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Full Length Research paper

Genetic Diversity and Possible Evidence of Recombination among *Banana Bunchy Top Virus* (BBTV) Isolates

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Banana bunchy top virus (BBTV) is the causal agent affecting banana production worldwide. Sequence analysis of replicase genes of BBTV isolates revealed that two Indian isolates under study, from Bhagalpur (Bihar), have one amino acid change (Alanine to Serine) at position number 95. The Stem Loop Common Region (CR-SL) was found to be highly conserved among BBTV isolates while CR-M exhibit greater homology with members of South Pacific group. Interestingly, DNA 2 CR-M of Bihar isolate (FJ605508) showed sequence homology with DNA 6 CR-M sequence indicating possible recombination between these two components. Such recombination event might occur in other genomic loci and could contribute towards viral evolution. Based on the phylogenetic studies (sequence alignment and phylogenetic tree) using all six DNA (DNA 1-6) including replicase and coat protein genes and CR-M region clearly indicates that our BBTV isolates belong to South Pacific group and are very close to Fiji and Myanmar isolates. On the basis of mix Phylogram of DNA components 1-6, we can assume that BBTV genome originated from a single component and during the course of evolution portion of DNA 3 and DNA 4 remained conserved.

Key Words: Banana bunchy top virus (BBTV), Replicase, Coat Protein, CR-M, CR-SL, Phylogenetic analysis.

INTRODUCTION

Banana - a fruit of great socio-economic significance is the largest fruit crop of India accounting ~35% of its total fruit production (FAO, 2007). Banana plantations are subjected to various natural calamities, but diseases, in particular, viral diseases constitute a major setback to this crop worldwide. Among viral infections, Banana Bunchy Top Virus (BBTV) is responsible for massive destruction/reduction in crop yield. Isometric virions of BBTV are approximately 18-20 nm in diameter (Iskra et al., 1989) consist of six single stranded circular DNA molecules (~1kb each) as part of its genome (Karan et al*.,* 1997). It belongs to genus Babuvirus and family *Nanoviridae* (Allen, 1987). It persistently transmitted by banana aphid *Pentalonia nigronervosa* (Thomas et al., 1991; Magee, 1927).

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Each component of the BBTV genome contains one large (monocistronic) transcriptional active open reading frame (ORF) except BBTV DNA 1 (Beetham et al., 1997), two conserved regions: CR-SL and CR-M, potential TATA box at 3' of the stem–loop and polyadenylation signal (Burns et al., 1995; Beetham et al., 1997, 1999). BBTV DNA 1 contains two ORF's - one master replication initiation protein (Rep) ORF (Hafner et al., 1997) and a small ORF internal to major ORF in a +2 reading frame (Beetham et al., 1997). CR-SL region extends up to 23 nucleotides at 5' end and 13 nucleotides at 3' of the stem-loop sequences of 31 nucleotides. CR-M is located 5' of the CR-SL and is between 71 and 92 nucleotides in length. TATA box (CTATAAATA) of 9 nucleotides is conserved in DNA 1, DNA 2, DNA 3 and DNA 4, while it exhibit minor sequence variability in DNA 5 (CTATTTAAA) and DNA 6 (CTATTAATA). Functions, with the exception of DNA 2 (predicted ten ORF's of varied size), have been assigned to all gene products of BBTV; DNA 1 encoding master replication initiation protein, DNA 3 coat protein (CP), DNA 4 movement

Table 1. Sequences of designed primers for Rep, CP gene and all six DNA components (DNA 1-6) of BBTV

Gene	Primer sequence		Product Size (~)
BBTV-Rep	F	5'- ATGGCGCGATATGTGGTATGC -3'	861bp
	R	5'- TCAGCAAGAAACCAACTTTATTCG -3'	
BBTV-CP	F	5'- ATGGCTAGGTATCCGAAGAAATCC -3'	513bp
	R	5'- TCAAACATGATATGTAATTCTGTTCTGG -3'	
BBTV DNA-1	F	5'- GGATGTTCACCATCAACAATCCC -3'	1111bp
	R	5'- TGCATACCACATATCGCGCCAT -3'	
BBTV DNA-2	F	5'- GTAACCGGTCAACATTATTCTGGC -3'	1058bp
	R	5'- CTTGACCTTCGGTCATATCACG -3'	
BBTV DNA-3	F	5'- ATCAAGAAGAGGCGGGTTGG -3'	1075bp
	R	5'- GGATTTCTTCGGATACCTAGCCAT -3'	
BBTV DNA-4	F	5'- GTATATTAAGCAGCTCGTGAGG -3'	1046bp
	R	5'- TTCGGTACCTCAAAGAGCAAAACC -3'	
BBTV DNA-5	F	5'- TGCCTGACGATGTCAAGAGAGAG -3'	1018bp
	R	5'- TAGCAGACCATTCCCAGAACTCC -3'	
BBTV DNA-6	F	5'- TGGAAGAAAGTCGCCTCGCAAGG -3'	1089bp
	R	5'- GCTCCAGAATCGACGCATGGTAC -3'	

protein, DNA 5 cell cycle control protein and DNA 6 encoding nuclear shuttle protein respectively (Vetten et al., 2005). Based on sequence analysis of BBTV DNA 1, 3 and 6, Karan et al. (1994, 1997) and Wanitchakorn et al. (2000) have categorized BBTV isolates under two groups viz. South Pacific group (isolates from Australia, Burundi, Egypt, Fiji, India, Tonga and Western Samoa) and the Asian group (Vietnam, Philippines and Taiwan).

In order to establish relationship within different components of BBTV in India, we performed genetic analysis based on replicase and coat protein gene of various isolates from different parts of India. Further, we analyzed genomic components (DNA 1-6) of BBTV Bihar isolate to establish origin of the virus in India.

Materials and Methods

Maintenance of virus culture and Total nucleic acid extraction

Banana plants exhibiting stunting of plant, yellowing of leaf margins and the presence of dark green streaks on the petioles, leaf lamina and leaf distortion symptoms associated with BBTV infection were selected for sampling. BBTV infected leaf and midrib samples were collected from diverse locations of banana growing belt of India. Virus cultures were maintained on banana plants grown under glass house condition and leaf tissues were stored at -80°C for long term storage. Genomic DNA was extracted from approximately 500mg of infected plant material following the method described by Doyle and Doyle (1987).

