EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES

PHYSIOLOGY & MOLECULARBIOLOGY

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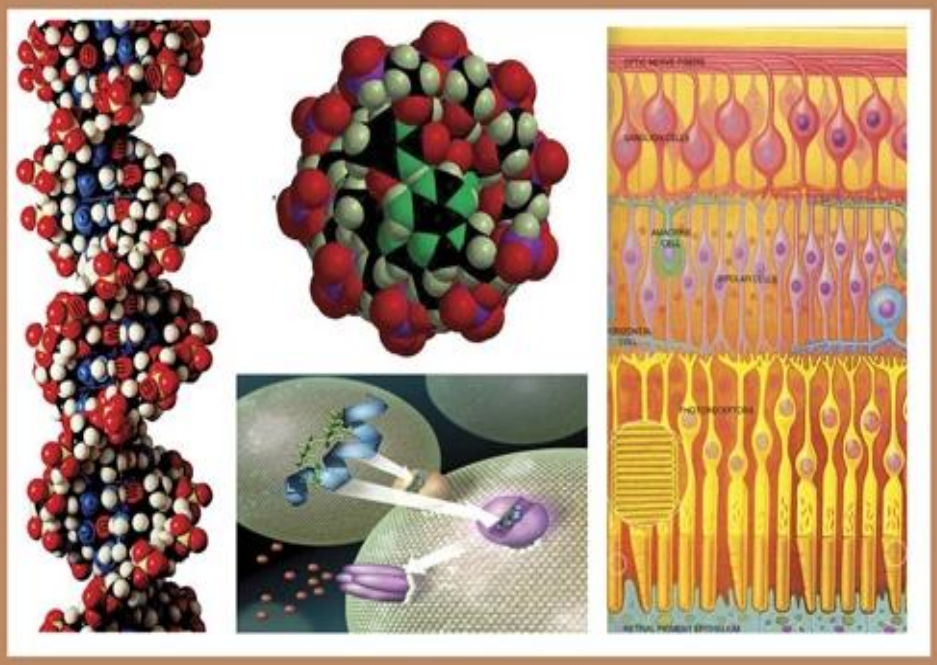

ISSN
2090-0767

WWW.EAJBS.EG.NET

Vol. 15 No. 1 (2023)

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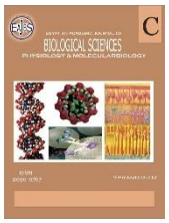
Citation: *Egypt.Acad.J.Biolog.Sci. (C.Physiology and Molecular biology) Vol. 15(1) pp455-473 (2023)*
DOI: 10.21608/EAJBSC.2023.301072

Egypt. Acad. J. Biolog. Sci., 15(1):455-473 (2023)

Egyptian Academic Journal of Biological Sciences
C. Physiology & Molecular Biology
ISSN 2090-0767
www.eajbsc.journals.ekb.eg



The Incidence and Molecular Characterization of Banana Bunchy Top Virus (BBTV) Infecting Banana (*Musa spp.*) in Ismailia governorate, Egypt

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ARTICLE INFO
Article History
Received:25/4/2022
Accepted:30/5/2023
Available:6/6/2023

Keywords:
Banana Bunchy Top Virus, Incidence, PCR, nucleotide sequences.

ABSTRACT

The banana is the most important fruit in Egypt. Banana bunchy top disease, caused by the banana bunchy top virus (BBTV), is one of the most damaging infections on banana plantations, resulting in significant crop losses. The current study was conducted to characterize the incidence of banana bunchy top disease (BBTD) and to study the molecular characteristics of BBTV from banana plantations in Ismailia. This study was conducted during the period 2019-2021 in three locations (Abu-Suwayr, El Tal-El kabir and Fayed) in Ismailia Governorate. The occurrence of BBTV was initially studied using visual inspection of external virus symptoms. TAS-ELISA, PCR and nucleic acid hybridization were used to detect the virus. Further molecular characterization of BBTV was performed based on Rolling Circle Amplification (RCA) and nucleotide sequencing. BBTD incidences ranging from 3.33 to 5.14% were observed in two localities Abu-Suwayr and El Tal-El kabir. While a relatively low incidence of 1.48% was observed in Fayed. Our results showed that the BBTD incidence is directly related to the percentage of winged aphids inside the aphid colonies regardless the percentage of the aphid's occurrence. The PCR product (973 bp) of BBTV- component 3 (DNA-S) was used for the creation of a non-radioactive Dig labeled- DNA probe for further use for BBTV detection using dot blot and Southern blot hybridization. The sequence comparison analysis of DNA-S component showed that the present Ismailia isolate (BBTV-3F) shared nucleotide identity 98.4%- 91.9 % with three isolates from Qalubya Egypt and shared 97.9% nt identity with east African isolates (Rwanda and Malawi). The phylogenetic analysis revealed that the DNA- component 3 (DNA-S) of the BBTV-3F isolate from Ismailia belongs to the South Pacific group (SPG) and is markedly separated from those of the Asian group (AG). This study is important for monitoring and early detection of BBTV threats and for rapid eradication of infected plants before the virus spreads and settles in a new area.

INTRODUCTION

Banana is one of the most essential fruits in Egypt and is cultivated in wide areas. Bananas are cultivated in more than 130 countries and are the fourth most important food crop in the world, behind maize, wheat, and rice. (Perrier *et al.*, 2011, Arimbawa *et al.*, 2022). In Egypt, Banana production exceeded 1292812 tons and the average crop was 18.53 Tons/Fadden. In Ismailia, Banana production exceeded 25432.56 tons and the average crop was 20.69 Tons/Fadden (Ministry of Agriculture. ARE. 2018).

Virus disease not only causes yield reductions but is also a major constraint to the exchange of germplasm (Kumar *et al.*, 2014, Sila *et al.*, 2020 and Rahayuniati *et al.*, 2021). Banana bunchy top virus (BBTV) is a highly infectious and destructive virus of the banana plantation. Furthermore, the disease can severely reduce the quality and marketability of fruit, resulting in huge economic losses when left uncontrolled (Rybicki and Pietersen 1999; Eman *et al.*, 2012 and Rybicki, 2015). Banana bunchy top disease is characterized by dark green streaks on the lower portions of the midrib of the leaf and the fresh infected leaves were stiff and erect, shorter and narrow with brittle, yellow and wavy edges (Allam *et al.*, 2013). As the disease progresses, leaf distortion, marginal leaf yellowing, J-hooks, (Morse code) reduced in size and gather at the top of the plant making a rosetting shape and the plants become 'bunched up', giving the distinct appearance and name (El-Dougdoug *et al.*, 2006 El-Dougdoug & El-Shamy,2011 and Arimbawa *et al.*, 2022). The virus is not transmitted mechanically but is transmitted by banana aphids (*Pentalonia nigronervosa*) Coquerel (Hemiptera, family *Aphididae*). The aphid transmits the virus effectively and quickly in a persistent, non-replicative manner. (Hooks *et al*., 2009; Foottit *et al.*, 2010; Chen and Hu, 2013). However, there is no correlation between the frequency of aphids and BBTD incidence. In addition, wild bananas and plantains may act as a reservoir of the virus (Niyongere, 2012). BBTV is a member of the genus Babuvirus, the family *Nanoviridae*. It is an isometric virus with a multi-component genome, consisting of 6 to 8 circular ssDNA molecules of approximately 1000 to 1100bp each (Stainton *et al.*, 2012). The BBTV components are identified as DNA-R (MRep), DNA-C (Clink), DNA-S (Coat protein), DNA-M (MP) and DNA-N (NSP) (Horser *et al.*, 2001 and Stainton *et al.*, 2012). The incidence of Banana bunchy top is widespread throughout the tropics and subtropics where the banana aphid is common. The virus can be extremely aggressive, reducing yield by up to 95 percent in susceptible varieties. Commercial and subsistence varieties experienced yield declines of up to 90%. The Banana bunchy top virus is active in many African, South American, and Asian countries, particularly in smallholder farming systems. The main countries affected are Egypt, India, China, South Africa, Brazil, and a number of countries in Central and South America. Banana bunchy top disease incidence, for example, is known to occur between 10 and 30 % in South Africa and between 14 and 74% in India (Elayabalan *et al.*, 2015; Das and Banerjee 2018). In Egypt, the rate of infections of BBTV in Alexandria reached up to 60% while it was 17.5, 13 and 2% in El-Menoufia, El-Qalyubia and Qena governorates respectively (EL-Afifi, 1984; Allam *et al.*, 2013 and Vishnoi *et al.*, 2009). Rolling circle amplification (RCA) is a new technique designed as a sensitive diagnostic method for the amplification of circular DNA templates such as plasmids and viruses having circular genomes including geminiviruses and nanoviruses (Rohina *et al.*, 2012 and Ali *et al.*, 2014). The present work aimed to assess the incidence, disease severity of BBTD and the *P. nigronervosa* occurrence in three locations in Ismailia Governorate (Abu-Suwyer, El Tal-El kabir and Fayed). Advanced and traditional tools, i.e., symptoms, serological (TAS-ELISA), and molecular (RCA, PCR and hybridization) techniques were used for virus detection.

