Molecular detection of DNA component 6 (DNA-N) of banana bunchy top virus isolated from Egypt

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Abstract

Banana bunchy top virus (BBTV) is considered the most serious disease affecting banana in Egypt.BBTV was isolated from infected banana(*Musa acuminata* cv Grandnain)obtained from EL-Behira, Egypt by*Pentalonianigronervosa* .Polymerase chain reaction (PCR) was used to detect BBTV in infected plants and banana aphid (*P.nigronervosa*) using specific primer of DNA-1 for BBTV. The results showed that amplified PCR product with the expected size 476 bp for both infected banana and aphid. PCR was used to detect component 6 of BBTV-DNA using specific primer at expected size 813 bp.The isolated component was cloned into PCR™-4-TOPO vector (3.956~kb) and were transformed using minipreperation method. Component-6 was sequenced to determine the phylogenetic with other published isolates. Phylogenetic tree showed the identity percentage of Egyptian isolate group of BBTV-component- 6 with component 6 of Indian, Taiwanese and Pakistani isolates group was 97%, whereas it was 84% in case ofChinese isolate group.

Key words: Banana viruses, BBTV, PCR,*Pentalonianigronervosa*, cloning,sequence,phylogenetic.

Introduction

Banana bunchy top disease is the most important that causes by *Banana bunchy top virus* (BBTV).Thesymptoms of BBTV include yellowing of leaf marginsand the presence of dark green streaks on the petioles, pseudo-stem and leaf lamina.(**El-Dougdoug and El-Shamy, 2011).**BBTV is transmitted by the banana aphid (*Pentalonianigronervosa*) in a persistent manner and also the virus is transmitted through infected plant suckers and other plant components used in banana propagation but is not sap transmissible **(Allam, *et al.* 2000).**Also the virus is successfully transmitted by mechanical method using syringe by injection the crude sap from infected samples in healthy samples **(Thabet, 2000).**BBTV which was classified as a member of luteovirus group **(Matthews, 1982)** is now Known

as the type spiecesssDNA ofBabuvirus group in the family Nanoviridae**(Hughes, 2004)**. The virus has an isometric particle with 18-20 nm in diameter and its genome consists of at least six components of circular single stranded DNA (cssDNA) each of about 1 kb **(Xie& Hu, 1995).** These cssDNA were initially known as BBTV DNA-1 to -6 but recently were renamed BBTV DNA-R, -U3, -S, -M, -C and –N, respectively **(Vettene*t al.*, 2005).** BBTV-R encodes a replication protein **(Hafnere*t al.*, 1997).** BBTV-U3 encodes unknown function protein while BBTV-S encodes the viral coat protein (CP)**(Beethame*t al.*, 1999).**DNA-M encodes a putative movement protein (MP)**,** BBTV-C encodes a protein that presumably facilities viral replication by switching the plant host cells into S-shapeand BBTV-N encodes a nuclear shuttle protein**(Wanitchakorne*t al.*, 2000).** This investigation aims to study the cloning, sequencing and analysisof the component-6 of BBTV DNA Egyptian isolate.

Materials and methods

Source of virus

Samples of infected banana plants showing visual symptoms of banana bunchy top disease (bunchy top, yellow at the margins and dark green streaks on leaf veins and midribs) were collected fromUm-Saber farm, Kafrbadr,Behira governorate, Egypt.

Isolation of virus

Virus was isolated using banana aphid according to **Allam, *et al.* (2000)**. Banana aphids (*Pentalonianigronervosa*) provided by Plant protection Dept., Fac. of Agric., Ain Shams Univ. were fed for 24 hrs. on the infected BBTV plants and then transferred to healthy banana plantsobtained from tissue culture (aged two months) (5 adult insects per each plant). After 24 hrs, the inoculated plants were sprayed with a systemic insecticide (Reldan 50% EC) to kill the aphids. The control and inoculated plants were kept in green-house for 60 days for showing visual symptoms of banana bunchy top disease and confirmed by PCR using specific primer for BBTV.