Polymerase chain reaction

PCR reactions for all the six components including coat protein and replicase gene of BBTV were performed using gene specific primers (Table 1) designed following sequence alignment of the BBTV sequences in the database in an automatic thermocycler TC-312 (TECHNE, UK). Standard PCR procedure, as described by (Burns et al., 1995), was employed to amplify DNA components and ORFs from the infected banana leaves. The amplification products (7µl each) were analyzed by agarose gel electrophoresis using 2% (w/v) agarose along with 100 bp ladder (Fermentas, Canada). Gels were photographed on a digital gel documentation system (BioRad, UK).

Cloning and sequencing of PCR amplicon

Amplified products of all the viral components were electrophoresed on 1.5% (w/v) agarose gel and eluted using DNA Extraction Kit (Fermentas, Canada) as per manufacturer's instructions. The eluted products were then ligated into pGEM-T® Easy vector (Promega, Madison, WI, USA). *E coli* XL1-Blue competent cells were transformed with the ligated products and transformed clones were selected using X-Gal as substrate on LA plates containing 50µg/ml ampicillin and 12.5µg/ml tetracycline. The transformants were confirmed through colony PCR and restriction digestion with *Eco*RI (Bangalore Genei Pvt. Ltd., India). Rep and CP clones of Bihar, Maharashtra and Karnataka were designated as BBTV BH, BBTV KAR and BBTV MH respectively. Clones were sequenced using T7 and SP6 universal primers in an automated sequencer (ABI Prism 310).

Sequence analysis and phylogenetic study

Sequence data of all the components obtained were compared with other reported isolates of BBTV from Indian subcontinent as well as with those reported from other parts of the world. Multiple nucleotide and deduced amino acid alignment for sequence comparison were carried out with isolates, reported for each component from various banana growing countries of the world using Clustal W (Version 1.82) and Bio-Edit (Version 7.0.0) (Hall, 1999) softwares. Phylogenetic trees were generated using

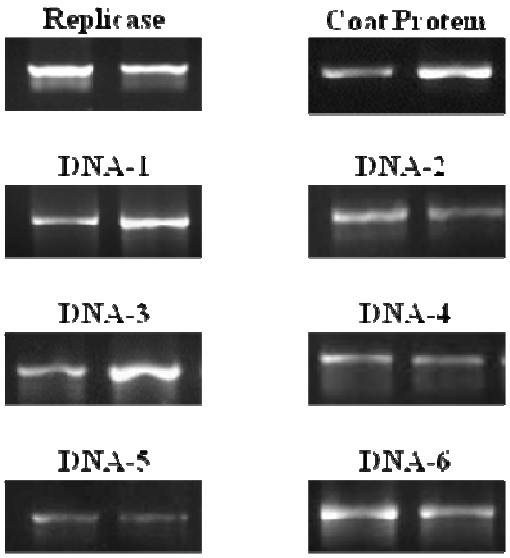

Figure 1 Agarose gel electrophoresis showing amplification of BBTV DNA components along with replicase and coat protein genes

Table 2. Accession number, isolates, nucleotide position of ORF and total number of predicted amino acids of sequenced component

Accession number	Isolates	Component/ ORF	ORF Position number/ total length(Nt.)	Predicted amino acids
FJ605506	Bhagalpur (Bihar)	DNA-1	102-962 / 861	286
DQ640741	Bhagalpur (Bihar)	Rep	861	286
EF584545	Hajipur (Bihar)	Rep	861	286
DQ640742	Maharashtra	Rep	861	286
FJ605508	Bhagalpur (Bihar)	DNA-2	143-376 / 234	77
FJ605507	Bhagalpur (Bihar)	DNA-3	228-740 / 513	170
DQ996466	Bhagalpur (Bihar)	CP	513	170
FJ168538	Hajipur (Bihar)	CP	513	170
EF584544	Maharashtra	CP	513	170
FJ609642	Bhagalpur (Bihar)	DNA-4	282-635 / 354	117
FJ609643	Bhagalpur (Bihar)	DNA-5	240-775 / 486	161
FJ609644	Bhagalpur (Bihar)	DNA-6	277-741 / 465	154

Neighbor-Net (Bryant and Moulton, 2004), a distance mode method incorporated in the program SplitsTree4 (version 4.10. 2008) (Huson and Bryant, 2006).

Results

Cloning and sequencing of Rep, CP gene and six DNA components of BBTV isolates

All the six components (DNA 1-6) were amplified (along with the replicase and coat protein gene) from the infected banana leaf samples (Figure 1). Different fragments were successfully cloned and screened by colony PCR as well as through restriction digestion. Sequencing of the cloned insert revealed that replicase was 861nt, coat protein gene was 513nt while DNA 1-6 components were 1111nt, 1061nt, 1075nt, 1046nt, 1018nt and 1090nt, respectively. Sequences of all the components along with replicase and coat protein gene were submitted to Genbank (Table 2).

Sequence analysis of different components of BBTV isolates

Complete sequences of all the components of BBTV Bihar (Bhagalpur) isolate along with sequence of replicase and coat protein gene of Hajipur, Bihar and Maharashtra isolates were analyzed. Sequence data

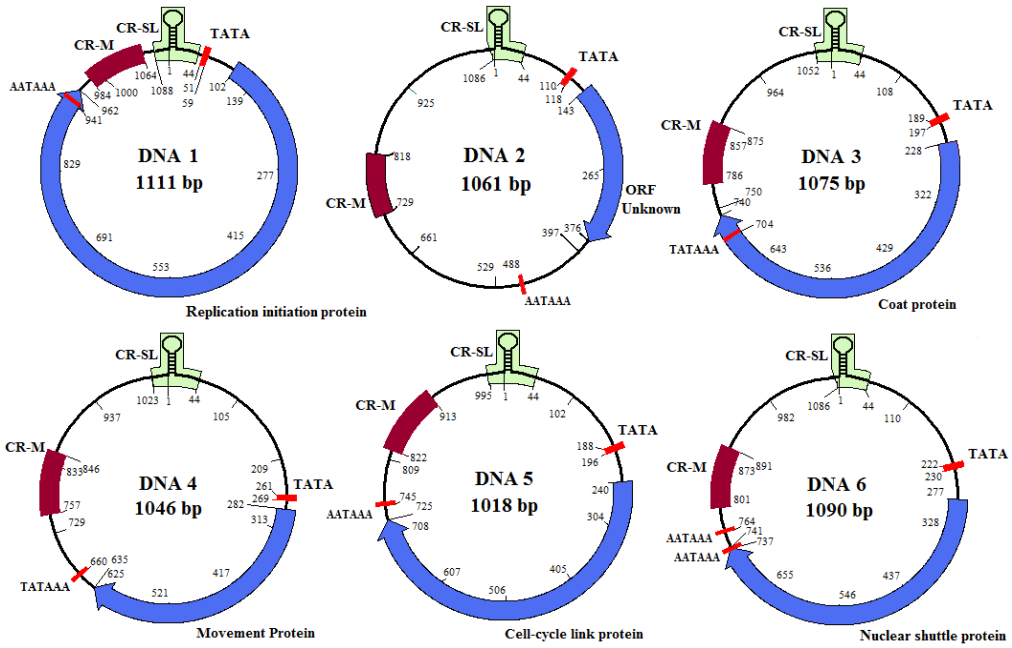

Figure 2 Genomic organization of all six BBTV DNA components of Bhagalpur (Bihar) isolate showing CR-SL, CR-M, TATA Box , Poly A signal and ORF

analysis of DNA components 1-6 revealed that they contained CR-SL, TATA Box, conserved Poly A (A/tATAAA) signal, CR-M and potential ORF (Figure 2). Complete sequences of replicase and coat protein gene were found to be of 861 and 513 nucleotides in size potentially encoding 286 and 170 amino acids respectively.