MATERIALS AND METHODS

Field Inspection And Sampling:

The incidence of BBTV was studied in Banana fields initially using visual examination for external virus symptoms. The current study was carried out between 2019 and 2021 to gather data on the incidence and disease severity of BBTD and the *P. nigronervosa* occurrence in three locations in Ismailia Governorate [Abu-Suwayr, El Tal-El kabir and Fayed]. A field with between 1600 and 3200 banana plantlets was inspected. Within each village, there was

a minimum of 500 metres between farms, and there were 10 kilometres between each community that was examined. A total of 480 banana plant samples were used for data collection from the three localities. The percentage and severity degree of infection was estimated by visual examination for BBTV symptoms.

Disease Incidence:

The BBTV incidence data were collected from the three commercial cultivars (Williams, Grand-Nain and Zief). The data had been collected and recorded from three locations in Ismailia Governorate during the period from 2019-2021. Thirty (30) plants per block of the banana area were randomly earmarked diagonally to track disease incidence. (Rao *et al.,* 2002). Sixteen farms from April to November were visited-per year. Thirty mats were randomly selected for data collection. Mats comprising one or more suckers with moderate to severe visible BBTD symptoms were considered infected. A mat that contained as a minimum one plant with visible BBTV symptoms was regarded as infected.

Percent disease incidence (PDI) was estimated according to the equation (Reddy *et al.,* 1983):

Percent disease incidence (PDI) = (Number of symptomatic plants/Total number of plants) x 100

Disease Severity:

A normal scale of 0 to 5 based on BBTD symptoms was used to determine the disease severity, where 0 indicates no BBTV symptoms, 1 indicates dark green streaks on the leaf veins, 2 indicates dark green streaks on the leaf midribs and petioles, 3 indicates marginal leaves chlorosis, 4 indicates dwarfing of leaves, and 5 indicates "bunchy top." aspect of the plant showing upright, crowded, and brittle leaves at the apex of the plant. (Niyongere, 2012).

Occurrence of *P. nigronervosa*:

Samples of the BBTV vector *Pentalonia nigr*onervosa Coq were collected from some fields. Later on, Laboratory analysis and identification were done at the plant protection research institute. All 30 mats were examined for the presence of *P. nigronervosa*, which was graded on a scale of 0 to 5: 0 indicates no aphid, 1 indicates a simple colony (no winged individuals), 2 indicates several simple colonies (no winged individuals), 3 indicates a large colony with one or more winged individuals, 4 indicates several colonies with winged individuals, and 5 indicates generalised colonies at the level of a banana plant's leaves and pseudo stem with numerous winged individuals (Niyongere, 2012).

Diagnosis of Banana Bunchy Top Virus:

Banana plant leaf samples (n=26) from symptomatic (n=16) and asymptomatic (n=10) plants were randomly collected from Etal-Elkbier and Abu-Suwayr of Ismailia Governorate as representative samples and utilized for BBTV detection using ELISA and nucleic acid hybridization.

A- Serological Testing:

Serodiagnosis was used to confirm visual inspection and bioassay for BBTV test detection. A total of 26 samples of banana plants either symptomless (n=10) or plants exhibiting dark green dot-dash spots on leaf laminae and severe rosetting with a bunchy top symptoms (n=16) were randomly collected from Etal-Elkbier and Abu-Suwayr of Ismailia Governorate. These samples were examined serologically using Agdia TAS-ELISA reagent set for BBTV (Agdia, Inc.). Plant samples that gave a positive reaction in the TAS-ELISA were used as a source of virus infection. Positive control (insect-inoculated banana plantlet that showed BBTD symptoms and kept in insect-proof cages at the glasshouse) and negative control (Healthy banana tissue culture virus-free plantlet) were used. ELISA-reader (CLINDIAG systems Co. LTD Microplate Reader) was used for measuring test reaction at 405 $_{nm}$ wavelength absorbance. Absorbance values more than twice the value of healthy control were considered positive.

B- Molecular Characterization And Detection Studies:

DNA Extraction and PCR of BBTV DNA-S Component:

BBTV-infected plant material was

collected from Ismailia, and DNA was extracted using DNeasy Plant Mini Kit (Qiagen). PCR amplification and sequencing were performed with two pairs of primers BBTV3c703 and BBTV2v775 according to Rezk *et al.,* 2005 (Table 1) . PCR reaction was performed in PCR thermal cycler (Biometra, Germany). Each reaction contained 100 ng of sample DNA, 0.25 mM dNTPs, 0.25 mM MgCl2, 2mM of each primer, and 0.5U of Taq DNA Polymerase. The amplification program was as follows: an initial denaturing step at 94°C for 3 min, followed by 35 cycles of 94°C for 1 min, 55°C for 1min and 72°C for 2min, and a final extension at 72°C for 10 min. Amplified PCR products were resolved on 1 % agarose gel stained with ethidium bromide (0.5 mg/ml), and photographed.

Table 1: Oligonucleotide sequences of the used primers.

Primers	Primer Sequences 5′-3′	Expected size
BBTV3c703	5'-CTTACTCCAGAACTACAATAGAATGCC-3'	973bp
BBTV2v775	5'-TACAAGACGCTATGACAAATGTACKGG-3	

Rolling Circle Amplification (RCA) using Klenow Fragment Enzyme:

Phi29 DNA polymerase is a polymerase isolated from the bacteriophage phi29 (F29) and used by RCA protocol. The enzyme appears to be structurally similar to the *E. coli* DNA Polymerase Klenow Fragment and includes a 3'-5'exonuclease activity. Therefore in this study, we replace the Phi29 enzyme with *E. coli* DNA Polymerase Klenow large fragment as an alternative enzyme for the RCA reaction. The DNA extract was subjected to RCA with modifications by using *E. coli* DNA Polymerase cloned Klenow Fragment, large fragment *E coli* DNA polymerase I (TAKARA BIO INC., Japan) Briefly, the reaction was carried out in 25µl total volume, 5µl (25 ng) of total DNA was mixed with 2µl of hexanucleotide primer (40ng/µl) and BSA (250mg/ml) denatured for 3 min at 95°C and cooled down on ice for 5 min. Then 2.5µl of 10 X Klenow reaction buffer and 1.25µl *E. coli* DNA Polymerase Klenow large fragment enzyme and 2.5µl dNTPS mix were added to the mixture. Amplification was performed from 3hr to O/N at 37°C and the reaction was terminated by heating for 10 min at 65°C to inactivate the enzyme.

DNA probe labeling, Southern and Dot Blot Hybridization:

cDNA probe was labeled with digoxigenin, using PCR- Dig labeling technique according to Roche, Boehringer Mannheim Corp., Indianapolis, IN, USA, protocol. The amplified DNA, which was previously produced in the PCR reaction, using forward (BBTV2v775) and reverse primer (BBTV3c703), was used as a template for PCR Dig labeling by Labeled dNTPs mixture. For Southern blot hybridization, the methods of Southern (1975) and Sambrook *et al.* (1989) were followed. PCR and RCA products for *BBTV* were gel electrophoresed and then blotted on a nitrocellulose membrane.

For dot blot hybridization, The BBTV PCR product and infected BBTV tissue were used as positive controls. While a healthy virus-free tissue culture banana plantlet was used as a negative control. A total of 26 samples of asymptomatic (n=10) and symptomatic banana plants (n=16) were randomly collected from Etal-Elkbier and Abu-Suwayr used to detect BBTV using dot blot hybridization technique. All tested banana tissues (0.5 g) were ground in 2.5 ml of denatured buffer containing 10% formaldehyde and 8X standardized saline citrate (SSC) (1X SSC is 150 mM NaCl, 15 mM sodium acetate, pH 7.0). The mix was then heated at 60 °C for 15 min and then kept on ice. Ten microliters of supernatant for each extract were spotted onto a nitrocellulose membrane (Boehringer Mannheim Corporation). The membrane was crosslinked using UV irradiation for 2 min (Loebenstein *et al.,* 1997). All tested banana

samples, positive controls and negative control were hybridized with BBTV-specific nonradioactive cDNA- Dig labeled probe according to Boehringer Mannheim Corp. instruction.

Nucleotide Sequencing and Phylogenetic Analysis:

The identification of the virus isolate was carried out based on DNA sequencing. The DNA amplicon was purified using a Geneaid PCR purification kit according to the manual protocol and stood up to DNA sequencing, using the DNA-S specific primers that were used for PCR amplification. The sequence was carried out using ABI 3730xl DNA Sequencer at the gene analysis unit, at the Analysis Company, Color Lab sequences facilities, (Egypt). The obtained DNA sequences corresponding to BBTV isolate (BBTV-3F) were deposited in the NCBI database under acc. no. (OP487706) and compared to that of other overseas GenBank reference BBTV isolates (Table 5). Multiple sequence alignment was accompanied using DNAMAM software v5.2.9 (Lynnon Biosoft, Quebec, Canada) and ClustalW of BioEdit software v7.2.5 program to obtain nucleotide sequence and deduced amino acid identities. Phylogenetic trees were constructed using the maximum-likelihood (ML) method at 1000 bootstrap trial values using the DNAMAM and BioEdit software.

