The Detection and Diversity of *Banana Streak Virus* Isolates in Egypt

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ABSTRACT

Banana plants from Williams' (AAA, Cavendish subgroup) banana (*Musa acuminate*) variety and from Pradica (AAB group) banana *(Musa* × *paradisiaca*) variety, collected from different locations in Egypt, were tested for the presence of *Banana streak virus* (BSV) using immunocapture-polymerase chain reaction (IC-PCR) and a specific antiserum for BSV. IC-PCR indicated the episomal presence of BSV in Williams and Pradica banana varieties. In addition, IC-PCR analysis on vitroplants collected from tissue cultures (TC) showed the significant role of TC in spreading out of BSV. PCR amplicons for the reverse transcriptase and RNaseH motifs of ORF III for four BSV isolates were cloned, sequenced and submitted to GenBank. The four accessions showed nucleotide-pairwise identity between 76-82% and amino acid similarity between 29-63% indicating high diversity. In addition, the four BSV isolates showed nucleotide identity between 64-88% with four BSV accessions, retrieved from the GenBank. Phylogenetic analysis based on the partial RT/RNaseH-sequence showed that the four Egyptian BSV isolates clustered together with two Egyptian sugarcane bacilliform badnavirus apart from other badnaviruses circumventing several viruses of BSV.

Key words: *Badnavirus*, Banana (*Musa* sp), *Banana streak virus* (BSV), IC-PCR, Virus detection, virus diversity, Egypt

INTRODUCTION

The current cultivated bananas and plantains developed originally from two wild species, *Musa acuminata* (AA genome) and *M. balbisiana* (BB genome) resulting in a chain of diploid, triploid, and tetraploid genomes (AA, AB, AAA, AAB), ABB , AABB, AAAB, ABBB) forming three types of food, dessert, cooking (including plantain) and beverage. Bananas and plantains (*Musa* spp.) include a wide range of dessert (AAB) and cooking (ABB) cultivars. Banana fruit are the fourth most important food commodity in the world, after rice, wheat, and maize, and represents a staple food and cash crop for millions of people in developing countries in South America, Southeast Asia, and Africa (Heslop-Harrison and Schwarzacher 2007).

Banana streak disease (BSD) was first reported in Ivory Coast in 1966 (Yot-Dauthy and Bové 1966). Since then the disease has been recorded worldwide (Lockhart and Jones, 2000). For two decade or so BSD was considered to be caused by variable isolates of a single virus, designated as *Banana streak virus*, BSV, (Lockhart, 1986). However, it has recently been discovered that some of its isolates that cause BSD differ biologically, serologically and genomically. Nowadays, the disease is considered to be caused by a complex of BSV (Geering *et al.,* 2005a; Harper *et al*., 2004, 2005, Gayral and Iskra-Caruana 2009). The International Committee on Taxonomy of Viruses (ICTV)

recognized only *Banana streak GF virus* (BSGFV), *Banana streak Mysore virus* (BSMyV) and *Banana streak OL virus* (BSOLV) as distinct badnaviruses for BSD (Hull *et al.*, 2005). A fourth species, *Banana streak acuminata Vietnam virus* (BSAcVNV), has recently been proposed based on full length sequence analyses (Lheureux *et al*., 2007). Though the name of BSV is no longer used, for the sake of simplification in this study, it will be referred to these viruses complex collectively as BSV.

BSV-infected *Musa* plants frequently express broken or continuous chlorotic or necrotic streaks on infected leaves and stems, stunting, pseudo-stem splitting, twisted leaves, severely malformed fingers, aberrant bunch emergence, reduced bunch sizes and occasional heart rot of the pseudostem and plant death (Gauhl and Pasberg-Gauhl, 1994; Lockhart and Jones, 2000; Harper *et al*., 2002). Disease-symptom expression of BSD is controlled, however, by many factors, as host genotype, virus isolates, level of management, and temperatures. These factors have complicated the diagnosis of BSD (Lockhart and Olszewski, 1993; Dahal, *et al*., 1998; Iskra-Caruana *et al*., 2010). Economic losses in infected banana may range between 6–100% (Dahal *et al*, 2000; Makokha, 2007).