CR-SL for DNA 1, DNA 3 and DNA 4 were found to be 73nt in length, while the same region for DNA 2, DNA 6 and DNA 5 was found to span a stretch of 48, 49 and 69nt, respectively (Figure 3). CR-SL region of all the components contains stem-loop with conserved nonanucleotide sequence 'TATTATTAC'. Besides, it also contains three putative iterated sequences (except DNA 2 having only two iterated sequences) corresponding to F1, F2 and R designated previously by Herrera-Valencia et al. (2006). Two of the three iterated sequences (F1 and F2) were found to be located within single nucleotide space at the 3' end of the stem-loop (DNA 1, 3, 4, 5 and 6) and just after in case of DNA 2. However, position of third iterated sequence (R) varies within different components, being located 89nt upstream of stem-loop region in case of DNA 2, 18 nt upstream from 5' end of stem-loop on the complimentary strand of DNA 1, 3, 4 and 5 and 9nt in case of DNA 6. Stem-loop sequence of DNA 6 found to be less identical compared to rest of the components.

Another conserved sequence, known as CR-M was found to be located at the 5' end of CR-SL in the non-coding part of each component. CR-M region were found to vary in length within different components, being 92 nucleotides in DNA 5, 91 in DNA 6, 90 in DNA 2, 3 and 4 and 81 in DNA 1 respectively. CR-M of each component except DNA 1, contains a near complete repeat of 16 nucleotides (ATACAAG/cACG/aCTATG/aA), present between position 4–19 and 21-36 (Figure 4). A potential TATA box situated at 3' to stem-loop and 5' to ORF were found in all six of the components of BBTV. TATA box (CTATAAATA) was found to be identical in DNA 1, 3 and 4, while DNA 2, 5 and 6 contain CAATAATTA, CTATTTAAA and CTATTAATA sequences respectively (Figure 2).

Interestingly, pair wise alignment of one component to another, DNA component 3 shows 66% sequence homology with DNA 4 on entire nucleotide basis while sequence identity matrix shows 96 % sequence homology between these two components on a continuous conserve stretch of 334 nucleotides that started from 5' of CR-M and extended upto 3' of CR-SL (Figure 5).

Genetic diversity and Phylogenetic studies of BBTV

Sequence of different components of BBTV genome including ORF of DNA 1 (Rep) and DNA 3 (CP), were analyzed using various bioinformatics tools in order to study their genetic diversity and establish phylogenetic relationship with other isolates belonging to two different groups *viz*., South pacific (India, Pakistan, Egypt, Australia, Burundi, Tonga, Myanmar and Fiji) and Asian group (Vietnam, Philippines, China, Japan Indonesia and Taiwan). Multiple Sequence alignment of different components of BBTV genome revealed that complete