RESULTS AND DISCUSSION

Banana Bunchy Top Disease Symptoms And Identification:

Disease symptoms usually appear about 25 days after inoculation in optimal conditions and this period is directly correlated with the age of the host plantlets. Infected plants were identified based on typical BBTD visual symptoms. These symptoms according to disease severity scale from 1 to 5 were dark green streaks on the veins (Fig.1 a), marginal chlorosis of the leaves (Fig.1 b), dwarfing of the leaves (Fig.2 c), dark green streaks midribs and petioles of the leaves (Fig.1 d&e), and extreme rosetting with a bunchy top appearance (Fig.1 f). In addition to this, there were dark green dot-dash spots and streaks on leaf lamina and pseudo stem with leaves showing excessive brittleness. These observations are similar to that obtained by (Saghir *et al.,* 2002; Lestari and Hidayat, 2020; Rahayuniati *et al.,* 2021; Arimbawa *et al.,* 2022 and Fatma *et al.,* 2022). The classical banana bunchy top virus symptoms were shown in Figure (1). In the field, distinctive BBTD symptoms are visible and distinguishable from other banana viruses.

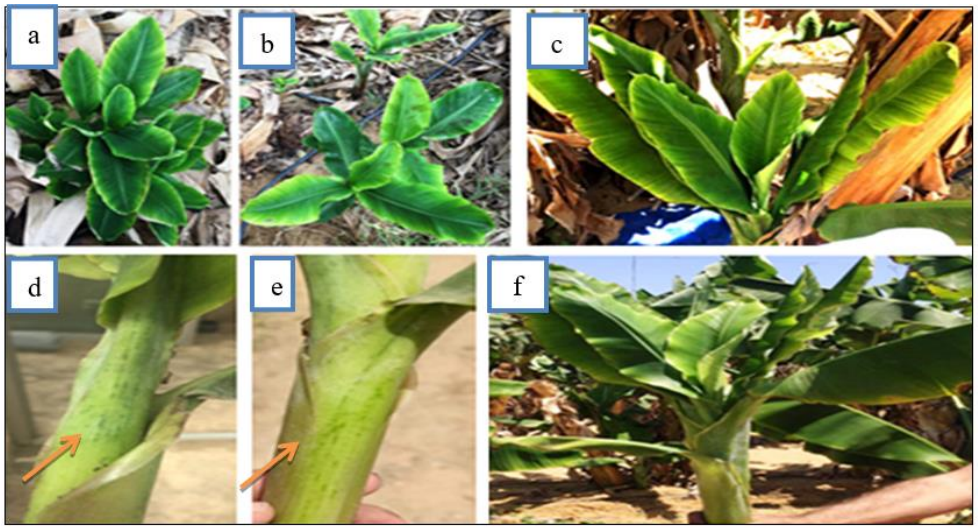


Fig.1: Naturally infected banana plants exhibited different viral symptoms. Dark green streaks on the leaf veins (a) , Marginal leaf chlorosis (b), dwarfing of leaves mixed with marginal chlorosis(c), dark green streaks and mottled on leaf midribs and petioles(d),(e) and bunchy top diseases (bunchy leaves) (f).

BBTV Incidence:

A total number of banana plantations were visited ranging from 1,600 to 3,200 per field. *The Banana bunchy top virus* was reported in all three surveyed localities. BBTV incidences ranging from 3.33% to 5.14% were observed in two localities Abu-Suwayr and El.Tal-El.kabir respectively. On the other hand, Fayed had a comparatively smaller incidence of 1.48% during the survey years (2019-2021) (Table 2). This implies that Fayed was later infected with infected plants in around 6,000 BBTD incident areas and using virus-free planting materials. This result showed a decrease in the incidence of BBTV compared to the incidence rate of BBTV in Alexandria, Menoufia, and Qalyubia governorates, which were 60, 17.5, and 13.5%, respectively (EL-Afifi, 1984; Allam *et al.*, 2013). Grande Naine, Williams and Zeif cultivars (belongs to "Cavendish" subgroup) are the most widely grown banana cultivars in Egypt, but are susceptible to the banana bunchy top virus. Cavendish cultivar is a significant cultivar that might have come from South China and form the backbone of the domestic industries in countries like Australia, India, China and South Africa (Ximba *et al.*, 2022). The disease incidence also varies among banana cultivars. However, the highest percentage of BBTD incidence was observed in El.Tal-El.kabir cv. Zief (10%) and cv. Grande Naine (10 %) during 2019. Our results are also in confirmation with those of (Soomaro and Khalid ,1992; Saghir *et al.*, 2002; Mwenda ,2010 ; Niyongere ,2012 and Elayabalan *et al.*, 2015). Waghmare *et al.*(2021) was reported that BBTV incidence ranging from 30-40% in Grande Naine (G-9) variety in India. This result is in agreement with (Lestari and Hidayat, 2020). While the lowest percentage of BBTD incidence was in Fayed cv. Williams (1%) and cv. Grande Naine (1.87%) in El.Tal-El.kabir during 2021 (Table 2). The lowest incidence was observed in plants sourced from tissue culture.

Table (2) BBTD incidence and occurrence of *P. nigronervosa* in three surveyed localities in Abu-Suwayr, El Tal-El kabir and Fayed (across 16 farms with 30 mats).

Location	Year	Cultivar	BBTD incidence(%)	Occurrence of *P. nigronervosa* (%)
Abu-Suwayr	2019	Williams	4.02%	20%
	2020	Grand Nain	2.6%	80%
		Williams	2.14%	40%
	2021	Williams	4.55%	19.23%
	Average		3.33%	39.8%
El.Tal-El.kabir	2019	Williams	3.12%	50%
		Grand Nain	10%	0
		Zief	10%	25%
	2020	Grand Nain	5.375%	0
		Williams	1.87%	50%
	2021	Williams	3.745%	50%
		Grand Nain	1.87%	50%
	Average		5.14%	32.14%
Fayed	2019	Williams	1.87%	50%
	2020	Williams	1.58%	50%
	2021	Williams	1%	0
	Average		1.48%	33.3%

Disease Severity:

A scale from 1 to 5 was used to valuate the level of the BBTD severity on infected mats. **(**Fig. 1**).** Scores 3 to 5, which are characterised by marginal leaf chlorosis to a bunchy top look, were more common than scores 1 or 2, which indicate the early symptoms exhibited by dark green streaks. In Ismailia governourate, the average severity of BBTD was 3.42% (i.e., 3-5), which was greater than the average severity of BBTV-infected plants at an early stage, which was 2.19% (i.e., 1-2). This result are in harmony with Niyongere *et al.*(2012). According to Table 2, the total average of BBTV incidence of 5.14% was detected in El Tal El Kabir and 4.39% of all examined mats exhibited advanced disease symptoms, which equate to a disease severity score ranging from 3 to 5 (Table 3). In Abu Sawyer, 5.89% of the assessed mats per location had high disease severity (i.e., 3-5). While in Abu-Suwayr, El Tal-El kabir, and Fayed, the percentage of surveyed mats with low disease severity (i.e. 1-2) was 2.87%, 2.69%, and 1.05%, respectively (Table 3). In addition, the difference in disease severity for each location could be attributed to apparently poor maintenance of banana plantations. This result is in agreement with those of (Niyongere *et al.,* 2012 and Rahayuniati *et al.,* 2021).

Table 3. BBTD severity (%) values for the three investigated localities ranged from 0 to 5.

Locations	Scores ranging from 0 to 5					
	0	1	2	3	4	5
Abu-Suwayr	91.24	2.54	0.33	1.55	0.48	3.86
Total	91.24	2.87		5.89		
El Tal El Kabir	93.1	1.54	1.145	1.69	-	2.7
Total	93.1	2.69		4.39		
Fayed	98.53	1.05	-	-	-	-
	98.53	1.05		-		
Average	94.29	1.7	0.49	1.08	0.16	2.18
The sum of score averages (%)		2.19%		3.42%		

Incidence of *P. nigronervosa* Aphids:

Pentalonia nigronervosa aphid has high host specificity for Musa spp. It is present in almost all banana-growing countries (Foottit *et al.,* 2010 Kumar *et al.,* 2011 and Niyongere *et al.,* 2012). In our study, we observed that the aphids were present in all surveyed localities across 16 farms with 30 mats. Aphids were most often observed near the base of banana plants. Occurrence of aphids (*P. nigronervosa*) Colonies on the banana plant; Aphids on the cigar leaf (Fig. 2 A), Aphids behind the leaf sheath (Fig. 2 B,C&D) and aphids upper the leaf (Fig.2 E). This result is in agreement with (Fatma *et al.,* 2022). The highest percentage of aphids occurrence was (80%) in Abu-Suwayr cv. Grande Naine during 2020. On the contrary, the lowest percentage of the aphid's occurrence was obtained upon cultivar *cv.* Williams (19.23%) in Abu-Suwayr in 2021 (Table 2). No aphids were found in the El.Tal-El.kabir cv. Grande Naine during 2019 and 2020 and in Fayed cv. Williams during 2021 (Table 4). Mwenda (2010) was found the aphid is still not widespread and to occur in only a few filed. The average incidence of aphids for all three years (2019-2021) was 39.8%, 33.3% and 32.14% in Abu-Suwayr, Fayed and El Tal-El kabir, respectively (Table 2).