BSV is transmitted in a semi-persistent manner by at least one of three species of mealybugs (*Hemiptera*; *Pseudococcidae*), of which *Planococcus citri* is the most prevalent (Meyer *et al.* 2008). BSV was detected in field-captured *Dysmicoccus brevipes* and *Saccharicoccus sacchari* (Kubiriba *et al.,* 2001). The most significant BSV transmission, though, occurs through vegetative propagation by exchanges of suckers and Tissue Culture (TC) vitroplants (Lockhart and Jones 2000).

The genome of badnaviruses typically contains three open reading frames (ORFs) except in *Taro bacilliform virus* (TaBV), *Cacao swollen shoot virus* (CSSV) and *Citrus yellow mosaic virus (CYMV)* which have four, five and six ORFs, respectively. The precise role of ORF1 and ORF2 has not been confirmed and the large polyprotein encoded by ORF3 contains domains associated with movement, the virus capsid, an aspartic protease, reverse transcriptase (RT) and ribonuclease H (RNase H) functions. Differences in polymerase (RT/RNase H) nt sequences of (20 %) and differences in other gene product sequences are the criteria followed by the ICTV to demarcate the species in the genus, Badnavirus (Fauquet *et al.*, 2005; King *et al.*, 2011).

Viruses causing BSD are pararetroviruses, have non-enveloped, bacilliform particles measuring *ca* 130-150 x 30 nm and containing a circular dsDNA genome of 7.4 kb (Lockhart, 1986). The genome replicates via an intermediate ssRNA and is converted back to dsDNA through the action of a virus-encoded reverse transcriptase.

The genome of BSV presents as either an episomal form (i.e.within intact virions) or in some cases as endogenous homologous viral sequences in the nuclear genome of certain interspesific triploid hybrids of *Musa* × *paradisiaca* (AAB genome) that resulted from crossing between the diploid *Musa balbisiana* (BB) and a tetraploid of *Musa acuminata* (AAAA). These hybrids with haploid B genome and endogenous BSV (eBSV) genome have the propensity of inducing episomal infection under certain circumstances of abiotic stress (Ndowora *et al*.,, 1999; Provost *et al*., 2006; Chabannes *et al*., 2013). Indeed dissemination of these triploid hybrids through TC vitroplants has led to worldwide-BSV outbreaks (Iskra-

Caruana, 2010; Chabannes *et al*., 2013). Additionally, eBSV sequence in banana can cause false positives upon detection with genome-based methods (e.g. nucleic acid hybridization, PCR amplification). Such nucleotide sequences of the eBSV characterized so far display very high levels of sequence identity with that of their cognate BSV species (Ndowora *et al*., 1999). Certain immuno-capture PCR (IC-PCR) methods, however, are in use now to overcome this problem through involving the use of *Musa* sequence tagged microsatellite site primers (Provost *et al*. 2006; Chabannes, *et al.*, 2013) or through the addition of a DNAse I digestion step within IC-PCR (Gambley, 2008).

In Egypt BSV was recorded for the first time in Giza governorate in 2005 (Abdel-Salam *et al.* 2005). For almost a decade, no thorough studies were made, in our laboratory, on this virus. The purpose of the present study is to examine the presence of BSV in certain governorates, its episomal nature, and measure diversity between the Egyptian BSV isolates and other foreign BSV through DNA sequence analysis.

MATERIALS AND METHODS

Virus Isolates:

Four isolates of BSV were obtained from commercial Williams' banana plantations in four governorates in Egypt including Giza, Sohag, Qalyubia, and Gharbia. According to isolate location, these isolates will be henceforward named as BSEV-Giza, BSEV-Sohag, BSEV-Qalyubia, and BSEV-Gharbia. Collected samples were from banana leaves showing collective symptoms of vein banding, chlorotic and necrotic streak symptoms. Samples were halved and a part was subjected to immediate processing and the other half was kept frozen at -86 ^{o}C. Other samples tested in the present study included vitroplants obtained from commercial TC laboratories at Badr city (Cairo Metropolitan city), Barrage-Qalyubia, and Shorba El-Khima city, Qalyubia. The origin of these vitroplants was unknown and whether they were from interspecific triploid hybrids or from Cavendish subgroup (AAA genome). A sample from Pradica banana *(Musa* × *paradisiaca*) variety showing short chlorotic streak symptoms was also tested for the presence of BSV. The latter sample was from banana plants brought from Qalyubia governorate and preserved at the greenhouse facilities in Cairo University.