Extraction of total nucleic acid

The total nucleic acids was extracted from fresh leaves samples of banana infected with BBTV isolate and aphid (*P.nigronervosa*) according to **(Dellaporta*et al.*, 1983).**

Detection of BBTV byPCR

Specific primers (Table 1) of DNA-1 for detection of BBTV were obtained fromvirology lab, Virus and Phytoplasma Dept., Plant Pathology Res. Inst. designed according to **Shamloul *et al.* (1999),** and the PCRwas conducted on a volume of 50 µl according to **Shamloul *et al.* (1999).**

Table 1. Primer for PCR detection of BBTV

primers	No. of bases	sequence	position	Amplified DNA (bp)
Viral sense of BBTV	21	5'-GTTCTCCAGCTATTCATCGCC-3'	569-589	476bp
Complementary of BBTV	21	5'-CATCATCGACGACGAAATGGC-3'	113-133	

The DNA template was amplified using the following program as described by **Shamloul *et al.* (1999).**

Gel electrophoresis

The PCR product was electrophoresed in 1% agarose gel in 1x TBE buffer at 120 V for 1 hour and stained with ethidium bromide (0.5 µl/ml) **(sambrook*et al.*, 1989).** The bands were photographed using UV lamp in gel-documentation (Bio Rad, Gel Doc XR system 170-8170).

Isolation of DNA component-6 from infected plant:

Polymerase chain reaction (PCR) technique was used for isolation of the component 6 of BBTV-DNA using specific primers designed for the component (table 2) according to **Rezk (2001).** The primers, called bbtv2v775 and bbtv6c549 were designed as specific primer for component 6. The primers were obtained from Invitrogen Company, Germany.

Cloning of the isolated compo-nent-6 of BBTV.

PCR amplified fragments of BBTV were cloned using the TA Cloning® Kit (Invetrogen, K4575-01, USA). The vector (PCR-4-TOPO) has ampicilin and kanamycin resistance genes for selection purposes and has incorporated a LacZ α gene in the location of the polylinker region. The fragments were ligated into PCR-4-TOPO plasmid as following:

Mixture of 1µl of fresh PCR product, 1µl of salt solution, 3µl of d.water and 1µl of TOPO vector was mixed gently and incubated for 5 minutes at room temperature (22^{o}c) and the tubes containing the reaction were placed in ice.

Competent cells of *E.coli* DH5α-T1 cells were treated with calcium chloride to facilitate the uptake of foreign DNA. The entire procedure was done under sterile conditions according to **sambrook*et al.* (1989).** After

performing the TOPO Cloning reaction, transformation will transform the plasmid (pCR ™ 4-TOPO) construct into the competent *E.coli* using one shot®, recombinant plasmids were extracted using mini-prep procedure as described by **sambrook*et al.* (1989)**.

Sequence, analysis of component DNA-6:

The isolated component from cloning using mini-preparation was sent to Sigma Company, Germany to sequence the components and analysis the sequence using DNAMAN software program to determine the phylogenetic with published sequence in Gene Bank.

Table (2): primers used in PCR amplification of component-6 of the isolated BBTV

Comp. No.	primer	Sequence of primer	Expected size band
BBTV6	bbtv2v775	5'-TACAAGACGCTATGACAAATGTACKGG-3'	813bp
	bbtv6c549	5'-CCGAATGGTACTATGAGTACTGGACGC-3'	

Results

Isolation of BBTV

Banana aphids (*P.nigronervosa*) which carried BBTV were used to inoculate healthy banana plants.Aphid inoculated plants were daily observed for 60 days. Typical symptoms of BBTV (bunchy top, yellow at the margins and dark green streaks on leaf veins and midribs) were appeared on all banana plants (fig. 1). The infected plants were confirmed for the presence of BBTV by the PCR technique.

Fig.1: Banana plants inoculated with BBTV isolate by banana aphids showing symptoms ofbanana bunchy top disease

BBTV detection byPCR

Total nucleic acids were extracted from both infected banana tissues and banana aphids (*P.nigronervosa*) for virus detection. As shown inFig. (2) the amplified PCR product with the expected size 476 bp for DNA of plant tissue and DNA of *P. nigronervosa* using primers (cBBTV-1 and hBBTV-1).

Identification of DNA component-6 of BBTV isolate:

The PCR was successfully to identify the (DNA-N) specific primers as shown in table(2).Data in Fig. (3) show that primers 2v775 (as viral primer) and bbtv6c549 (as complimentatry primer) were used to amplify component DNA-6 with the expected size (813bp) as shown in Fig. (3).