Colonies of *P. nigronervosa* were found on mats of both symptomatic and asymptomatic. *P. nigronervosa* was found in simple colonies (scoring 1 to 2) in 4.9% of the mats examined, and winged individuals (scores 3 to 5) were seen in an average of 5.98% of the mats (Table 4 and Fig. 2). According to the location, Mats containing winged aphids (scoring 3–5), the potential

vectors that transmit BBTV from one plant to another, differed with 12, 6, and 0% of the mats surveyed in Abu-Suwayr, El.Tal-El.kabir and Fayed, respectively. While the mats contain simple colonies (scoring 1 to 2), with 9, 5 and 1% of mats surveyed in Abu-Suwayr, El.Tal-El.kabir and Fayed, respectively (Table 4). This result showed that the BBTD incidence is directly related to the percentage of winged aphids inside the aphid colonies regardless of the percentage of the aphid's occurrence.

Table 4. Topology of *P. nigronervosa* colonies in the three surveyed localities with scores ranging from 0 to 5.

Locations	Scores from 0 to 5					
	0	**1**	**2**	**3**	**4**	**5**
Abu-Suwayr	79	6	3	5	4	3
		9		**12**		
El Tal-El kabir	89	1	4	3	1	2
		5		**6**		
Fayed	99	1	-	-	-	-
		1		**0**		
Average	89	2.6	2.3	2.66	1.66	1.66
The sum of score averages (%)		**4.9%**		**5.98%**		

-: not found or (0).

Fig. 2: Occurrence of aphids (*P.* nigronervosa) Colonies on the banana plant; Aphids on the cigar leaf (A), Aphids behind the leaf sheath(B),(C) and (D) and aphids upper the leaf (E).

The Relationship between BBTV Incidence And Aphids Occurrence:

Looking at the rate of disease incidence percentage during 2019 and comparing it with the percentage of aphids occurrence in the three localities under study, we found that although Fayed area had the lowest percentage of disease incidence (1.87%) as illustrated in Figure 3, it was the highest ratio (50%) of aphids occurrence (Fig 4). This result are in harmony with (Waghmare *et al.*, 2021) Similarly, in 2020, despite the increase in aphid infestation (60%) in the Abu-Suwayr area, we noticed a decrease in the incidence of the disease from 4.02 to 2.37% (Fig. 3 and 4). On the contrary, although the incidence of aphids decreased in the Abu- Suwayr area from 60 in 2020 to 19.23% in 2021 (Fig. 3), the incidence of BBTD increased from 2.37 to 4.55, respectively. (Fig 4). The sum of average scores of BBTD severity percentage from 3 to 5 was higher (3.42%) than that of severity index from 1 to 2 which was 2.19% for the Ismailia (Table 3). The difference in disease severity for each location can be attributed to the fact that banana plantations appear to be poorly maintained (Niyongere *et al.*, 2013 and Ximba *et al.*, 2022).

The aphid *P. nigronervosa* is the only vector known to transmit BBTV and reproduce efficiently on bananas (Foottit *et al.*, 2010 and Niyongere *et al.*, 2012). The *P. nigronervosa* vector was observed in all of

the banana fields that was surveyed in the three locations. Aphids were found in abundance in the region, with simple colonies and winged aphids, which contribute to the disease transmission. (Table 4). The efficiency of BBTV transmission depending on temperature and age of plants and the titer of the virus in the aphids. The weather might affect the vector survival, migration of the aphids. (Elayabalan *et al.*, 2015 and Rahayuniati *et al.*, 2021).

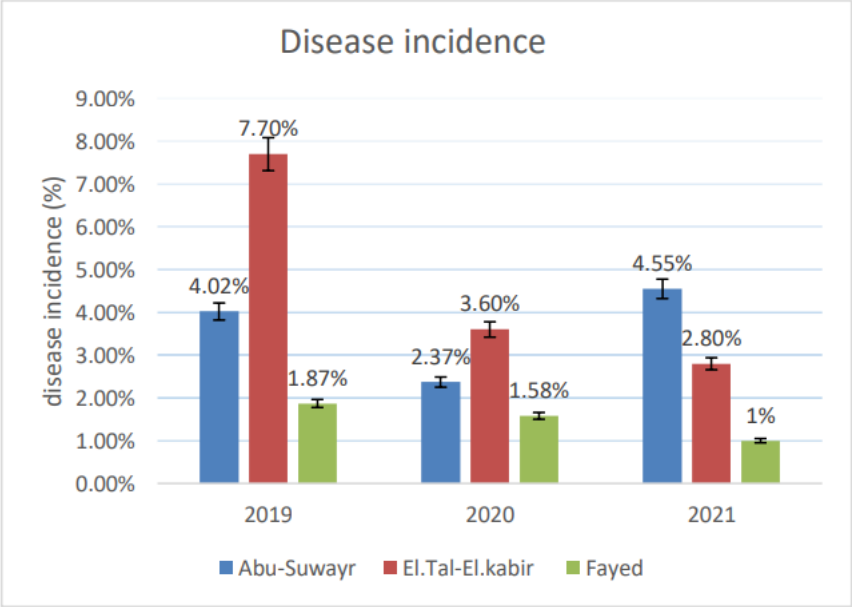


Fig 3 : The BBTD incidence during the period from 2019 to 2021 in the three localities, Abu-Suwayr, El Tal-Elkabir and Fayed.

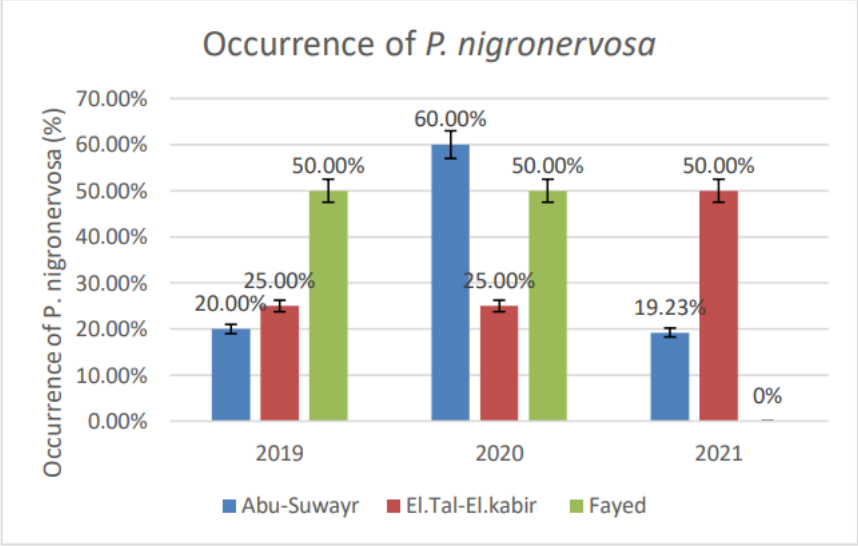


Fig 4: The percentage of Aphids Occurrence during the period from 2019 to 2021 in the three localities, Abu-Suwayr, El Tal-Elkabir and Fayed.

Diagnosis of Banana Bunchy Top Virus Using ELISA:

Based on the variability in BBTV symptom expression, detection methods that are more sensitive have been developed. In this study, the enzyme-linked immunosorbent assay (TAS-ELISA) was used for BBTV detection. All samples from

asymptomatic plants (n= 10) tested negative but thirteen out of 16 samples from symptomatic plants tested positive. This results could be as a results of low virus concentration. The TAS-ELISA is commonly used for BBTV detection, although this may be of limited sensitivity especially where there is very low virus concentration in banana tissues (Kolombia *et al.*, 2021).

PCR of BBTV DNA-S Component:

BBTV-infected plant material was collected from Ismailia, and DNA was extracted using DNeasy Plant Mini Kit (Qiagen). The most sensitive methods currently available are based on polymerase chain reaction (PCR) undertaken to amplify specific DNA sequences of the virus (Su *et al.*, 2003). A sample of Banana plant exhibiting dark green dot-dash spots on leaf lamina and severe rosetting with a bunchy top collected from Etal-Elkbier of Ismailia Governorate was examined using PCR. The PCR using BBTV-specific primers gave a 973 bp amplified product corresponding to the BBTV-DNA-S component as shown in (Fig. 5A lane 2) as reported by (Rezk *et al.*, 2005). Whereas no amplification was detected with the healthy banana plant (Fig. 5 A lane 1). Although Leaf extract of banana has an inhibitory factors (e.g. phenolics) on the method and the oxidation of banana extract increasing inhibition of PCR (Lestari and Hidayat, 2020); Our result suggested that the PCR amplification using DNA-S specific primer is efficient for rapid detection of BBTV infection. Numerous studies demonstrated the reliability of PCR as a BBTV detection tool (Lestari and Hidayat, 2020; Rahayuniati *et al.*, 2021; Abdel Razek *et al.*, 2022).