Immunocapture PCR (IC-PCR)

IC-PCR was performed using a rabbit polyclonal antiserum prepared for BSV-Egypt, BSEV (Abdel-Salam *et al*., 2005). Sterile polypropylene thin-walled 0.2 ml microfuge tubes were coated overnight at 4^{o}C or for 2 hr at 37^{o}C with 25 µl of BSV polyclonal antiserum diluted 10^{-2} in sodium carbonate coating buffer (15 mM sodium carbonate, 34 mM sodium bicarbonate, pH 9.6), then washed three times with 100 µl of PBST washing buffer (136 mM NaCl, 1.4 mM KH_2PO_4, 2.6 mM KCl, 8 mM Na_2HPO_4, 0.05% Tween-20, pH 7.4,) and incubated overnight at 4°C with 50 µl of sap extract. Plant extracts were prepared by macerating 0.1 g of leaf tissue in 1 ml of citrate extraction buffer (0.05 M citrate pH 7.0, 0.5 mM EDTA, 1% skim milk powder, 0.5% glycerol, 0.05% Tween-20, and 0.05% of 2-mercaptoethanol) and clarified by centrifugation at 14,000 x g for 5 min. The tubes were washed as before. To circumvent any possible presence of integrated DNA sequence for BSV in tested tissues, the tubes were then incubated at 37^{o}C for 30 min with 3 U

of DNAse I (Invitrogen) prepared in 1 × DNAse I buffer (Invitrogen) in a total reaction volume of 50 µl. The DNAse I was removed by washing tubes with 100 µl washing buffer. The tubes were then rinsed with 100 µl sterile water, and the complete 25 µl PCR mix was added directly to the tube using degenerate primers for BSV.

Badna FP (5'ATGCCITTYGGIITIAARAAYGCI CC3') and Badna RP (5' CCAYTTRCAIACISCICCCCAICC3') were designed based on the consensus sequence of the RT and RNase H coding regions to amplify a 580 bp product (Yang *et al.,* 2003).

PCR reaction mixes of 25 µl contained 1 µl of each primer (10 pm), 0.5 µl of 10 mM dNTPs mix, 5 µl of 5X GoTaq DNA polymerase reaction buffer (Cat No. M8301, Promega, Madison, WI, USA), 2.5 µl of 25 mM $MgCl_2$, 0.25 µl of 5 U/µl Taq DNA polymerase (M8301, Promerga) and 14.75 µl of water. PCR cycle conditions, as described by Yang *et al,* (2003), were an initial denaturation step at 94 °C for 4 min, followed by 40 cycles (94°C for 30 s, 50°C for 30 s, 72°C for 30 s) followed by a final elongation step of 5 min at 72 °C. Ten µl/well of PCR products were analyzed by standard electrophoresis on a 1.0% agarose gel in 0.5× TAE (40 mM Tris–acetate, 1 mM EDTA, pH 8.3) followed by ethidium bromide staining and visualization under UV light.

Cloning, sequencing, and analysis

The PCR products were cut from the gel and purified using QIAEX II gel extraction kit (Qiagen) and then cloned into pTZ57R/T vector using InsT/Aclone PCR product cloning kit #1214 (Fermentas). Plasmids were transformed in *E. coli* JM 109 cells using standard molecular techniques. Colonies were tested for the presence of PCR inserts in plasmids using Badna F/P primers. Plasmids were purified from positive bacterial clones using Qiaprep Spin Miniprep Kit (cat. no. 27104). Extracted DNAs were sequence in both directions and sequences were submitted to GenBank. Four accessions viz., KM624615, KM624616, KM624617, and KM624618 were assigned for BSV isolates from Sohag, Giza, Qalyubia, and Gharbia, respectively.

The four sequences were compared for matching sequences in the Genbank databases, at http://www.ncbi.nlm.nih.gov**.,** after removing primer positions, using the BLAST (NCBI). Percentage identities in both nucleotides and amino acids were determined using the Blastn and blastp of GenBank, respectively. The encoded amino acid sequences were translated from the nucleotide sequences, with Standard genetic code, using the EditSeq program in DNASTAR. Translated amino acids of the four Egyptian BSV isolates were aligned with amino acid sequences of other members of the *Caulimoviridae* using ClustalW method (Thompson *et al*., 1994). Phylogenetic relationships of BSV tested isolates and other badnaviruses were inferred from multiple sequence alignment using the Neighbor-Joining method (Saitou and Nei, 1987) and a consensus tree was generated following 500 rounds of bootstrapping (Rzhetsky and Nei, 1992; Dopazo, 1994) using MEGA 6 program (Tamura *et al.*, 2013). The evolutionary distances were computed using the p-distance method (Nei *et al*., 2000) and are in the units of the number of amino acid differences per site. Names of *Caulmoviridae* sequences and their accession numbers in the GenBank, used in the present study were depicted in Table (1).