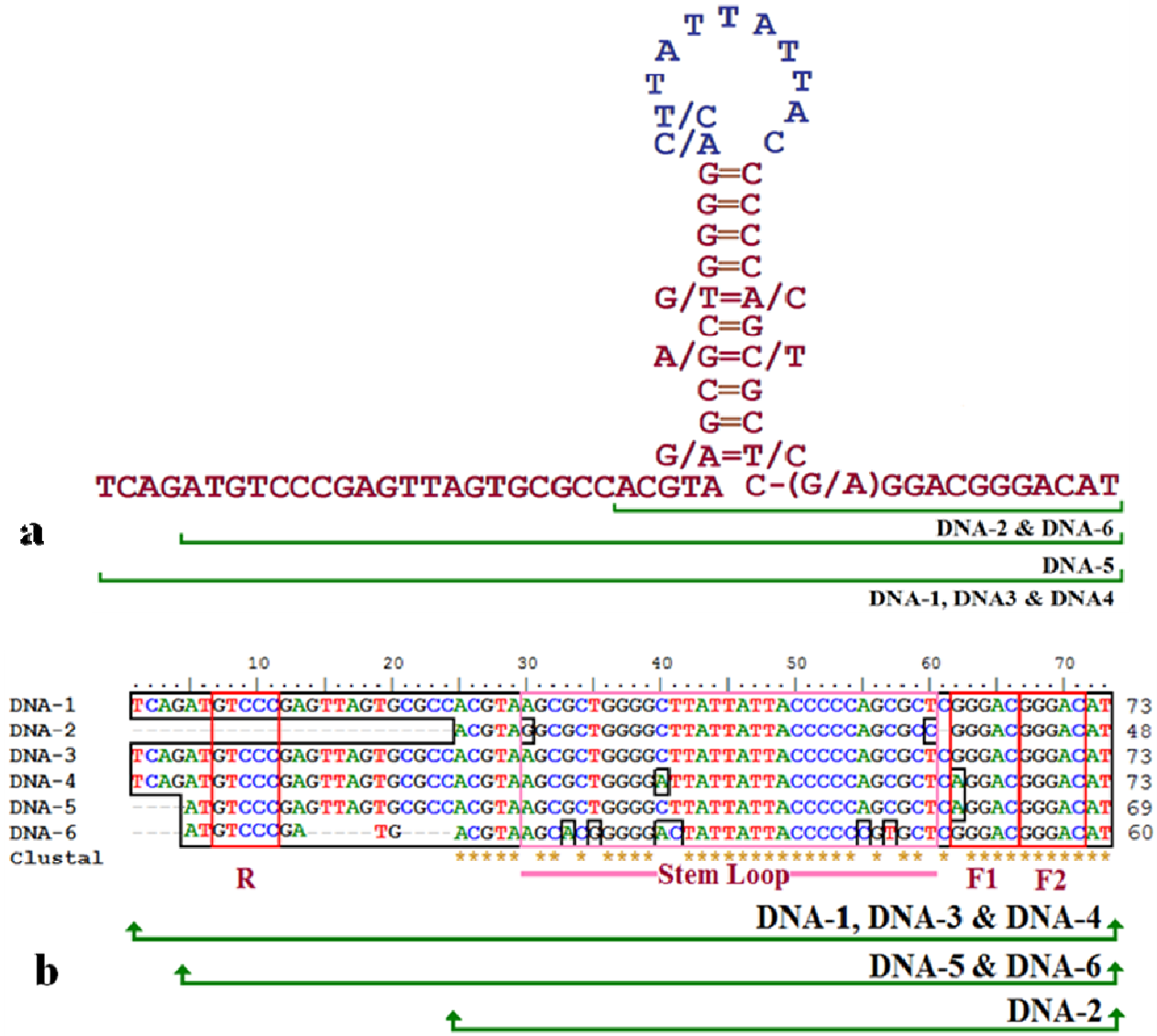

Figure 3 Stem-loop common region (CR-SL) of all six BBTV components showing conserved between each other within the isolate from Bhagalpur (Bihar) (a) Diagrammatic view of CR-SL and (b) Sequence homology a of CR- SL (Pink box contain stem-loop region and red box contain iterated sequences)

sequence of DNA 1 (FJ605506) shares 97.8-99 % and 88.3-90% sequence identity with members of South Pacific group and Asian group respectively, while replicase (FJ605506, EF584545, DQ640741 and DQ640742) was found to be 98-99.6% identical between members of South Pacific group and 91-93.9% identity with Asian group. Sequence comparison of replicase at amino acid level showed greater identity (98.6-100% with South Pacific group and 94-95.1% with Asian group) than nucleotide sequences (Table 3).

Sequence alignment of DNA 3 (FJ605507) showed 97-98.4% and 84.5-86.8% identity with isolates of South Pacific group and Asian group respectively. Sequence of coat protein gene (FJ605507, DQ996466, EF584544 and FJ168538) along with its putative amino acid sequence revealed 98.6-100% and 99.4-100% identity within South Pacific group and 91.8-94.1% and 96.4-99.4% within members of Asian group. Nucleotide sequence of DNA 2 (FJ605508) shares 89.6-96% and 70.8-80.9%, DNA 4 (FJ609642) 93.3-97.7% and 75.6-79.1%, DNA 5 (FJ609643) 97.4-99.2% and 85.5-88 % and DNA 6 (FJ609644) 94.7-98% and 82.9-84.8% identity between South Pacific and Asian group respectively (Table 3).

Sequence of all six components (DNA 1-6) including replicase and coat protein gene of BBTV isolates, representing both Asian and South Pacific isolates were used for generating phylogenetic trees both individually as well as in combination of DNA 1-6 using Neighbor Net

Table 3. Percent sequence identity at nucleotide and amino acid levels of BBTV DNA components and its ORF among Indian and other isolates belonging to South pacific group and Asian Group.

Sequences	Isolates under study	Indian isolates	South Pacific group	Asian group
Replicase (nt)	99.0-99.6	99.0-99.7	98.0-99.6	91.0-93.9
Replicase (aa)	99.3-100	99.3-100	98.6-100	94.0-95.1
Coat Protein (nt)	99.4-98.6	98.6-100	98.6-100	91.8-94.1
Coat Protein (aa)	99.4-100	99.4-100	99.4-100	96.4-99.4
DNA-1 (nt)	----------	98.4-98.9	97.8-99.0	88.3-90.0
DNA-2 (nt)	----------	86.2-96.0	86.2-96.0	70.8-80.9
DNA-3 (nt)	----------	97.6-98.3	97.0-98.4	84.5-86.8
DNA-4 (nt)	----------	97.3-97.9	93.3-97.9	75.6-79.1
DNA-5 (nt)	----------	97.4-99.1	97.4-99.2	85.5-88.0
DNA-6 (nt)	----------	94.7-97.8	94.7-98.0	82.9-84.8

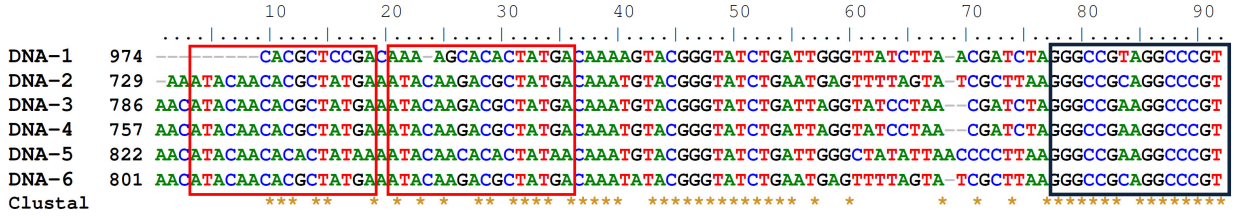

Figure 4 Major common region (CR-M) organization of all six BBTV components showing nucleotide position and its clustal consensus with each other. Red box contain 16 nucleotide direct repeat while, blue box containing GC rich region can also form 13 nucleotide stem loop structure (5 nucleotide stem and three nucleotide loop)

joining method in SplitTree4 software with 1000 bootstrap. Two other nanovirus viz. Milk Vetch Dwarf Virus (MVDV) and Faba bean necrotic yellows virus (FBYNV) was used as out group members (Figure 6 and 7) (Only replicase gene phylogenetic tree is shown in the figure).

Phylogenetic analysis of Major Common Region (CR-M) and Stem Loop Common Region (CR-SL)

Analysis of CR-SL and CR-M regions revealed CR-SL to be highly conserved between all reported isolates, while as CR-M found to be conserved within groups but shows less identity between members of South Pacific and Asian group. Phylogenetic tree for CR-M of 142 different sequences from various regions was generated using Neighbor Net Joining method in SplitTree4 software with replicates of 1000 bootstrap. Interestingly, CR-M region of DNA 2 used in this study was found identical with DNA 6, but not with reported sequences of DNA 2 (Figures 8 and 9).

DISCUSSION

Banana, a major staple food crop for approximately 400 million people are under a constant threat of large number of biotic and abiotic stresses (Msogoya and Grout 2008). However among biotic factors, viruses in particular Banana bunchy top virus (BBTV) represents a major constrain, limiting its production worldwide. BBTV is the sole member of the genus *Babuvirus* in the family *Nanoviridae*, having isometric virion of approximately 18-20nm and is transmitted by an aphid vector, *Pentalonia nigronervosa* besides being transmitted by vegetative planting materials.