Rolling Circle Amplification (RCA):

Rolling circle amplification (RCA) is an isothermal enzymatic reaction in which a short starting material of DNA or RNA is amplified to generate long single-stranded DNA or RNA using a circular DNA template and specialized DNA or RNA polymerases. Therefore, in this study, we replace the Phi29 enzyme with *E. coli* DNA Polymerase Klenow large fragment as an alternative enzyme for the RCA reaction. High molecular weight smear product was generated (Fig 6A). This result is due to the fact that the RCA amplicon is a chain of hundreds of tandem repeats that are complementary to the BBTV circular DNA components as well as that it mixed with plant chromosomal DNA (Ali *et al*., 2014).

Nuclic Acid Hybridization Using Non-Radioactive Probe:

a.Southern Blot Hybridization:

PCR poducts for BBTV were gel electrophoresed and then were blotted on nitrocellulose membrane and hybridized with non-radioactive probe specific for BBTV DNA-S component. The BBTV DNA probe was clearly hybridized with the PCR product that was amplified using specific primers from the BBTV-infected plant and showed a signal with the same Intensity (Fig. 5 B). No signal appears with the PCR product of a healthy banana plant. RCA products were run on the gel and transferred to nitrocellulose membrane and this membrane was hybridized with DNA-S specific probe, a smeared signal corresponding to DNA-S component was observed after hybridized with a specific probe (Fig. 6 B). No signal appeared with RCA DNA of the healthy banana plant. Based on our result, biorecognition, sensing and imaging can easily be made by hybridizing RCA products with complementary dig-labeled probes. Furthermore, the visibility of bands in Southern and dot blot hybridizations depends on the concentration of nucleic acid and the use of the RCA increasing nucleic acid concentration and consequently increase the sensitivity of the BBTV detection.

b.Dot Blot Hybridization:

Dot blot hybridization on a nitrocellulose membrane was used for the direct detection of BBTV-DNA in large numbers of infected banana tissues (Fig. 7). All of the tested samples, as well as positive controls from BBTV PCR-amplicon and infected tissues (Fig. 7, E-3), were hybridized using the previously prepared, non-radioactive Dig-labeled DNA probe. Six

samples from asymptomatic plants were negative, but all samples from symptomatic plants (n = 16) and samples from asymptomatic plants (n = 4) tested positively. The infected samples (positive samples) with BBTV showed strong color signals whereas the uninfected banana tissues showed no signals as shown in Figure 7 **(**from tracks A-1 to E-2). Allam *et al.* (2013) used ^{32}P-labelled radio-active pBT338-insert to detect Egyptian BBTV isolate in different parts of plants. Dot blot hybridization was more sensitive than Southern blot hybridization, and hybridization of midrib, roots, meristems was higher than those from corm, leaves and pseudostem.

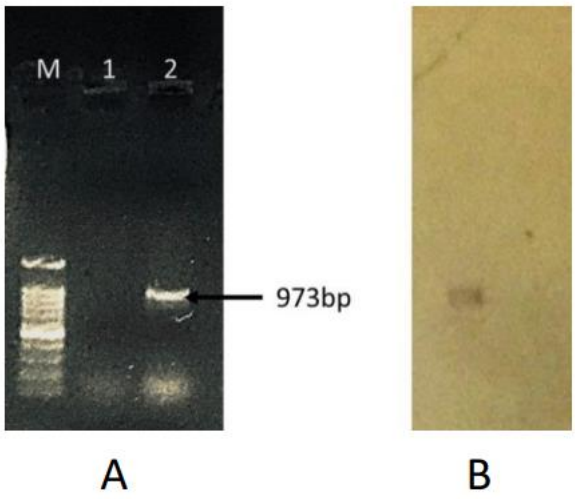


Fig.5: A: 1% agarose gel electrophoresis analysis of the amplified product of BBTV DNA-S component using BBTV3c703 and BBTV2v775 specific primers. M: 100 bp DNA marker, Lane 1: PCR amplified product from virus-free tissue culture banana plantlet (healthy control), Lane 2: inverse PCR from BBTV DNA-S component from a banana infected plant. **B:** Southern blot using the cDNA dig-labeled probe.

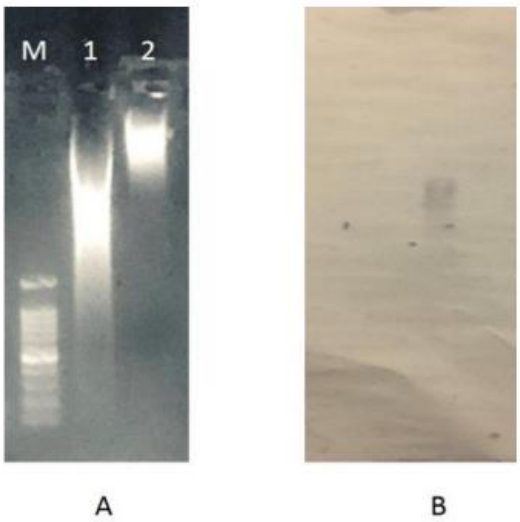


Fig. 6: A: 1% agarose gel electrophoresis analysis of the Rolling Circle Amplification (RCA) of BBTV DNA components using hexamer random primer and BBTV3c703 specific primers. M: 100 bp DNA marker. Lane 1: RCA of BBTV sample. Lane 2: RCA OF virus-free tissue culture banana plantlet (negative control). **B:** Southern blot using the DNA-S dig labeled probe.

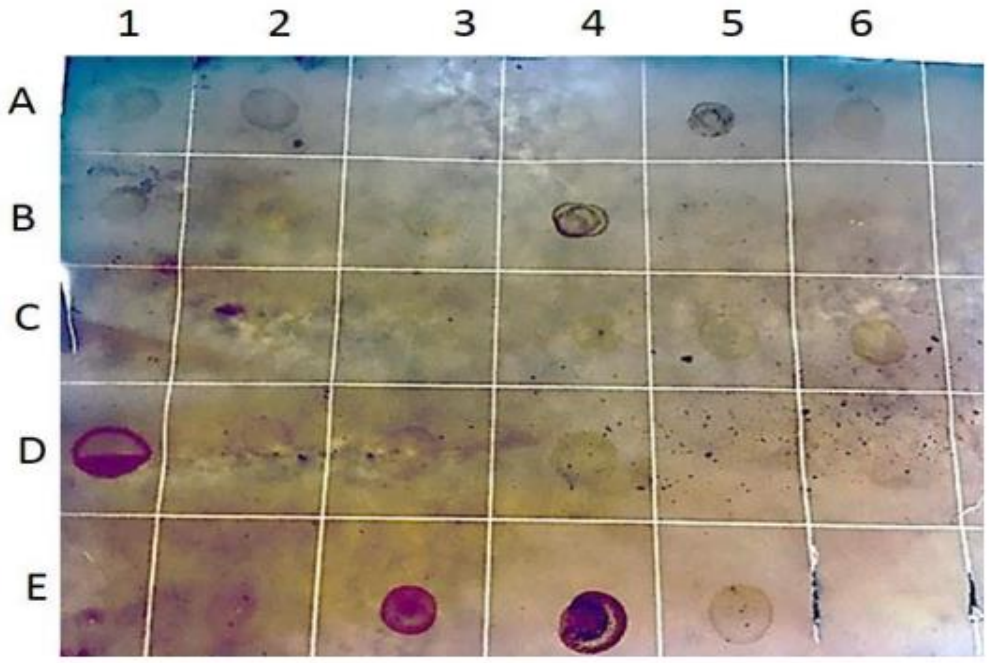


Fig. 7: Dot blot hybridization on nitrocellulose membrane. The membrane hybridized with a specific non-radioactive BBTV –DNA –Dig labeled probe. Dot blot extracts from field-collected banana plants are on tracks A-1 to E-2. Track E-3 is a PCR-amplified product, Trak E-4 and Trak E-5 are dot blots from the BBTV positive control, and Track E-6 is a PCR-amplified product from a healthy control.

