and Italy is severely constrained by *Tomato yellow leaf curl virus-Sardinia* (TYLCV). All of these new viruses are recombinants (Umaharan *et. al.* 1998). In India, Kirthi *et al* (2002) have detected recombination between strains of *Tomato leaf curl virus* from Bangalore. Girish and Usha (2005) have analysed recombination events in TLCV DNA-A. Recombinations within DNA-A of all four experimental biotypes were studied using the RDP3 program. Results of recombinations within DNA-A genome of *okra yellow vein mosaic virus* are reviewed in figure 4. From figure 4 and 5, it is observed that DNA-A sequence of Delhi biotype shows a sequence tract A portion where the recombination has occurred the sequence. Figure also reproduces that there is recombination between 766 nt and 1022 nt with a probability of 3.158. The present figure also represents that there is a major recombination between the Delhi and Madurai biotypes with a tract of Aurangabad biotype as a
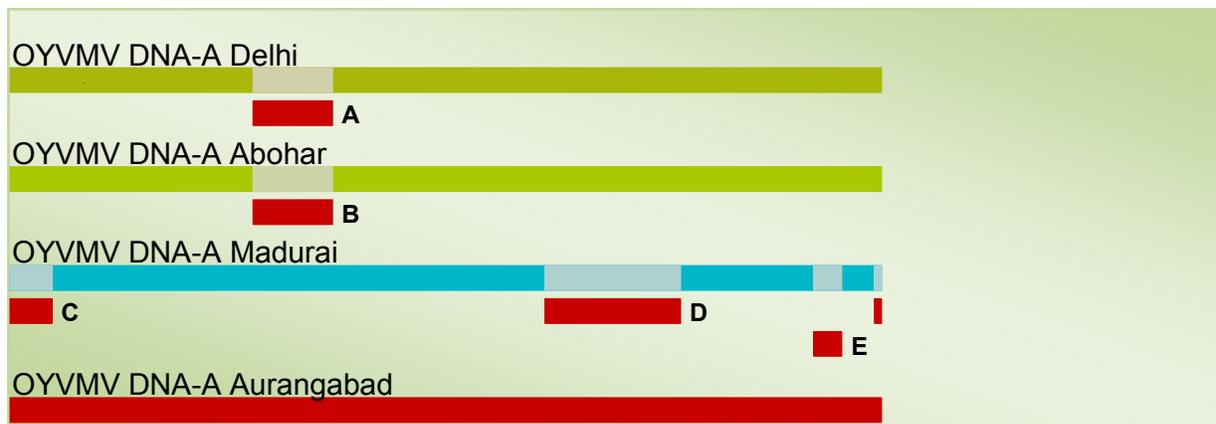
Fig. 4. The schematic display of recombination. Shows sites recombinations within DNA-A Genome sequence of OYVMV biotypes. The piece of sequence from major parent and piece of sequence from minor parent is highlighted. Piece of minor parent shows the site of recombination.
A: Site of recombination within DNA-A of Delhi Biotype
B: Site of recombination within DNA-A of Abohar Biotype.
C, D & E: Sites of recombination within DNA-A of Madurai Biotype

As seen in table, there are two conserved regions of 29 and 24 nucleotides present in the AC2 gene. From table we also understand that there are three conserved regions amongst four biotypes within the AC3 gene which produces Replication Enhancer Protein. Results also highlight great similarity within three conserved regions from AC1 gene which codes for Rep Protein.