Sequence data analysis of different BBTV DNA component (DNA 1-6) revealed them to be 1111, 1061, 1075, 1046, 1018 and 1090 nucleotides in length, with each DNA component comprising a CR-SL, TATA Box, conserved Poly A (A/tATAAA) signal, CR-M and a potential ORF. Besides, sequences analysis of replicase and coat protein gene revealed them to be of 861 and 513 nucleotides, potentially encoding 286 and 170 amino acids bearing proteins respectively.

Findings of CR-SL sequence analysis in the present study conflict with the recent reports by Amin et al. (2008) and Vishnoi et al. (2009) for DNA 1, DNA 3 and DNA 4 in being larger by 4nt than reported 69nt. CR-SL region of all the components contains stem-loop having conserved nonanucleotide sequence 'TATTATTAC'. Besides, it also contains three putative iterated sequences (except DNA 2 having only two iterated sequences), occurring as

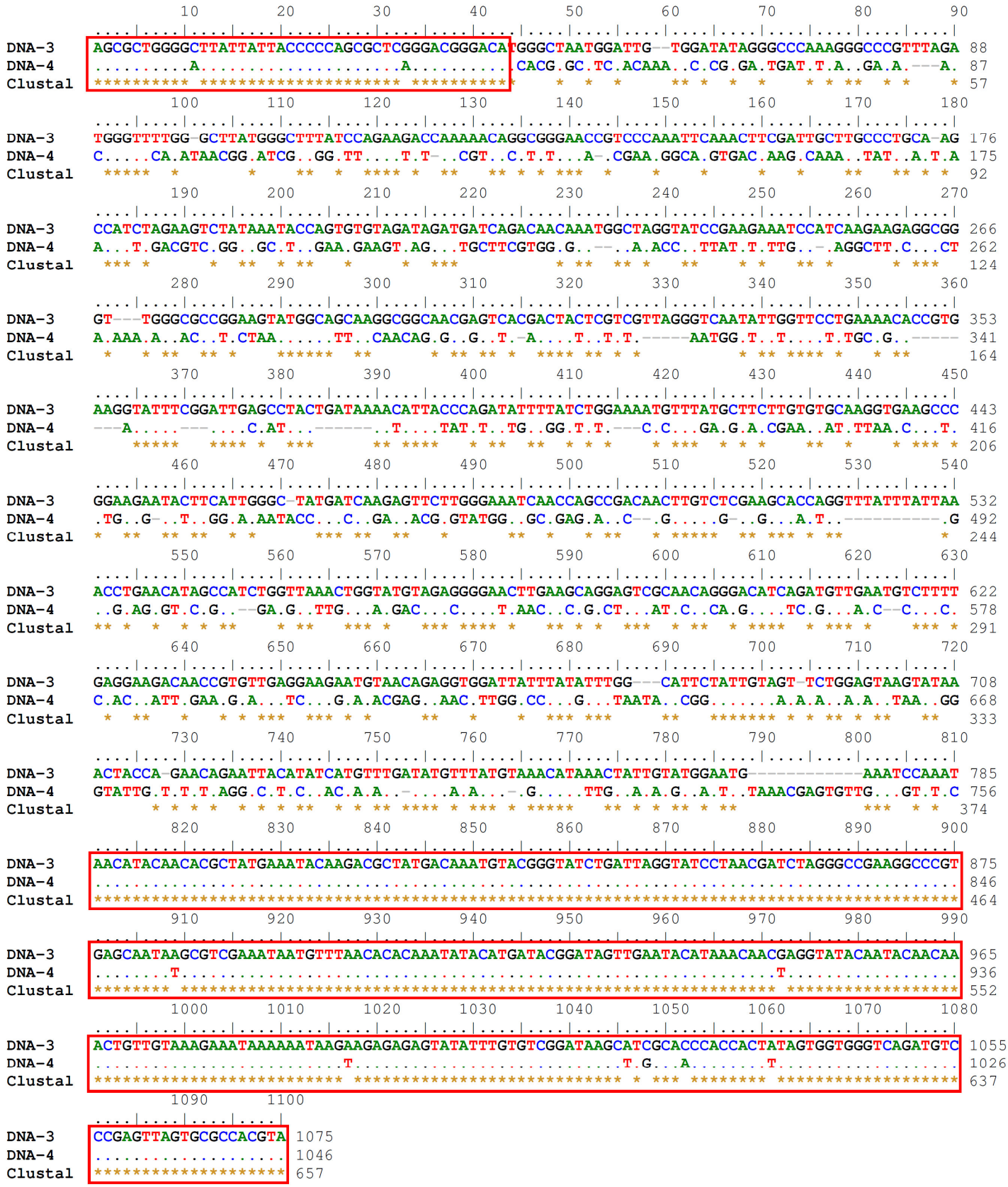

Figure 5 The nucleotide sequences alignment of DNA 3 and DNA 4 components showing 96% identity in the region starting 5' of CR-M to 3' of CR-SL (Red box