Table (1): *Caulimoviridae* members used in the present study

Virus Name	Acronym of virus isolates	GenBank Accessions	Country*	Reference
Banana streak OL virus	BSOLV	AJ002234	Nigeria	Harper and Hull 1998
Banana streak gold finger virus	BSGFV	AY493509	Ecuador	Unpublished
Banana streak Mysor virus	BSMyV	AY805074	Australia	Geering *et al.*(2005b)
Banana streak accuminata Vietnam virus	BSAcVNV	AY750155	Vietnam	Lheureux *et al.* (2007)
Banana streak China Virus	BSCV	FJ594880	China	Unpublished
Banana streak CA virus	BSCAV	HQ593111	Kenya	James *et al. 2011*
Banana streak Cuba virus	BSCV- E239-72	KF386746	Cuba	Javer-Higginson *et al.* (2014)
Banana Streak Uganda A viurs	BSUgAV	AJ968453	Uganda	Harper *et al.*(2005)
*Banana streak Egypt virus***	BSEV- Giza	KM624616	Giza-Egypt	This study
Banana streak Egypt virus	BSEV- Sohag	KM624615	Sohag-Egypt	This study
Banana streak Egypt virus	BSEV- *Qalyubia*	KM624617	Qalubia-Egypt	This study
Banana streak Egypt virus	BSEV- Gharbia	KM624618	Gharbia-Egypt	This study
Cacao swollen shoot virus	CSSV	L14546	France	Hagen *et al.* (1993)
Rice tungro bacilliform virus	RTBV	D10774	Japan	Hay *et al*. (1991)
Commelina yellow mottle virus	ComYMV	X52938	USA	Medberry *et al*. (1990)
Citrus yellow mosaic virus	CYMV	AF347695	India	Huang and Hartung (2001)
Sugarcane bacilliform Morocco virus	SCBMV	M89923	Morocco	Bouhida *et al.*(1993)
Sugarcane bacilliform Ireng Maleng virus	SCBIMV	AJ277091	Australia	Geijskes *et al.* (2002)
Sugarcane bacilliform Guadeloupe virus	SCBGV-R570-78	FJ439815	Guadeloupe	Muller *et al.* (2011)
Sugarcane bacilliform India Virus	SCBIV-B091	JN377533	India	Karuppaiah *et al.* (2013)
*Sugarcane bacilliform Egypt Virus***	SCBEV-Giza	KM591919	Giza-Egypt	This study
Sugarcane bacilliform Egypt Virus	SCBEV- Assiut	KM591920	Assiut-Egypt	This study

*Virus-source according to GenBank ** Egyptian badnaviruses were marked in gray

RESULTS

Symptomatology

The observed collected symptoms from commercial banana fields in four governorates, though from supposedly one variety, viz. Williams' banana variety, were diverged. The most prevalent symptom manifestation ranged from chlorotic to necrotic streak on leaves that run from the leaf midrib to the margin. As shown in Figure (1-A, B), small banana plants in nursery exhibited vein banding. Older leaves may exhibit broad range of streak patterns that range from being fine, long, and narrow (Fig.1-D), short discontinuous (Fig. 1-E), to broad chlorotic and necrotic streak (Fig. 1-F). Upon disease progress, large chlorotic streak can be observed parallel to secondary veins (Fig. 1-G) which develop farther to necrotic streak (Fig. 1-H, I).

Fig. (1): Field symptoms observed upon BSV infection on banana. A & B, vein banding developed on upper (A) and lower surface (B) of young leaves; C, healthy leaf; D, fine, long, and narrow streak; E, short discontinuous chlorotic streak; F, broad chlorotic and necrotic streak; G, large chlorotic streak; H, necrotic streak further detailed in (I) as banded necrotic streak (see arrows); J, late stage of chlorotic and necrotic streak with torn leaf lamina; K, pseudostem splitting; L, dark brown streak on pseudostem after removing leaf sheath

Upon farther disease progress, leaf lamina become torn (J). Infected stems develop pseudostem splitting from leaf sheath with conspicuous dark brown necrotic streak (Fig. 1-k, L). Short discontinuous streak similar to that described on Williams' variety in addition to leaf chlorosis and necrosis were the main symptoms developed on Pradica banana variety.