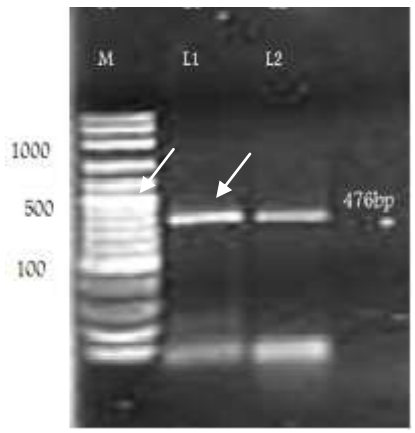

Fig. (2): Agarose gelelectrophoresis analysis of the PCR products for detection of BBTVin banana plant tissue and the aphid(*P.nigronervosa*).

M= DNA marker
L1= Amplified fragment of DNA of BBTVobtained from plant tissue
L2= Amplified fragment of DNA of BBTVobtained from insect.

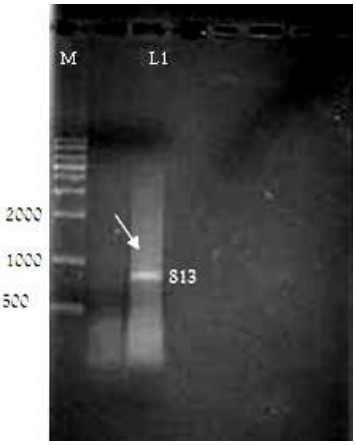

Fig. (3): Agarose gel electrophoresis of the PCR amplified the component-6 from banana plantsinfected with BBTV using specific primer

M= DNA marker
L1= Amplified fragment of DNA-6 of BBTV.

Cloning of DNA-6

PCR amplified products were cloned using the Cloning® Kit (Invetrogen, K4575-01, USA). The fragment of component DNA-6 of BBTV were ligated into PCR™-4-TOPO recombinant DNA plasmid vector (with molecular weight 3.956~kb) and transformed into competent cells of *E.cloi* DH5α-T1 (one shot®). Fig. (4)show the primers bbtv2v775 (as viral primer) and bbtv6c549 (as complimentatry primer) were used to amplify component 6 with the expected size (813 bp) as shown in Fig. (4 L1).

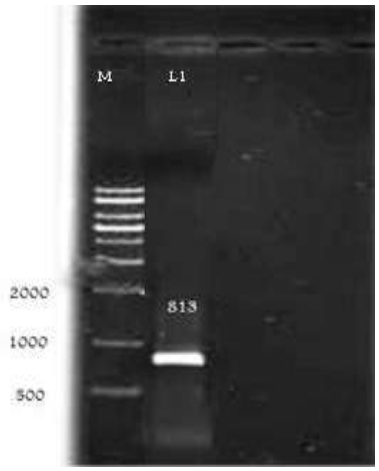

Fig. (4): Gel electrophoresis of the PCR amplified the component-6 from recombinant plasmid isolated by Mini-prep. using specific primer.

M= DNA marker
L1= Amplified fragment of DNA-6 of BBTV.

Sequence, analysis components DNA-6:

Component DNA-6 of Egyptian BBTV isolate was sequenced and analyzed to compare and determine the phylogenetic of these components with other BBTV isolatesin Gene Bank.

BBTV-DNA-component 6:

Egyptian isolate of BBTV-DNA-component-6 was amplified 813 bp fragment using Specific primers (in table 2). The 813 nt. of BBTV-6 was sequenced and analyzed. Fig.(5) showed that the sequence of Egyptian isolate of BBTV-component-6 was compared with component 6 of Chinese, Indian and Pakistani isolates. Phylogenetic tree as in fig.(6) showed the identity percentage of component-6 of

Egyptian isolate group with component 6 of Indian, Taiwanese and Pakistani isolates group was 97% and with Chinese isolate group was 84%. The sequence of Egyptian of BBTV-component-6 was translated to amino acids fig.(7).Twenty amino acids were translated for component 2. The percentages of amino acids were Arginine (10.7%), Serine (9.2%), Leucine (8.5%), Isoleucine (8.1%), Lysine (8.1%), Threonine (6.5%), Glycine (5.2%), Alanine (4.8%), Tyrosine (4.4%), Glutamic acid (3.7%), Asparagine (3.3%), Aspartic acid (3.3%), Glutamine (3%), Proline (3%), Methionine (2.6%), Phenylalanine (2.6%), Tryptophan (2.6%), Cysteine (2.2%), Histidine (2.2%) and Valine (1.8%)