Detection of recombinations within DNA-A and β-DNA genomes using Recombination Detection Program (RDP3) tool

Recombination can provide selective advantage in the evolution of viruses within strains, species, genera and

minor parent. Second strand from Figure 4 belongs to Abohar biotype. RDP3 tool showed that DNA-A sequence of Abohar biotype have a sequence tract B portion where the recombination has occurred within the sequence. Figure imitate that there is recombination between 766 nt and 1022 nt with a probability of 2.469 (Figure 6). The present figure also informs that there is a major recombination between the Abohar and Madurai biotypes with a tract of Aurangabad biotypes as a minor parent. From the Figure 4, third strand belongs to Madurai biotype. The bioinformatics program RDP3 showed that DNA-A sequence of Madurai biotype have three sequence
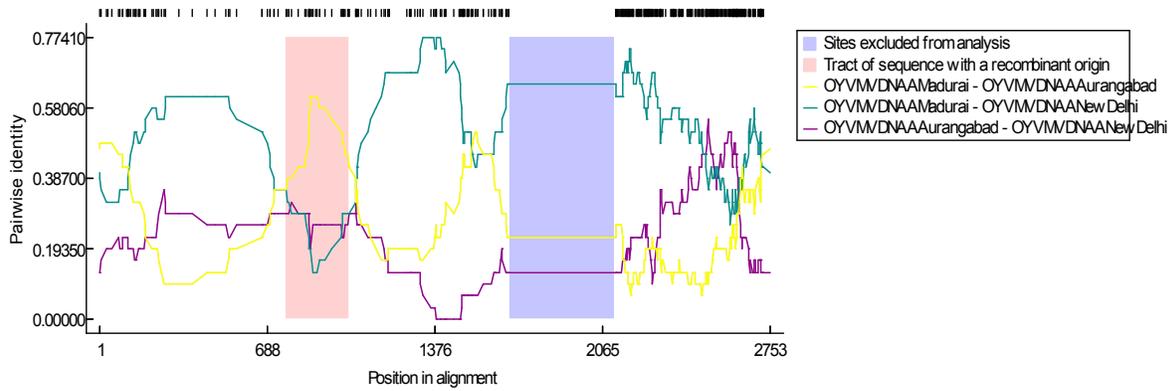
Fig. 5. : The schematic display of nucleotides where the recombination is occurred. Tract of sequence with recombinations within DNA-A genome of OYVMV Delhi biotype.
Beginning   : 766 nt; Ending   : 1022 nt; Daughter   : OYVMV Delhi; Major Parent   : OYVMV Madurai (88.3%); Minor Parent   :   OYVMV Aurangabad; Probability   : 3.158
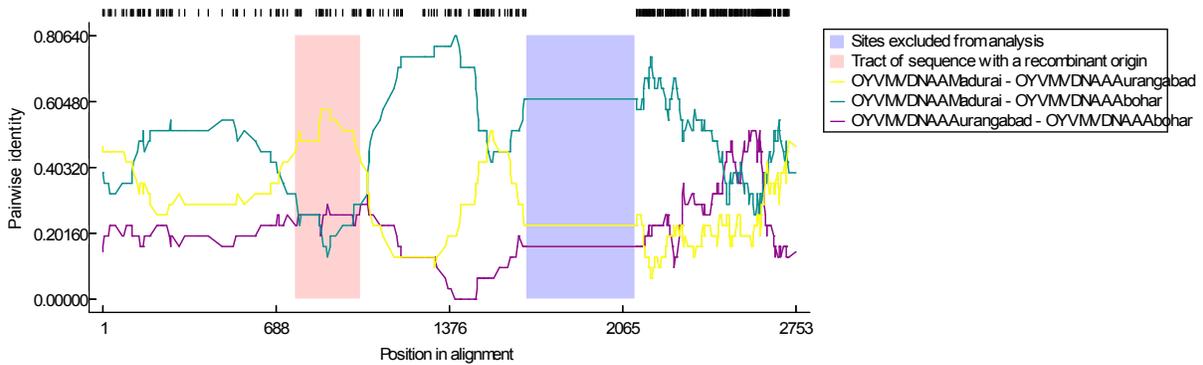
Fig. 6. The schematic display of nucleotides where the recombination is occurred. Tract of sequence with recombinations within DNA-A genome of OYVMV Abohar biotype.
Beginning   : 766 nt
Ending   : 1022 nt
Daughter   : OYVMV Abohar
Major Parent   : OYVMV Madurai (89.7%)
Minor Parent   : OYVMV Aurangabad
Probability   : 2.469
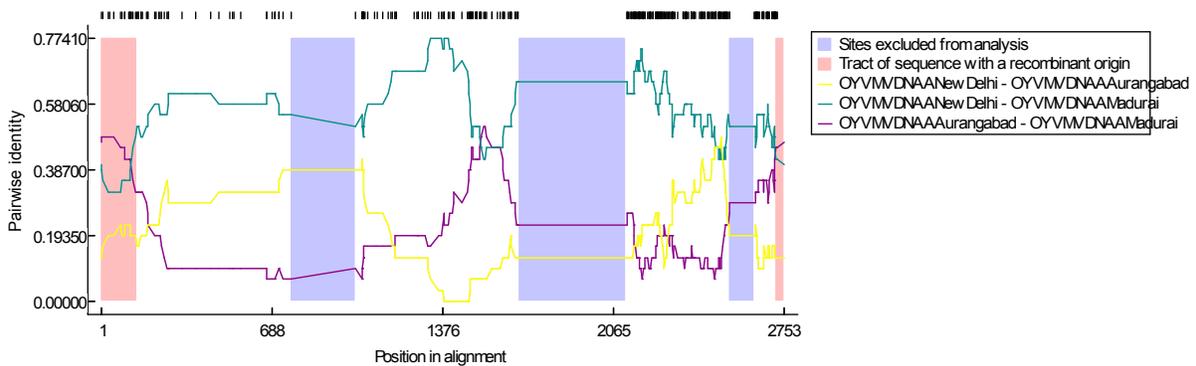
Fig. 7. The schematic display of nucleotides where the recombination is occurred. Tract of sequence with recombinations within DNA-A genome of OYVMV Madurai biotype.
Beginning   : 2712 nt
Ending   : 139 nt
Daughter   : OYVMV Madurai
Major Parent   : OYVMV Delhi (91.9%)
Minor Parent   : OYVMV Aurangabad (94.8%)
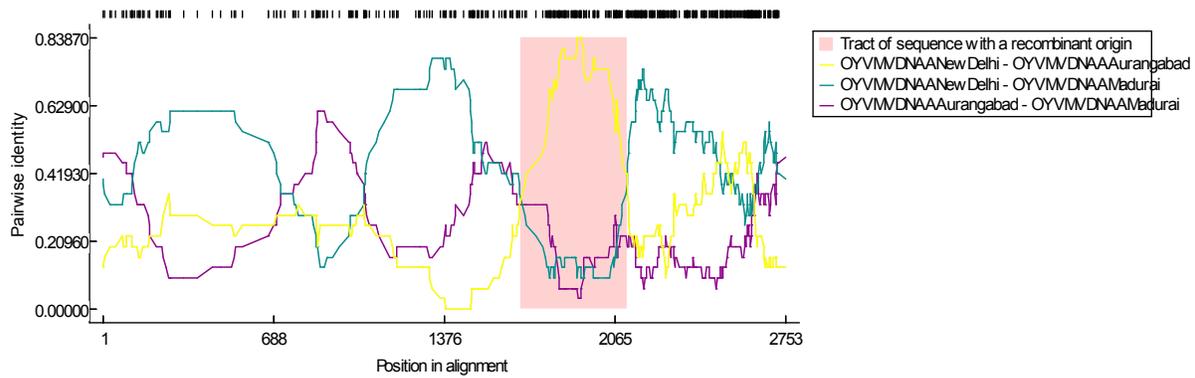
Fig. 8. The schematic display of nucleotides where the recombination is occurred. Tract of sequence with recombinations within DNA-A genome of OYVMV Madurai biotype.