Nucleotides and Phylogenetic Analysis of BBTV DNA-S Component:

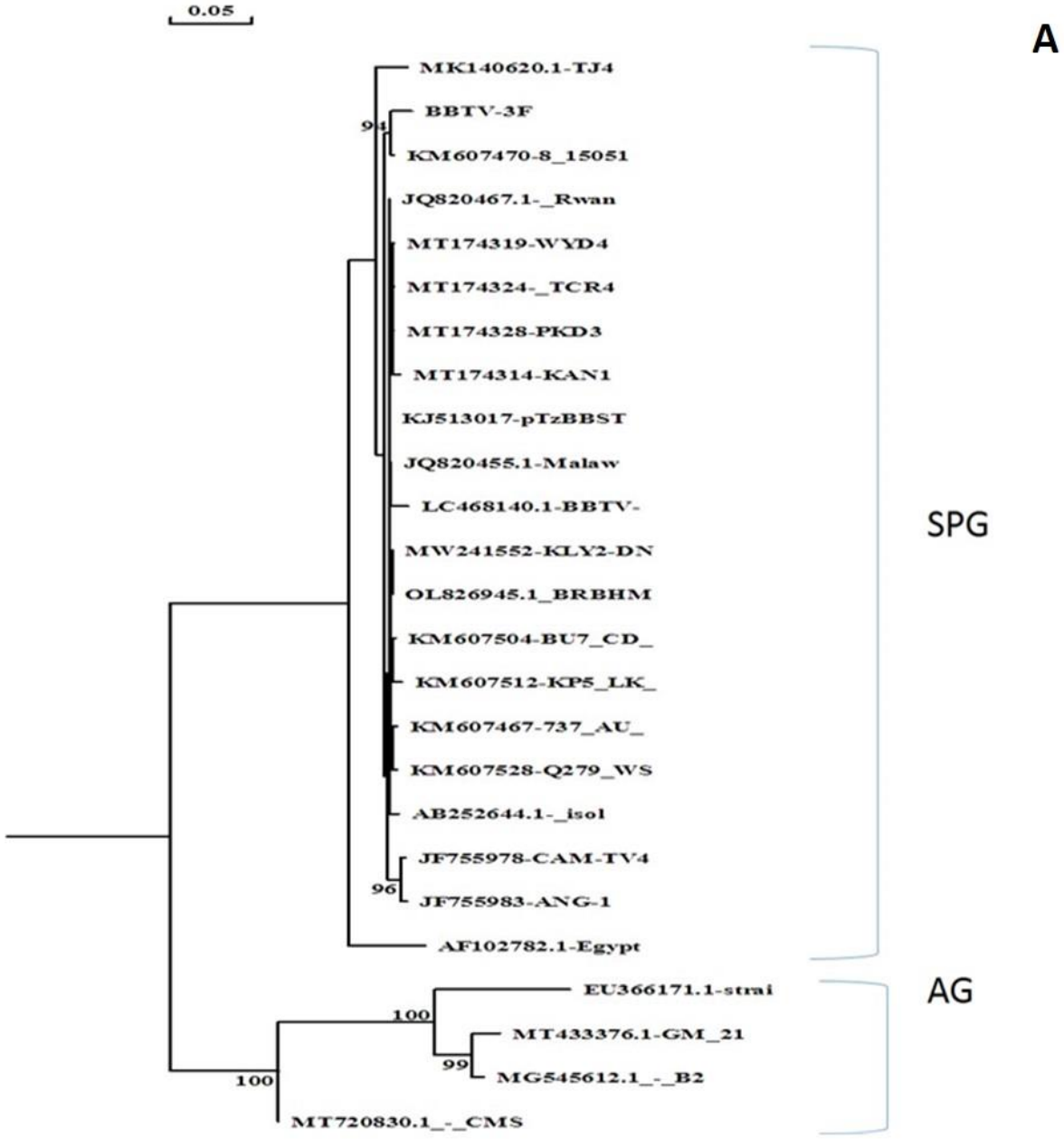
Components of BBTV isolates broadly fall into two geographically well-defined phylogenetic groups, the South Pacific group (SPG) and the Asian group (AG) (Karan *et al.,* 1994, Wanitchakorn *et al.,* 2000). These two groups have also been reffered as the Pacific Indian Ocean (PIO) and the Southeast Asia (SEA) groups, respectively (Yu *et al.,* 2012), here we will use their original names SPG and AG groups according to Karan, *et al.,* 1994 and Stainton *et al.,* 2015. The sequence comparison analysis was carried out with the present Ismailia isolate named BBTV-3F with accession No. (OP487706) and other BBTV isolates (DNA-S component) available in GenBank (Table 5). The highest nucleotide identity 98.4% was detected with 8_150510_EG_2010 (KM607470) isolate infecting Banana from Egypt. The Egyptian isolate of the current study BBTV-3F (Accession No. OP487706) from Ismailia governorate shared 96.7% and 91.9 % with two Egyptian isolates (AF102782.1) and LC468140.1) from Qalyubia Egypt as indicated in Table 5. The Ismailia isolate (BBTV-3F) shared 97.9% nucleotide identity with isolates Rwanda 142 (JQ820467) and Malawi 73 (JQ820455) from Rwanda and Malawi respectively (Table 5).

Table 5: The Accession numbers and origin for DNA-S of *BBTV isolates* used in the present study and the nucleotide sequence identities with the present (BBTV3F) isolate.

No.	BBTV group	Isolate name	Accession No.	Country	% Identities
1	South Pacific group (SPG)	BBTV-3F	OP487706	Egypt	100%
2		BBTV-DNA-3 Egy	LC468140	Egypt	96.7%
3		8_150510_EG_2010	KM607470	Egypt	98.4%
4		Egyptian isolate	AF102782	Egypt	91.9%
5		KLY2	MW241552	India	97.3%
6		KAN1	MT174314	India	96.8%
7		PKD3	MT174328	India	97.1%
8		WYD4	MT174319	India	97.1%
9		pTzBBSTri7	KJ513017	India	97.6%
10		TCR4	MT174324	India	97.1%
11		BRBHM1	OL826945	India	97.3%
12		BU7_CD_2012	KM607504	Congo	97.4%
13		KP5_LK_2003	KM607512	Sri Lanka	96.9%
14		ANG-1	JF755983	Angola	95.7%
15		CAM-TV4.1	JF755978	Cameroon	96.4%
		Cameroon	GQ249344	Cameroon	96.5%
16		737_AU_1997	KM607467	Australia	97.2%
17		Q279_WS_1989	KM607528	Samoa	97.2%
18		Rwanda 142	JQ820467	Rwanda	97.9%
19		TJ4	MK140620	Pakistan	95.7%
20		Malawi 73	JQ820455	Malawi	97.8%
21		MY03	AB252644	Myanmar	97.1%
22	Asian group (AG)	TW3	EU366171	Taiwan	91.1%
23		CMS16	MT720830	Philippines	91.8%
24		B2	MG545612	China	91.0%
25		GM_216024	MT433376	Indonesia	89.9%

This result revealed that the BBTV-3F DNA-S had a high nucleotide identity with the East African isolates. Whereas, the lowest nucleotide identity 89.9% - 91.8% was found with Southeast Asian (AG) isolates TW3, GM-216024 and B2 (EU366171, MT433376 and MG545612) from Taiwan, Indonesia and China respectively. The homology tree (data not shown) showed that the BBTV-3F isolate had a low heterogeneity (97% homology) to the South Pacific group including KAN1, WYD4, PKD3 and TCR4 isolates from the Indian subcontinent previously described by Karan *et al.* (1994) and Stainton *et al.* (2015). The Indian subcontinent isolates are considered both the major location for BBTV dispersion to other parts of the world and the main recipient location of virus introductions. These include one dispersal event from the Indian subcontinent to Egypt between 1929 and 1936 (Stainton *et al.*, 2015). The deduced amino acid sequence comparison showed that the Ismailia BBTV-3F isolate shared 96.4% - 94.03 % with the Asian group isolates (Taiwan and Indonesia respectively) despite its low nucleotide identity (89.9-91.8).The rooted Maximum likelihood (Ml) phylogenetic analysis tree for nucleotide sequence comparison of DNA-S of BBTV3F isolate with the GenBank BBTV reference isolates (Fig 8A) indicated a close relationship between the present Ismailia BBTV isolate (BBTV-3F) and 8_150510_EG_2010 isolate from Egypt with accession No. (KM607470). The Topology of the phylogenetic tree was similar to the topology of the homology tree hence the isolate BBTV-3F clustered together with the 8_150510_EG_2010 isolate (KM607470) from Egypt (Fig. 8A) and grouped in a monophyletic group with the South Pacific isolates including the Egyptian isolates with accession No. LC468140 and AF102782.1 (Fig.8A). Abdel Razek *et al.,* (2022) reported that the Phylogenetic trees of the Egyptian isolate (BBTV-DNA-3 Egy with accession No. LC468140.1) confirmed the genetic relationships among the high percentage of similarity among the compared overseas BBTV isolates. Phylogenetic analysis of the BBTV-3F isolate together with existing overseas BBTV isolates sequences in GenBank (Table 5) confirmed the grouping of BBTV into two large groups, the Asian group (AG) and the South Pacific group (SPG) (Karan *et al.*, 1994 and Stainton *et al.*, 2015). The present results showed that the BBTV3F isolate from Ismailia governorate,

Egypt belongs to the South Pacific group (SPG) including WYD4, TCR4, PKD3, KAN1, pTzBBSTri7 from India and Rwanda 142, Malawi 73 isolates previously described by Yu *et al.* 2012, Hyder *et al.* (2007). Our results came in harmony with Abdel Razek *at al.*, (2022). Whereas it is in contrast to Stainton *et al.*, (2015) results that the isolate 8_150510_EG_2010 from Egypt belongs to the Asian group rather than the South Pacific group. The neighbor joining (NJ) phylogenetic analysis for the deduced amino acid sequence comparison showed that the present Ismailia (BBTV-3F) isolate and 8_150510_EG_2010 (KM607470) isolate from Egypt distinctly grouped together in a separate cluster and proposes that the two Egyptian isolates may have evolved from a recent common ancestor (Fig.8 B).