Comparison between bases composition of component 6 sequence for BBTV Egyptian isolates and four different isolates published in GenBank was done to determine C+G and A+T ratio in Table (3), data showed that total base pair of Egyptian, Chinese, Indian, Pakistani and Taiwanese BBTV isolates component-6 was 549 bp, 545 bp, 551 bp, 545 bp, 551 bp respectively with molecular weight 20.3 KDa, 20.2 KDa, 20.4 KDa, 20.2 KDa and 20.4 KDa respectively. The percentage of Guanine (G) of Egyptian, Chinese, Indian, Pakistani and Taiwanese BBTV isolates component-6 was 25.1%, 24.9%, 25%, 25.1% and 25% respectively. Cytosine (C) was 19.9%, 19.3%, 20%, 20%, and 20% respectively. Adenine (A) was 29.5%, 29.7%, 28.9%, 29.4% and 28.9% respectively. Thymine (T) was 25.5%, 26%, 26.1%, 25.5% and 26.1% respectively. G+C was 45%, 44.2%, 45%, 45.1% and 45% respectively. A+T was 55%, 55.7%, 55%, 54.9% and 55% respectively.

Fig.(5): Comparison between the sequence of Egyptian isolate of BBTV component-6 with component 6 of Chinese, Indian Pakistani and Taiwanese isolates.

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Egypt comp6     AGCACGGGGG ACTATTATTA CCCCCCGTGC TCGGGACGGG ACATGACGTC
China comp6     AGCACGGGGG ACTATTATTA CCCCCCGTGC TCGGGACGGG ACATGACGTA
India comp6     AGCACGGGGG ACTATTATTA CCCCCCGTGC TCGGGACGGG ACATGACGTC
Pakistan comp6  AGCACGGGGG ACTATTATTA CCCCCCGTGC TCGGGACGGG ACATGACGTC
Taiwan comp6    AGCACGGGGG ACTATTATTA CCCCCCGTGC TCGGGACGGG ACATGACGTC

Egypt comp6     AGCAAGGATT ATAATGGGCT TTTTATTAGC CCATTTATTG AATTGGGCCG
China comp6     AGCATAGATT ATAATGGGCT TTTTAAAGCC CATATAAGGG AAGTGGGCCG
India comp6     AGCAAGGATT ATAATGGGCT TTTTATTAGC CCATTTATTG AATTGGGCCG
Pakistan comp6  AGCAAGGATT ATAATGGGCT TTTTATTAGC CCATTTATTG AATTGGGCCG
Taiwan comp6    AGCAAGGATT ATAATGGGCT TTTTATTAGC CCATTTATTG AATTGGGCCG

Egypt comp6     GGTTTT.GTC ATTTTACAAA AGCCCGGTCC AGGAT.AAGT ATAATGTCAC
China comp6     GGTTTGAGAC ATATTTCGAA AGCCCGG.CT TGGAA.AAGG ATAAAGTCAC
India comp6     GGTTTT.GTC ATTTTACAAA AGCCCGG.TC CAGGATAAGT ATAATGTCAC
Pakistan comp6  GGTTTT.GTC ATTTTACAAA AGCCCGG.TC CAGGATAAGT ATAATGTCAC
Taiwan comp6    GGTTTT.GTC ATTTTACAAA AGCCCGG.TC CAGGATAAGT ATAATGTCAC

Egypt comp6     GTGCCGAATT AAAAGGTTGC TTCGCCTCGA AGAAACCTAA ATTGAGGTTG
China comp6     GTTCCGAAT. AATAGGTTGC TTCGCCGCGA AGCAACCTAA TAAATTGTTG
India comp6     GTGCCGAAT. AAAAGGTTGC TTCGCCTCGA AGAAACCTAA TTTGAGGTTG
Pakistan comp6  GTGCCGAAT. AAAAGGTTGC TTCGCCTCGA AGAAACCTAA TATGAGGTAG
Taiwan comp6    GTGCCGAAT. AAAAGGTTGC TTCGCCTCGA AGAAACCTAA TTTGAGGTTG