Beginning	: 1683 nt
Ending	: 2114 nt
Daughter	: OYVMV Madurai
Major Parent	: OYVMV Delhi (90.2%)
Minor Parent	: OYVMV Aurangabad
Probability	: 1.485
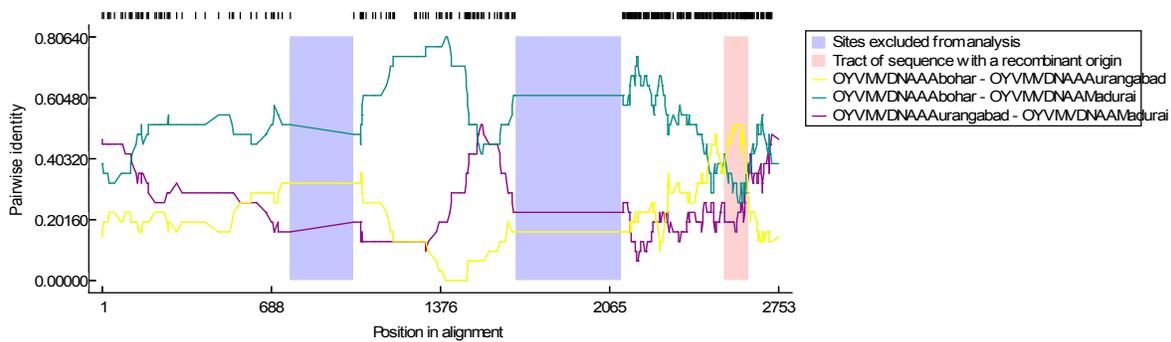
Fig. 9.The schematic display of nucleotides where the recombination is occurred. Tract of sequence with recombinations within DNA-A genome of OYVMV Madurai biotype.