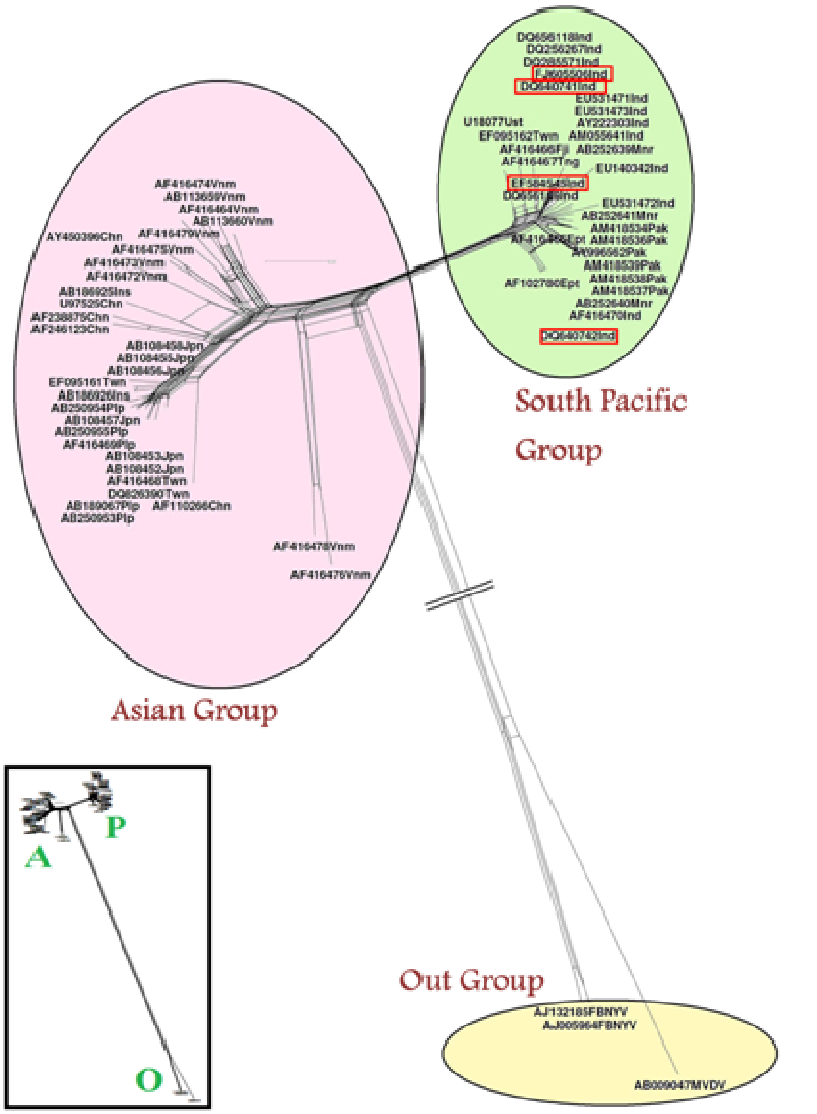

Figure 6. Phylogram, drawn by Neighbor Net Joining Bootstrap method (bootstrap analysis with 1000 replicates) in SplitTree (version 4), illustrating phylogenetic relationship based on multiple alignments of the Replicase (DNA1 ORF) nucleotide sequences of 61 isolates of BBTV including 2 isolates of MVDV and one isolate of FBYNV as an out group. Red Box indicates sequences under study. The inset showing the relative branch length of sequences of BBTV and other nanoviruses (A: Asian, P: South Pacific, O: Out Group)

tandem repeat with the sequence 'GGGAC' in case of F1 and F2 whereas it is 'GTCCC' (reverse complimentary of F1 and F2) in case of third iterated sequence R. However, in case of DNA 4 and DNA 5, F1 iterated sequences were found to posses 'A' in place of 'G' (A→G, transition) at the beginning of the sequence. Mutational studies of the iterated sequences have revealed that F2 iteron play an important role in the replication of BBTV. F1 and R iterons plays an important role in 'Rep' recognition and speculated to function as enhancers for the BBTV replication process (Herrera-Valencia et al., 2006). CR-M is located at the 5' end of CR-SL in the non-coding part of each component contains a 15 nucleotides GC rich region (GGGCCGC/atAGGCCCGT), sharing similarity between all components except A→C transversion at position 84 of CR-M in DNA 2 and 6 and A→T transversion in DNA 1. This GC rich region can potentially form a small stem-loop structure with 5 nucleotides stem with a loop of 3 nucleotides.

The complete nucleotide sequence of DNA 1 was found to be 1111 nt with an ORF of 861 nucleotides potentially encoding 286 amino acid replicase protein (~33.58 kDa). These results are in line with the finding of

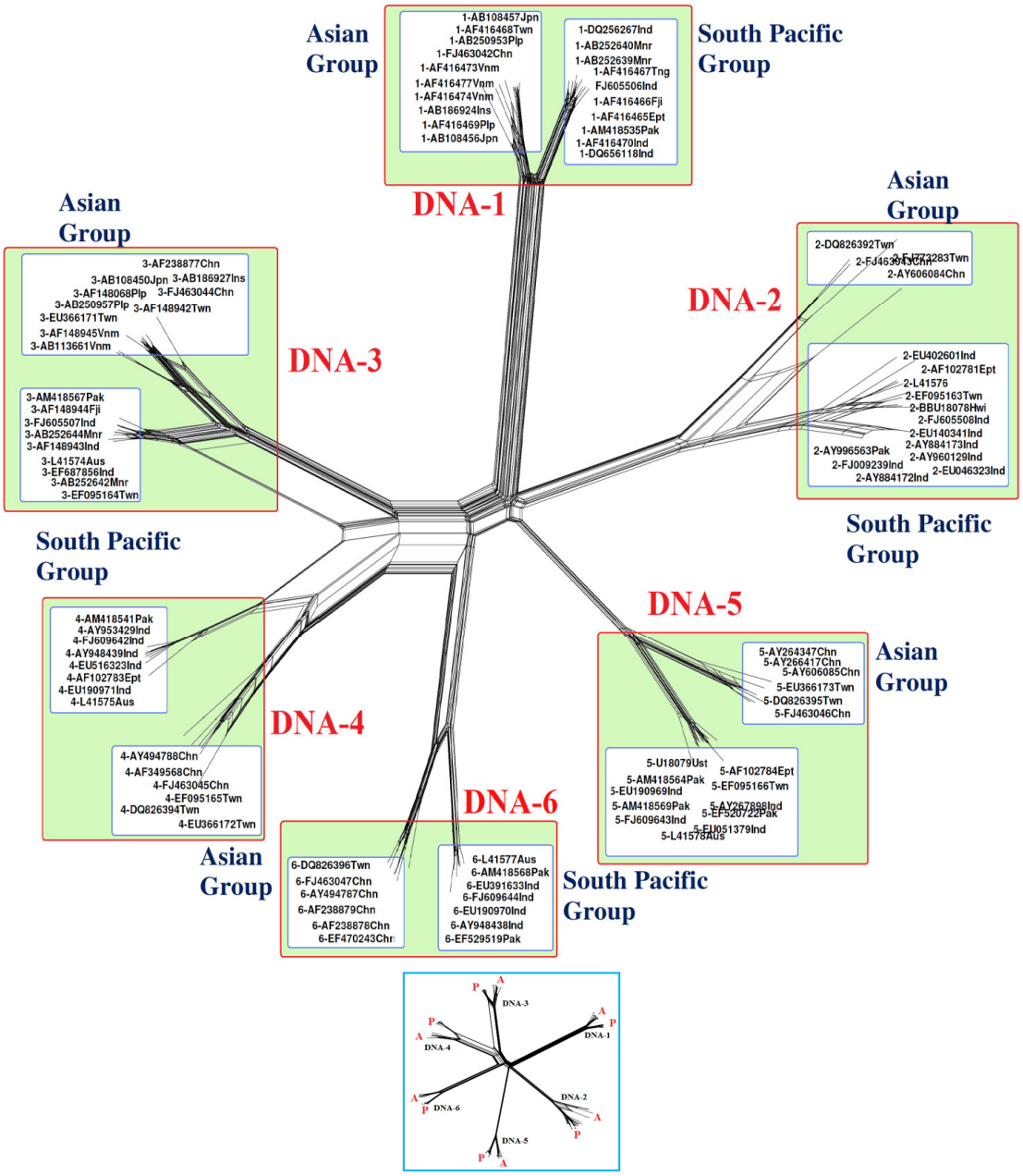

Figure 7 Phylogram, drawn by Neighbor Net Joining Bootstrap method (bootstrap analysis with 1000 replicates) in SplitTree (version 4), illustrating phylogenetic relationship based on multiple alignments of DNA 1-6 nucleotide sequences of different isolates BBTV representing Asian group and South Pacific group. Inset showing the relative branch length of group