Egypt comp6     CGTATTCAAT ACGCTACCGA ATATCTATTA ATAAGCGAGT CTCTGCCGAA
China comp6     CGTATTCAAT ACGCAACTAA AAGTCTATTA ATATGCCTGT CTCTGCCGAA
India comp6     CGTATTCAAT ACGCTACCGA GTATCTATTA ATATGTGAGT CTCTGCCGAA
Pakistan comp6  CGTATTCAAT ACGCTACCGA ATATCTATTA ATATGTGAGT CTCTGCCGAA
Taiwan comp6    CGTATTCAAT ACGCTACCGA GTATCTATTA ATATGTGAGT CTCTGCCGAA


Egypt comp6     AACAATCAGA GCGAAAGCGG AAAGCAGAAG CGATGGATTG GGCGGAATCA
China comp6     TA.AATCAGA GCGTATGCG. .AAGCAGAAG CGATGGATTG GGCAGAATCA
India comp6     AC.AATCAGA GCGAAAGCA. .AAGCAGAAG CGATGGATTG GGCGGAATCA
Pakistan comp6  AC.AATCAGA GCGAAAGCA. .AAGCAGAAG CGATGGATTG GGCGGAATCA
Taiwan comp6    AC.AATCAGA GCGAAAGCA. .AAGCAGAAG CGATGGATTG GGCGGAATCA


Egypt comp6     CAATTCAAGA CTTGTACTCA TGGATGCGAT TGGAAGAAGA TATCATCGGA
China comp6     CAATTCAAGA CATGTACCCA TGGCTGTGAT TGGAAGACGA TATCATCGGA
India comp6     CAATTCAAGA CTTGTACTCA TGGATGCGAT TGGAAGAAGA TATCATCGGA
Pakistan comp6  CAATTCAAGA CCTGTACTCA TGGATGCGAT TGGAAGAAGA TATCATCGGA
Taiwan comp6    CAATTCAAGA CTTGTACTCA TGGATGCGAT TGGAAGAAGA TATCATCGGA


Egypt comp6     TTCAGCCGAT AATCGACAAT ATGTACCATG CGTCGATTCT GGAGCTGGAA
China comp6     TTCATCGGAA AATCGGCAAT ATGTACCTTG CGTCGACTCT GGTGTTGGAA
India comp6     TTCAGCCGAT AATCGACAAT ATGTACCATG CGTCGATTCT GGAGCTGGAA
Pakistan comp6  TTCAGCCGAT AATCGACAAT ATGTACCATG CGTCGATTCT GGAGCTGGAA
Taiwan comp6    TTCAGCCGAT AATCGACAAT ATGTACCATG CGTCGATTCT GGAGCTGGAA


Egypt comp6     GAAAGTCGCC TCGCAAGGTA CTTCTTAGAT CTATTGAAGT TGCGTTTAAC
China comp6     GAAAGACGCC TCGCAAGGTA CTTCTTCGAT CTATCGAAGT TGTATTCAAT
India comp6     GAAAGTCGCC TCGCAAGGTA CTTCTTAGAT CTATTGAAGC TGTGTTTAAC
Pakistan comp6  GAAAGTCGCC TCGCAAGGTA CTTCTTAGAT CTATTGAAGC TGTGTTTAAC
Taiwan comp6    GAAAGTCGCC TCGCAAGGTA CTTCTTAGAT CTATTGAAGC TGTGTTTAAC

Egypt comp6     GGAAGCTT......CAGCGG AAATAATAGG AACGTTCGTG GATTTCTCTA
China comp6     GGAAGTTT......TAAAGG GAATAATCGG AATGTTCGTG GCTTCTTATA
India comp6     GGAAGCTTAA GCTTCAGCGG AAATAATAGG AACGTTCGTG GATTTCTCTA
Pakistan comp6  GGAAGCTT.. ....CAGCGG AAATAATAGG AACGTTCGTG GATTTCTCTA
Taiwan comp6    GGAAGCTTAA GCTTCAGCGG AAATAATAGG AACGTTCGTG GATTTCTCTA

Egypt comp6     CGTATCGATC AGAGACGATG ACGGAGAAAT GCGTCCAGTA CTCATAGTAC
China comp6     CGTATCAATC CGAGACGATG ATAGAACATG GCGTCCAGTA CTTATAGTAC
India comp6     CGTATCGATC AGAGACGATG ACGGAGAAAT GCGTCCAGTA CTCATAGTAC
Pakistan comp6  CGTATCGATC AGAGACGATG ACGGAGAGAT GCGTCCAGTA CTCATAGTAC
Taiwan comp6CGTATCGATC AGAGACGATG ACGGAGAAAT GCGTCCAGTA CTCATAGTAC