Immunocapture PCR (IC-PCR)

IC-PCR (Fig.2) amplified 580 bp amplicons for BSV-RT/RNase H motifs from vitroplants obtained from three different TC laboratories, from

four BSV isolates, and from BSV-infected Pradica banana. No PCR amplicons were obtained from healthy *M acuminate* sap.

Sequence analysis

Sequence analysis involved comparing the local BSEV accessions collected from four governorates with other foreign BSV and other members of the *Caulimoviridae* (Table 1), on the nucleotide and\or amino acid levels, in the RT/RNaseH motifs, present in full or partial sequences, for these viruses. The four accessions had nucleotide-pair wise identity between 76-82% and low amino acid similarity between 29-63% which indicates wide genetic diversity among them. BSEV-Giza, BSEV-Sohag and BSEV-Qalyubia had nucleotide identity between 81%-82%; while BSEV-Qalubia and BSEV-Gharbia had 76% nucleotide identity (Table 2). Results in Table (3) showed that BSEV-Giza and BSEV-Sohag had respectively nucleotide sequence identity of 86% and 88% with BSV from Cuba. While BSEV-Qalyubia and BSEV-Gharbia had, on the other hand, moderate to low nucleotide sequence identity ranging from 79% to 64% with BSV from Cuba, Uganda, Kenya and China.

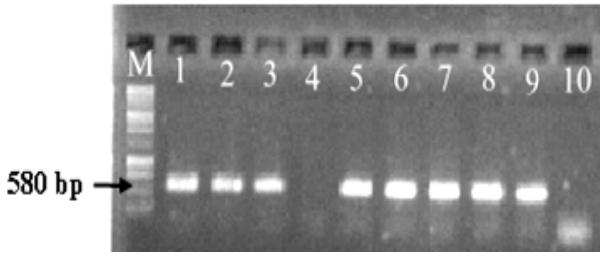

Fig. (2). IC-PCR performed on leaf extracts from lanes: Vitroplants from Badr City (1), Barrage-Qalyubia (2), Shorba El-Khima (3); 4 and 10, Healthy *M. acuminate* var. Williams; 5, 6, 7, 8, BSV-infected banana from Giza, Sohag, Qalyubia, and Gharbia; 9, BSV-infected Pradica banana variety. M, 1 kb DNA ladder (Promega).

Table (2): Nucleotide (NT) sequence identity and amino acid (AA) similarity (in bold) between the pair wise combinations of partial RT/RNase H sequence of four tested Egyptian BSV (BSEV isolates created by NCBI blastn and blastp respectively.

Percent NT Identity and AA similarity				
BSEV Isolates	**Giza** ***Acc No.***	**Sohag** ***Acc No.***	**Qalyubia** ***Acc No.***	**Gharbia** ***Acc No.***
Giza, *Acc No.KM624616*	100 **100**	81 **34**	76 **63**	77 **62**
Sohag, *Acc No.KM624615*		100 **100**	82 **29**	76 **37**
Qalyubia, *Acc NoKM624617*			100 **100**	76 **33**
Gharbia, *Acc No.KM624618*				100 **100**

Table (3): Nucleotide (NT) sequence identity between RT/RNase H sequences of four tested Egyptian BSV (BSEV) isolates against four corresponding isolates retrieved from the GenBank.

BSV Acc. No.\ Country	**Percent NT Identity**			
	BSEV Giza ***Acc No. KM624616***	**BSEV Sohag** ***Acc No. KM624615***	**BSEV Qalyubia** ***Acc No. KM624617***	**BSEV Gharbia** ***Acc No. KM624618***
KF386746\Cuba	86	88	77	79
AJ968453\Uganda	70	76	70	67
HQ593111\Kenya	69	74	69	74
FJ594880\China	64	72	74	72

Phylogenetic tree depicting the relationships of the tested BSEVes with other badnaviruses based on amino acid sequences of RT/RNase H using the Neighbor-Joining method was illustrated in Figure 3. All BSEVes and SCBEVes clustered in one clade apart from RTBV, and another clade for the foreign badnaviruses circumventing other BSV and SCBV; illustrating that BSEVes and SCBEVes are distinctive genomically. Similarly, Ugandan BSVes clustered separately from the clade circumventing the other BSVes (Iskra-Caruana *et al*., 2014).