Harding et al. (1991) and Burns et al. (1995). However, sequences reported from other regions (namely Vietnam, China, Taiwan, Indonesia, Philippines and Japan) were only 1103-1104 nt in length. Full length nucleotide sequence of DNA-2 was found to be 1061 nt in the isolate under study. DNA 2 of other reported sequences vary from 1035 nt (Pakistan isolate) to 1067 nt (China isolate). Similar to other reports, ORF size of DNA 2 was 234 nucleotides encoding a protein of 77 amino acid residues. Function of ORF DNA 2 protein is unknown. Sequence of DNA 3 reflected that it is 1075 nt long, potentially encoding coat protein of 170 amino acid through the ORF size of 513 nt. Sequence data of DNA 4 revealed it to be 1046 nt long with a predicted ORF size of 354 nt encoding Movement protein of 170 residues (~13.82 kDa). Length of DNA 4 is known to vary between 1037-1050 nt. The complete nucleotide sequences of DNA 5 was 1018 nt encoding retinoblastoma (Rb) binding

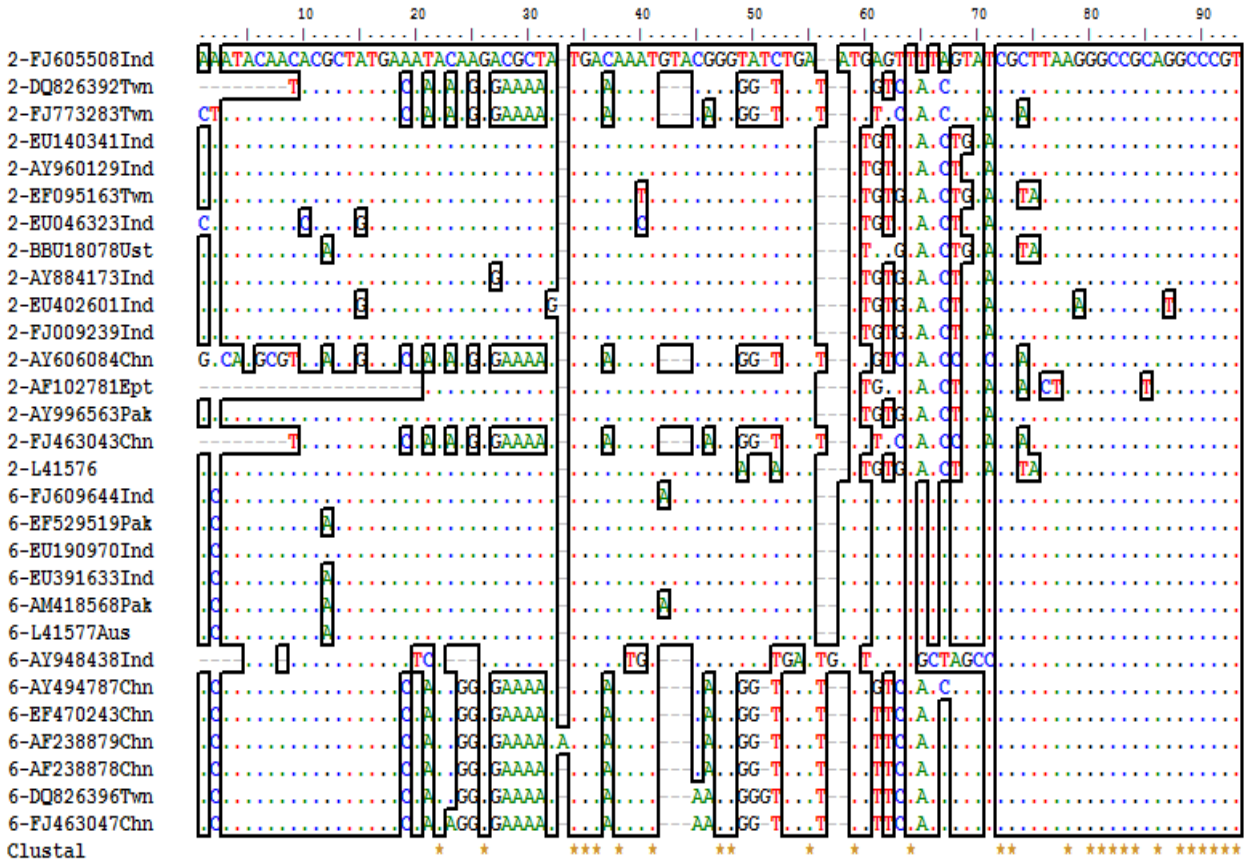

Figure 8 The nucleotide sequences alignment of CR-M of DNA components 2 and 6 showing South Pacific group sequences are different with Asian group, while sequences within the same group are much conserved within components. Position number 58-71 of DNA 2 of Bhagalpur (Bihar) isolate was found identical with DNA 6 of South Pacific group but not with DNA 2 and shows possible recombination. (Before Accession number 2 and 6 indicates DNA 2 and DNA 6)

like protein of 161 amino acid residues (~19.07kDa). Similar to other reports, DNA 6 was 1090 nt long putatively encoding Nuclear shuttle protein of 154 amino acids residues. However, Amin et al. (2008) reported the DNA 6 (1096 nt) with an ORF of 471 nt encoding protein of 156 amino acid residues having deduced molecular mass of 17.5 kDa. However, contrary to the sequences reported by Amin et al. (2008) (EF529519, Pakistan) and Vishnoi et al. (2009) (EU391633, India: Lucknow) where an insertion of 6 nucleotide direct repeat representing 2 additional amino acids (nucleotide position 453-458) have taken place in these two sequences, we did not observe such nucleotide insertions.

Phylogenetic analysis was performed by multiple sequence alignment of isolates under study with other isolates reported from India as well as other parts of the world available in GenBank using BLAST search program (Altschul et al., 1997) for sequence identification. Phylogenetic studies using replicase and coat protein gene as well as complete nucleotide sequences of (DNA 1-6) of various isolates reported from India and other parts of the world revealed that the isolates under study belong to South Pacific group and are very close to Fiji and Myanmar isolates. One of the Taiwan isolate usually falls in South Pacific Group while others remain in Asian group. Analysis shows the concentrated and divergent evolution of Banana bunchy top virus. These results are line with the earlier finding (Hu et al., 2007).