Egypt comp6     CATTCGG
China comp6     CATTTGG
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continue

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India comp6     CCTTCGG
Pakistan comp6  CATTCGG
Taiwan comp6CCTTCGG
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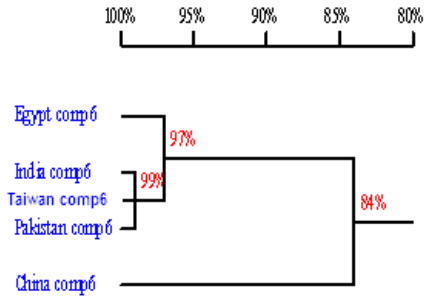

Fig. (6): The phylogenetic tree of BBTV- component-6 of Egyptian isolate with component-6 of Chinese, Indian, Taiwanese and Pakistaniisolates.

Fig.(7): Nucleotide sequence of the Egyptian isolate of BBTV-6 and the encoded amino acids sequences.

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1         TACAAGACGCTATGACAAATGTACGGGTATCTGAATGAGTTTTAGTATCGCTTAAGGGCC
1          Y  K  T  L  *  Q  M  Y  G  Y  L  N  E  F  *  Y  R  L  R  A

61        GCAGGCCCGTTAAAAATAATAATCGAATTATAAACGTTAGATAATAATCAGAGATAGGTG
21         A  G  P  L  K  I  II  E  L  *  T  L  D  N  N  Q  R  *  V

121       ATCAGATAACATAAACATAAACGAAGTATATGGCGGTACAATAATAAAATAAGTTAAAAA
41         I  R  *  H  K  H  K  R  S  I  W  R  Y  N  N  K  I  S  *  K

181       AAAAAACATATGAATACTAATCTCTGATTGGTTCAGAAGAAAGGCCCACCAACTAAAAGG
61         K  K  H  M  N  T  N  L  *  L  V  Q  K  K  G  P  P  T  K  R

241       TGGGGAGAATGTCCCGATGACGTAAGCACGGGGGACTATTATTACCCCCCGTGCTCGGGA
81         W  G  E  C  P  D  D  V  S  T  G  D  Y  YY  P  P  C  S  G

301       CGGGACATGACGTCAGCAAGGATTATAATGGGCTTTTTATTAGCCCATTTATTGAATTGG
101        R  D  M  T  S  A  R  I  I  M  G  F  L  L  A  H  L  L  N  W

361       GCCGGGTTTTGTCATTTTACAAAAGCCCGGTCCAGGATAAGTATAATGTCACGTGCCGAA
121        A  G  F  C  H  F  T  K  A  R  S  R  I  S  I  M  S  R  A  E

421       TTAAAAGGTTGCTTCGCCTCGAAGAAACCTAAATTGAGGTTGCGTATTCAATACGCTACC
141        L  K  G  C  F  A  S  K  K  P  K  L  R  L  R  I  Q  Y  A  T


481       GAATATCTATTAATAAGCGAGTCTCTGCCGAAAACAATCAGAGCGAAAGCGGAAAGCAGA
161        E  Y  L  L  I  S  E  S  L  P  K  T  I  R  A  K  A  E  S  R

541       AGCGATGGATTGGGCGGAATCACAATTCAAGACTTGTACTCATGGATGCGATTGGAAGAA
181        S  D  G  L  G  G  I  T  I  Q  D  L  Y  S  W  M  R  L  E  E


601       GATATCATCGGATTCAGCCGATAATCGACAATATGTACCATGCGTCGATTCTGGAGCTGG
201        D  I  I  G  F  S  R  *  S  T  I  C  T  M  R  R  F  W  S  W

661       AAGAAAGTCGCCTCGCAAGGTACTTCTTAGATCTATTGAAGTTGCGTTTAACGGAAGCTT
221        K  K  V  A  S  Q  G  T  S  *  I  Y  *  S  C  V  *  R  K  L

721       CAGCGGAAATAATAGGAACGTTCGTGGATTTCTCTACGTATCGATCAGAGACGATGACGG
241        Q  R  K  *  *  E  R  S  W  I  S  L  R  I  D  Q  R  R  *  R

781       AGAAATGCGTCCAGTACTCATAGTACCATTCGG
261        R  N  A  S  S  T  H  S  T  I  R
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Table (3): Comparison between bases composition of component-6 and fourdifferentisolatespublished in GenBank.