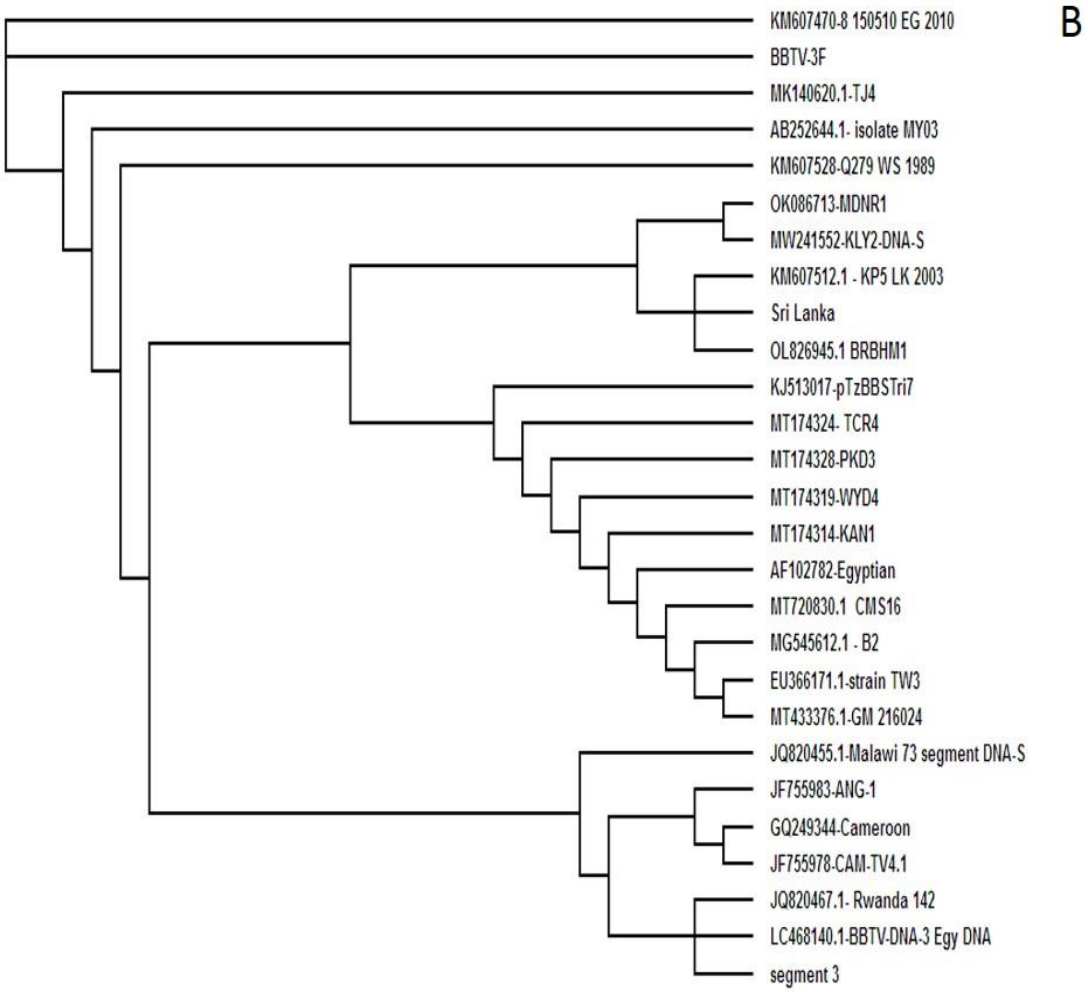


Fig. 8. A: The rooted Maximum likelihood (Ml) Phylogenetic tree showing the relationship of DNA-S component nucleotide sequence of BBTV-3F isolate from Ismailia to BBTV GenBank reference isolates belonging to the two main groups (AG) and (SPG) as defined by Karan *et al.,* (1994) and Stainton *et al.,* (2015). Numbers close to the nodes represent the bootstrap values obtained from 1000 replications (values ≥ 90 are shown). **B**: the neighbor-joining (NJ) phylogenetic tree of the present BBTV-3F isolate with other reference BBTV GenBank isolates based on the deduced amino acid sequences for BBTV-component S.

CONCLUSIONS

The current study was conducted in Abu-Suwayr, El Tal-El kabir and Fayed, Ismailia Governorate to characterize incidence of BBTV and molecular characteristics of BBTV from banana plantations. Abu-Suwayr and El Tal-El kabir are two localities in Ismailia Governorate that experienced BBTV incidences ranging from 3.33 to 5.14%. The molecular characteristics of BBTV were studied using TAS-ELISA, PCR and nucleic acid hybridization. The PCR product (973 bp) of BBTV- component 3 (DNA-S) was used for the creation of a non-radioactive Dig labeled-DNA probe for BBTV detection. The sequence comparison analysis showed that the present Ismailia isolate (BBTV-3F) shared nucleotide identity 98.4%-91.9 % with three isolates from Qalubya Egypt and 97.9% nt identity with east African isolates (Rwanda and Malawi). This study is important for surveillance and early detection of BBTV threats and for rapid eradication of infected plants before the virus spreads and settles in a new area.

REFERENCES

Ali, M. M.; Li, F.; Zhang, Z.; Zhang, K.; Kang, D.; James, A.; Ankrum, X.; Le, C. and Zhao, W. (2014). Rolling circle amplification: a versatile tool for chemical biology, materials science and medicine. *Chemical. Society. Reviews*, 43: 3324-3341.

Allam, E.K.; Dale, J.L.; EL-Afifi, S. I.; Harding, R.M. and Sadik, A.S. (2013). Nucleic acid hybridizations as a radioactive tool for rapid detection of banana bunchy top virus. *Pakistan Journal of Biotechnology, 10 (1) 1 – 9.*

Arimbawa, I. M.; Wirya, G. N. A. S.; Sudiarta, I. P. (2022). First report of banana bunchy top virus on heliconia (*Heliconia spp.*) in Bali, Indonesia. *Journal of Trop Plant Pests and Diseases,* 22: 77-82. https://doi.org/10.23960/jhptt.12277-82.

Chen, Y. and Hu, X. (2013). High-throughput detection of banana bunchy top virus in banana plants and aphids using real-time TaqMan® PCR. *Journal of Virological Methods,* 193: 177–183.

Das, T. and Banerjee, A. (2018). Distribution, molecular characterization and diversity of *banana bunchy top virus* in Tripura, India. Virus Disease, 29(2):157–166.

EL-Afifi, S. I. (1984). Identification of viruses infecting banana in Egypt. *Nine International Congress for Statistics Computer Science, Social and Demo-graphic Research,* 31: 461-482.

Elayabalan, S.; Subramaniam, S. and Selvarajan, R. (2015). Banana bunchy top disease (BBTD) symptom expression in banana and strategies for transgenic resistance: A review. *Emirates Journal of Food and Agriculture, 27 (1): 55-74.* doi: 10.9755/ejfa.v27i1.19197.

El-Dougdoug, K.A.; Hazaa, M.M.; Hanan, H.A. and Sabah, A. E M. (2006). Eradication of banana virus from naturally infected banana plants. 1. Biological and Molecular detection of cucumber mosaic virus and banana bunchy top virus. *Journal of Applied Sciences Research, 2 (12): 1156-1163.*

El-Dougdoug, Kh. 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,* Vol. 5(32), pp. 5923-5932, 30.

Eman, H. ES.; Mahfouze, S.A.; Shaltout, A.D.; El-Dougdoug, Kh. A. and Sayed, R.A. (2012). Chemical mutation of *in vitro* cultured shoot tip of banana cv. grand-nain for resistance some virus diseases. *International Journal of Virology,* 8(2):178–190.

Fatma A.R. S., Samar E-M. S.; Ibrahim, S. D. ; El-Arabi, T. F.; Mahmoud, E. K.; Sadik A. S. (2022). Bioinformatics analyses of the complete DNA genome of an Egyptian isolate of banana bunchy top virus. *Egyptian Academic Journal of Biological Science (G. Microbiolog),* Vol.14 (2) pp.1- 18

Foottit, R.G.; Maw, H.E.L.; Pike, K.S. and Miller, R.H. (2010). The identity of *Pentalonia nigronervosa* Coquerel and *P. caladii* van der Goot (Hemiptera: Aphididae) based on molecular and morphometric analysis. *Zootaxa*, 2358: 25–38.