It was found that CR-SL and TATA regions are highly conserved between all reported isolates except DNA 4 in which TATA region diversify between two groups. Sequences alignment of DNA 1-6 CR-M was found to be conserved within groups but shows less identity between members of South Pacific and Asian group. These finding corroborates with the previous studies by Hu et al. 2007. Both isolates of Bhagalpur (Bihar) were found to have a change in amino acid at position 95 (Alanine to Serine) of Replicase gene. This change is correlated with change already reported in one member each of South Pacific group (Fiji) and Asian group (China). However, changes at other positions in the nucleotide of replicase and coat protein gene ORF does not lead to change in amino acid and maintain the conserved identity.

A mix Phylogram based on the DNA components 1-6

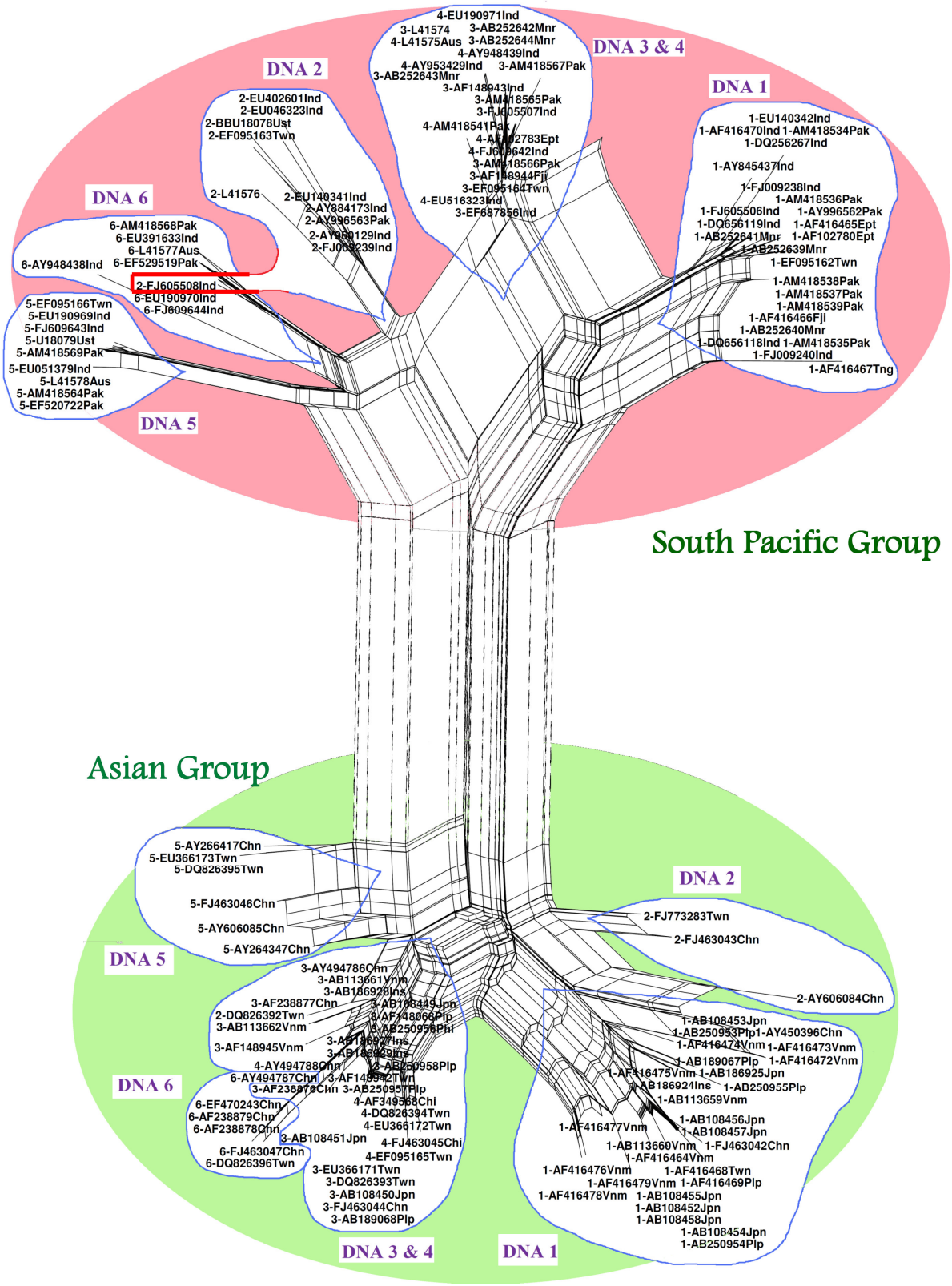

Figure 9 Phylogram, based on multiple alignments of CR-M nucleotide sequences of 140 isolates of BBTV (Before Accession number 1to 6 indicates DNA 1 to 6). Bhagalpur (Bihar) isolate of DNA 2 CRM was found into DNA 6 clade of South Pacific group.

showed that DNA 3 and DNA 4 are closer to each other than the remaining components (DNA 1, 2, 5 and 6). Based on the above findings we can assume that BBTV genome originated from a single component and during evolution some portion of DNA 3 and DNA 4 remained conserved (Figure 5 and 7). CR-M of 142 different isolates (DNA 1-6) was also used for generating phylogenetic tree of BBTV. The results showed two clades of South Pacific and Asian group (Figure 9) similar to our results obtained using complete sequences. In both groups, sequences from the same components cluster separately while DNA 3 and DNA 4 falls together in one cluster. Interestingly, CR-M of DNA 2 (FJ605508) cluster with DNA 6 in Asian group.

Plants elicit defense responses in response to pathogen entry including RNAi (Chellappan et al., 2004). BBTV, like other viruses, can also induce and become target of host RNAi pathway(s). In order to replicate and sustain the virulence, certain viruses might incorporate variability within its genome. This can be achieved by employing several mechanism(s) including recombination and can be achieved through recruitment of host proteins. Intriguingly, we observed that DNA 2 CR-M of Bihar isolate (FJ605508) at position number 58-71 has no any significant identity with DNA 2 of any other isolate and it was found to be 100% identical with the CR-M sequences of DNA 6 of South Pacific group and shows possible recombination between these two components. Considering the fact that the CR-M region does not encode for any protein, the insertion might not have any impact on viral replication. However, we cannot exclude possibility of any such events in other region(s) of the genome which may be relevant in viral pathogenesis.

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