Isolates	Total bp	M.W KDa	G		C		A		T		G+C		A+T	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Egypt	549	20.3	138	25.1	109	19.9	162	29.5	140	25.5	247	45	302	55
China	545	20.2	136	24.9	105	19.3	162	29.7	142	26	241	44.2	304	55.7
India	551	20.4	138	25	110	20	159	28.9	144	26.1	248	45	303	55
Pakistan	545	20.2	137	25.1	109	20	160	29.4	139	25.5	246	45.1	299	54.9
Taiwan	551	20.4	138	25	110	20	159	28.9	144	26.1	248	45	303	55

Discussion

BBTV was isolated from naturally infected banana plants which grown in Behira governorate. Banana aphid (*P. nigronervosa*)was used to isolate the virus from infected plants to healthy banana plants after 24 hrs acquisition-feeding period and 24 hrs inoculation-feeding period. The symptoms of BBTV (bunchy top, yellow at the margins and dark green streaks on leaf veins and midribs) were appeared after two months from insect transmission. These results are in agreement with that obtained by **Thabet, 2000; Hooks *et al.*, 2009;Selvarajan*et al.*, 2011and Watanabe *et al.*, 2013.**

Detection of BBTV in infected banana samples using polymerase chain reaction (PCR) with specific primers showed that the amplifying of BBTV at 476bp. The similar result was mentioned by **Shamloul *et al.*, 1999 and Rezk, 2001.**

PCR succeeded to amplify DNA-6 of BBTV using specific primers. The component DNA- 6 was amplified at 813bp. The same result was obtained by **Rezk, 2001**.

Isolated component-6 was cloned and ligated into PCR™-4-TOPO vector (3.956~kb) (recombinant plasmids) and transformed into competent cells of *E.cloi* DH5α-T1 (one shot).The colonies which had component-6grown on S.O.C medium had the recombinant DNA. Component 6 was cloned before by**Mathiyazhagan*et al.*, 2011 andHuang *et al.*, 2011.**On the other hand **Zheng *et al.* (2005)** used *Agrobacterium tumefaciens* to transform the component 1**.**

Component 6 of Egyptian isolate (813nt) was sequenced and analysed. Data showed that the identity percentage of Egyptian isolate group of BBTV-component- 6 with component 6 of Indian, Taiwanese and Pakistani isolates group was 97% and with Chinese isolate group was 84%. On other hand, **He *et al.* (2000)** reported that the idintity of component-6 of Chinese isolate of BBTV was 85.5% with Australian isolate, but **Huang *et al.* (2008)**

mentioned that the identity of BBTV component-6 of Chinese isolate was 70.78% and 56.24% with Australian and Indian respectively, but**Radha*et al.* (2009)** mentioned that the idintity of component-6 of Indian isolate was 99% with south pacific group. While **Mathiyazhagan*et al.* (2011),**mentioned that the component 6 of BBTV isolate of Tamil Nadu, India shared 97% of nucleotide sequence identity with an Australian isolate and 95% with a Pakistan isolate of BBTV

References

Allam, E. K.; Othman, B. A.; El-Sawy, A. and Thabet, S. D. (2000). Purification and production of Banana Bunchy Top Virus (BBTV) antiserum. J. Appl. Sci. Zagazig Univ., Zagazig, Egypt.

Beetham, P. R.; Harding, R. M. and Dale, J. L. (1999). Banana bunchy top virus DNA-2 to 6 are monocistronic. Arch Virol. vol. 144. 89-105.

Dellaporta, S. L.; Wood, J. and Hicks, J. B. (1983). A plant DNA miniprepration: version II. Plant. Mol Biol. Rep. 1: 19-21.

El-Dougdoug, K. A. and El-Shamy, M. M. (2011). Management of viral disease in banana using certified and virus tested plant material. African Journal of Microbiology Research. 5: 32, 5923-5932.

Hafner, G. J.; Harding, R. M. and Dale, J. L. (1997).A DNA primer associated with banana bunchy top virus. Journal of General Virology; vol 78: 2, 479-486.

He, Z. F.; Li, H. P.; Xiao, H. G. and Fan, H. Z. (2000). Cloning and sequencing of DNA component 1 of two BBTV strains. ActaPhytopathologicaSinica. 30: 4, 364-369.