Beginning	: 2532 nt
Ending	: 2627 nt
Daughter	: OYVMV Madurai
Major Parent	: OYVMV Abohar (91.3%)
Minor Parent	: OYVMV Aurangabad
Probability	: 1.971

tracts C, D & E portion where the recombination has occurred within the sequence. Figure also imitate that there is recombination between 2712 nt to 139 nt with a probability of 3.389 (Figure 7). During RDP scanning, tract of Delhi biotype was found to be a major parent and a tract of Aurangabad biotype as a minor parent. While from figure 8, it is also observed that there is recombination between 1683 nt to 2114 nt with a probability value of 1.485. During RDP scanning, tract of Delhi biotype was found to be a major parent and a tract of Aurangabad biotype as a minor parent. Apparently from figure 9 it seemed that there is recombination between 2532 nt to 2627 nt with a probability value of 1.971. During RDP scanning, tract of Abohar biotype was found to be a major parent and a tract of Aurangabad biotype as a minor parent. During this scanning, DNA-A genome sequence of Aurangabad biotype was used as a nome sequences from all other biotypes were scanned against DNA-A genome sequence of Aurangabad biotype.

Prasanna and Rai (2007) carried out the recombination breakpoint analysis using Recombination Detection Program. They detected the frequency of recombination in tomato-infecting Begomoviruses of South and Southeast Asia. Vadivukarasi *et.al.* (2006) also studies the sequence and recombination analyses of the geminivirus replication initiator protein. To understand the frequent evolution of new geminiviruses, recombination detection analysis was carried out using RDP3. The recombinant regions predicted by both RDP and GENECONV were verified by constructing NJ tree, using ClustalW program. Seventy-seven potential/putative interspecies recombination events were predicted by both RDP and GENECONV. Geminiviruses infecting plants of the *Malvaceae, Asteraceae, Solanaceae, Euphorbiaceae, Cucurbitaceae* and *Fabaceae* families were included in recombination analysis. Among their predicted 77 events, 42 take place in the region spanning the 5′ half of IR and the 5' end of *Rep* gene adjoining it. Thirty events take place only in the

5´ region of *Rep* gene; three events in the IR alone and only two events were predicted in the 3´ part of *Rep* gene. The length of the region of recombination ranges from 43 to 468 nucleotides.

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