Furuya N.; Kawano S.; Natsuaki K. T. (2005). Characterization and genetic status of Banana bunchy top virus isolated from Okinawa, Japan. *Journal of General Plant Pathology*, 71: 68–73. DOI 10.1007/s10327-004-0162-3

Hooks, C.R.; Fukuda, S.; Perez, E.A.; Manandhar, R.; Wang, K.H.; Wright, M.G. and Almeida, R.P. (2009). Aphid transmission of

banana bunchy top virus to bananas after treatment with a bananacide. *Journal of Economic Entomology, 102: 493-499.*

Horser, C.L.; Karan, M., Harding, R.M., Dale, J.L. (2001). Additional repen coding DNAs associated with banana bunchy top virus. *Archives of Virology*,146:71–86

Hyder, M.Z.; Raza, S.Q.; Hameed, S.; Kahlid, S.; Naqvi, S.M.S. (2007). Phylogenetic relationship of TJ1 isolate of banana bunchy top virus from Pakistan by DNA-R sequence analysis. *Canadian Journal of Plant Pathology*, 29: 63–68.

Karan, M.; Harding, R. M. and Dale, J. L. (1994). Evidence for two groups of banana bunchy top virus isolates. *Journal of General Virology*, 75: 3541–6.

Kolombia, Y. ; Oviasuyi, T. ; Ayisah, K. D. ; Gonh-Goh, A. A.; Atsu, T. *et al.* (2021). First report of banana bunchy top virus in banana (Musa spp.) and its eradication in Togo. *Plant Disease*, 105: 3312. https:// doi.org/10.1094/PDIS-03-21-0473-PDN.

Kumar, P. L.; Hanna, R.; Alabi, O. J.; Soko, M. M.; Oben, T. T. and Vangu, G. H. (2011) *Banana bunchy top virus* in sub-Saharan Africa: Investigations on virus distribution and diversity. *Virus Research,* 159 (2), 171–182.

Kumar, P.L.; Selvarajan, R.; Iskra-Caruana, M L. and Chabannes, M. (2014). Biology, etiology, and control of virus diseases of banana and plantain R.Hanna. Adv Virus Res. 2015;91:229-69. http://dx.doi.org/ 10.1016/bs.aivir.2014.10.006.

Lestari, S.M . and Hidayat, S.H. (2020). Survey and detection of banana bunchy top virus in Java. *IOP Conference Series: Earth and Environmental Science.* (583): 012022. doi:10.1088/1755-1315/ 583/1/012022.

Loebenstein, G.; Akad, F.; Filatov, V.; Sadvakasova, G.; Manadilova, A.; Bakelman, H.; Teverovsky, E.; Lachmann, O. and David, A. (1997). Improved detection of potato leafroll *Luteovirus* in leaves and tubers with a Digoxigenin-labeled cRNA probe. *Plant Disease*. 81: 489-491

Mwenda, E. (2010).Occurrence and incidence of banana bunchy top disease in major banana growing regions of Zambia . M.Sc. thesis of science in agronomy University of Zambia.128 pp.

Niyongere, C., (2012). Occurrence, characterization and screening for resistance to banana buncy top virus in Burundi, Democratic Republic of the Congo and Rwanda. PHD, thesis. Philosophy in Horticulture in the Jomo Kenyatta University of Agriculture and Technology. pp 139.

Niyongere, C.; Losenge, T.; Elijah, M.A.; Nkezabahizi, D.; Blomme, G.; Lepoint, P. (2012). Occurrence and distribution of banana bunchy top disease in the Great Lakes region of Africa. *Tree and Forestry Science and Biotechnology,* 6 (1) pp: 102-107.

Niyongere, C.; Losengeb, T.; Atekab, E. M.; Ntukamazinaa, N.; Ndayiragijea, P. ; Simbarec, A.; Cimpayec, P.; Nintijec, P.; Lepointdand P.; Blommee G. (2013). Understanding banana bunchy top disease epidemiology in Burundi for an enhanced and integrated management approach. *Plant Pathology*, 62,562–570. Doi: 10.1111/j.1365-3059.2012.02676.x

Perrier, X., De Langhe, E.; Donohue, M.; Lentfer, C.; Vrydaghs, L. and Bakry, F. and *et al.,* (2011). Multidisciplinary perspectives on banana (*Musa spp*.) domestication. *Proceedings of the National Academy of Sciences of the United States of America*, 108: 11311–11318.

Rahayuniati, R.F.; Hartono, S.; Somowiyarjo, S.; Subandiyah, S. and Thomas, J.E. (2021). Characterization of banana bunchy top virus on Sumatra (Indonesia) wild banana. *Biodiversitas Journal of Biological Diversity*, 22(3):1243-1249.

Rao, S.A.; Abdul, L.K.; Muhammed, A.K.; Muhammed, A.R. and Khalid, I.R. (2002). Occurrence and incidence of banana bunchy top disease in southern part of Sindh Pakistan. *Journal of Plant Pathology*, Vol 1(2-4) pp: 74-75.

Reddy, D.V.R.; Amin, P.W.; Mcdonald, D. and Ghanekar, A.M. (1983). Epidemiology and control of groundnut bud necrosis and other diseases of legume crops in India caused by tomato spotted wilt virus. In: R.T. Plumb, J.M. Thresh (eds.): *Plant Virus Epidemiology: The Spread and Control of Insect-Borne Viruses*, pp. 93-102.

Rezk, A. A.; Mazyad, H. and Shalaby A. A. (2005). Detection methods for viruses of banana, citrus, cucumber, grape, potato, stone fruits, and tomato. Virus detection: Banana bunchy top virus (BBTV). Developed by MERC Scientists.2-11

Rohina, B.; Javed, F.; Ahmed, R. and Mansoor, S. (2012). Use of rolling circle amplification for the identification of unknown components of banana bunchy top virus from Pakistan. *Pakistan Journal of Life and Social Sciences*, 10(2): 91-97

Rybicki, E. P. (2015). A top ten list for economically important plant viruses, *Archives of Virology*, 160: 17–20.

Rybicki, E. P. and Pietersen, G. (1999). Plant virus disease problems in the developing world. *Advances in Virus Research*, 53: 127–75.

Saghir, A.R.; Mazhar, A.Q.; Abdul, L.K. and Mahammad, A.K. (2002). Occurrence and incidence of banana bunchy top disease in southern part of Sindh. *Plant Pathology journal*, 1: 74-75

Sambrook, J.; Fritsch, E. R. and Maniatis, T. (1989). Molecular cloning: A laboratory manual (2nd ed.). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Sila, S.; Abadi, A.L.; Mudjiono, G. and Astono, T.H. (2020). *Banana bunchy top virus* (BBTV) on wild banana species in Kutai Kartanegara Regency. *EurAsian Journal of Biosciences*, 14, 5843-5847.

Soomaro, M.H.; Khalid, S. and Aslam, M. (1992).Outbreak of banana bunchy top virus in Sindh, Pakistan. *FAO Plant Protection Bulletin*, 40:95-99.

Southern, E. M. (1975). Detection of specific sequences among DNA fragments separated by gel electrophoresis. *Journal of Molecular Biology, 98 (3): 503–517.*

Stainton, D.; Kraberger, S.; Walters, M.; Wiltshire, E. J.; Rosario, K. and Halafihi, M. (2012). Evidence of inter-component recombination, intra-component recombination and re assortment in banana bunchy top virus. *Journal of General Virology, 93, 1103*–1119. DOI 10.1099/vir. 0.040337-0

Stainton, D.; Martin, D. P.; Muhire, B. M.; Lolohea, S.; Halafihi, M.; Lepoint, P. *et al.* (2015). The global distribution of banana bunchy top virus reveals little evidence for frequent recent, human-mediated long distance dispersal events. *Virus Evolution*, 1(1): 1–16. doi: 10.1093/ ve/vev009.

Su, H.J.; Tsao, L.Y.; Wu, M.L. and Hung, T.H. (2003). Biological and molecular categorization of strains of banana bunchy top virus. *Journal of Phytopathology* 151, 290-296. Sun, S.K. (1961). Studies on the bunchy top disease of bananas. Special Publication *College of Agriculture, Taiwan University*, 10,

82-109.136.

Vishnoi, R.; Shri, K.R. and Vivek, P. (2009). Molecular characterization of an Indian isolate of banana bunchy top virus based on six genomic DNA components. *Virus Genes*, 38, 334–344.

Waghmare, S. S.; Adat, S. R.; Mohite, V. K.; Waghule A. A. and Patale, S. S. (2021). Study of bunchy top of banana virus (BBTV) and its control by integrated disease management (IDM). International Journal of Current Microbiology and Applied Sciences, 10(10): 416-429. https://doi.org/10.20546/ijcmas.2021.1010.047.

Wanitchakorn, R.; Harding, R.M. and Dale, J.L. (2000). Sequence variability in the coat protein gene of two groups of banana bunchy top isolates. *Archives of Virology*, 145, 593-602.

Ximba, S.P.F.; Tshabalala, J.; Gubba, A. and Jooste, A.E.C. (2022). Monitoring the distribution of banana bunchy top virus in South Africa: a country-wide survey. *Archives of Virology*, 167(6):1433-1441. doi: 10.1007/s00705-022-05451-5.

Yu, N.-T.; Zhang, Y.-L.; Feng, T.-C.; Wang, J.-H.; Liu, Z.-X .*et al.* (2012). Cloning and sequence analysis of two banana bunchy top virus genomes in Hainan, *Virus Genes*, 44:488–94.