Hooks, C. R. R.; Fukuda, S.; Perez, E. P.; Manandhar, R.; Wang, K. H.; Wright, M. G. and Almeida R. P. P. (2009). Aphid Transmission of Banana Bunchy Top Virus to Bananas After Treatment With a Bananacide. Journal of Economic Entomology 2009.vol. 102(2):493-499.

Huang, Y. H.; Zeng, J. W.; Wu, Y. L.; Xia, R. and Yi, G. J. (2011).CR-M: an important nucleotide acid sequence to distinguish Banana bunchy top virus isolates from two groups. ActaHorticulturae;vol 897, 485-488.

Huang, Y. H.; Mei, M.; Zeng, J. W.; Zhou, B. R.; Wu, Y. L.; Xia, R. and Yi, G. J. (2008). Cloning of a DNA component of Banana bunchy top virus and analysis of its characteristic sequences. Journal of Fruit Science.vol 25: 6, 881-886.

Hughes, A. L. (2004). Birth-and-death evolution of protein-coding regions and concerted

evolution of non-coding regions in the multi-component genomes of nanoviruses. Mol. Phylogenet. Evol. vol. 30: 287-294.

Mathiyazhagan, K.; Sankarasubramanian, H.; Neelakandan, K.; Duraisamy, S. and Ramasamy, S. (2011). Molecular characterisation of coat protein and nuclear shuttle protein genes of Banana bunchy top virus from Western Ghats in India. Archives of Phytopathology and Plant Protection.vol 44: 5, 405-411.

Matthews, R.E.F. (1982). Classification and nomenclature of viruses. Intervirology 17: 1-200.

Radha, V.; Raj, S. K. and Vivek, P. (2009). Molecular characterization of an Indian isolate of Banana bunchy top virus based on six genomic DNA components. Virus Genes; vol 38: 2, 334-344.

Rezk, A. A. (2001). Studies on some Banana Viral Disease M. Sc. thesis, Faculty of Agriculture, Ain Shams Univeristy, Cairo, Egypt. 108 pp

Sambrook, J.; Fritsch, E. F. and Maniatis, T. (1989). Molecular cloning: a laboratory Manual, vol. 3. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Selvarajan, R.; Sheeba, M.M. andBalasubramanian, V. (2011). Simultaneous detection of episomal Banana streak Mysore virus and Banana bunchy top virus using multiplex RT-PCR. Current Science, 2011.vol.100, (1), p. 31-34

Shamloul, A. M.; Hadidi, A.; Madkour, M. A. and Makkouk, K. M. (1999). Sensitive detection of banana bunchy top and faba bean necrotic yellows viruses from infected leaves, in vitro tissue cultures, and viruliferous aphids using polymerase chain reaction. Canadian Journal of Plant Pathology; vol 21: 4, 326-337.

Thabet, S. D. (2000). Production of virus-free plants. Ph.D., thesis, Faculty of Agriculture, Ain Shams Univeristy, Cairo, Egypt. 160 pp

Vetten, H. J.; Chu, P. W. G.; Dale, J. L.; Harding, R.; Hu, J.; Katul, L.; Kojima, M.; Randles, J. W.; Sano, Y. and Thomas, J. E. (2005). Nanoviridae. Virus taxonomy, English report of the ICTV. Elsevier/Academic Press. pp 343-352

Wanitchakorn, R.; Harding, R. M. and Dale, J. L. (2000). Functional analysis of proteins encoded by Banana bunchy top virus DNA-4 to -6. J. Gen Virol. vol.81. 299-306.

Watanabe, S.; Greenwell, A. M. and Bressan A. (2013). Localization, Concentration, and Transmission Efficiency of Banana bunchy top virus in Four Asexual Lineages of

Pentalonia aphids. Viruses 2013.vol. 5, 758-775.

Xie, W. S. and Hu, J. S. (1995). Molecular cloning, sequence analysis, and detection of banana bunchy top virus in Hawaii. Phytopathology.vol 85: 3, 339-347.

Zheng, Y.; Li, H. P.; Xiao, H. G. and Fan, H. Z. (2005). Cloning and nucleotide sequence analysis of DNA-5 of Banana bunchy top virus NSP strain. ActaPhytopathologicaSinica. vol 35: 4,