SCBEV-Giza and -Sohag formed sub-cluster alone apart from BSEV-Qalyubia and –Gharbia. Both Egyptian BSEVes and SCBEVes, however, united in one node. Likewise, BSOLV clustered with SCBGV R570-78.

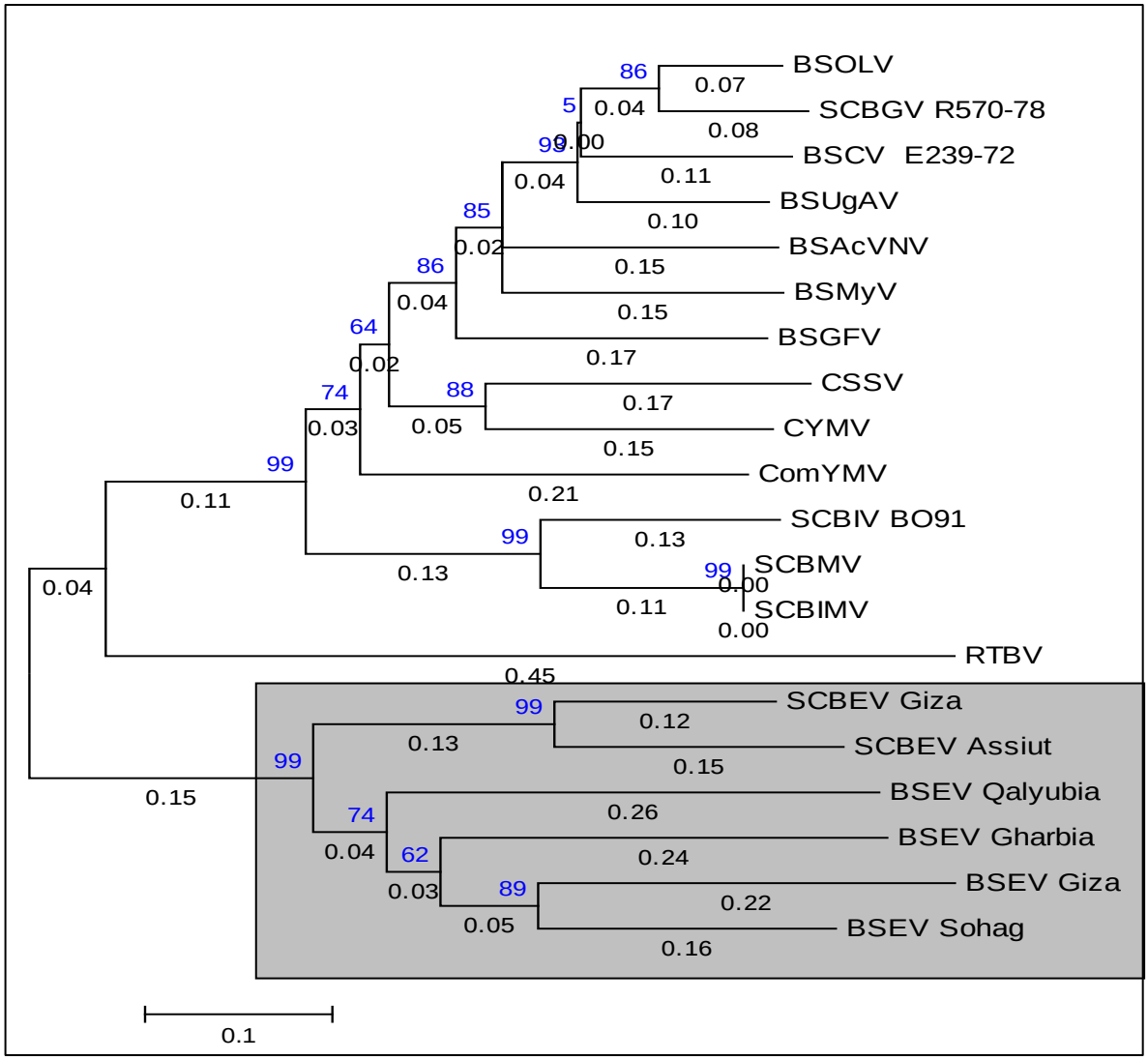

Fig. (3). Phylogenetic tree depicting the relationships of BSEV tested isolates with other badnaviruses based on amino acid sequences of RT/RNase H using the Neighbor-Joining method. The dendrogram was bootstrapped 500 times (score are shown on nodes). The shaded box indicates the Egyptian badnaviruses from banana and sugarcane. The analysis involved 20 amino acid sequences. Names of *Caulmoviridae* sequences and their accession numbers in the GenBank, used in the present study were depicted in Table (1).

DISCUSSION

This study confirms the previous report (Abdel-Salam *et al*., 2005) on the occurrence of BSV in Egypt by serological and molecular techniques.

Symptomatology

The detection of BSD by symptoms alone is unreliable as symptomless infections can occur and any of several viruses may be involved (Dahal *et al*., 1998). The described above symptoms; though diverged, were however typical to those mentioned by Daniells *et al*., 1998; Harper *et al*. (2002), Furuya *et al*. (2012).

The divergence in disease-symptom expression on one banana variety, though grown in different geographic locations extending from lower to upper Egypt may reflect environmental effect especially temperature. Dahal *et al*. (1998) showed the effect of cool temperature (e.g. 22°C) in stimulating symptom expression. However, in our case severe streak and mild streak symptoms can be observed in the same field which indicates that in addition to temperature effect the presence of different viruses causing BSD may offer a plausible explanation to symptom diversity. Indeed such case was observed by Harper *et al*. (2002) who indicated that no correlation was found between the disease severity or type of symptoms and the banana cultivar or locality within the country. Further, several authors showed that BSD is caused by the episomal infection of a wide variety of banana streak viruses that revealed great genetic diversity, with up to 30% nucleotide divergence among BSV isolates infecting the same Musa host plant (Geering *et al*., 2005b; Harper *et al* , 2004, 2005; Jaufeerally-Fakim *et al.* 2005; Iskra-Caruana *et al*., 2010.

Immunocapture PCR (IC-PCR)

In the present study, an additional DNAse I digestion step (Gambley, 2008) was involved in the IC-PCR to circumvent ultimately the presence of any eBSV sequences that might have entrapped on the polypropylene thin-walled tubes during IC-PCR analysis. Provost *et al*. (2006) have used instead a multiplex IC-PCR for the detection of BSV by implementing *Musa* sequence tagged microsatellite site primers in combination with BSV species-specific primers to monitor possible contamination by *Musa* genomic DNA with eBSV; under optimized IC conditions included limiting the IC step time to 3 h at room temperature to eliminate background amplification of eBSV sequences during IC-PCR. The present IC-PCR method using DNAse I removed any traces of eBSV; whereas, the described method of Provost *et al* (2006) relied on limiting the IC time. Therefore, any prolonged incubation in the latter method would result in high background and erroneous detection results.

In the present study, IC-PCR detected BSV in vitroplants obtained from three different TC laboratories, from four BSV isolates, and from BSV-infected Pradica banana. No PCR amplicons were obtained from healthy *M acuminate* sap. Similarly, Provost *et al*. (2006) and Chabannes *et al*. (2013) detected no eBSV sequence in healthy *Musa acuminate* (AAA genome) upon using IC-PCR since eBSV sequences are present only in banana with B genome. The genomic nature of these vitroplants was ambiguous and whether they were from interspecific triploid hybrids or from Cavendish subgroup (AAA genome). Should they were reproduced from Williams' banana; BSV incidence would be just a virus passage through TC. On the other hand, if being triploid with haploid B

genome i.e. AAB one would assume a situation of reactivation of eBSV sequence through TC. Never the less, the obtained results indicate the importance of indexing vitroplants for the presence of BSV.

Similarly, the detection of IC-PCR to BSV in the four isolates of BSV infecting Williams' banana variety indicates the episomal nature of BSV in these virus isolates.

Detection of BSV in Pradica banana variety (AAB genome), showing streak symptoms, with IC-PCR indicates its presence as an episomal entity. Yet one can not tell whether or not its episomal nature was due to horizontal transmission by mealybug or through eBSV reactivation.

Sequence analysis

Having used the 80% nucleotide identity threshold in the RT/RNase H domain, conserved in badnaviruses, to discriminate badnavirus species as stated by the ICTV (Fauquet *et al*., 2005; King *et al.*, 2011), it appears that at least two of the four local BSEV accessions represent different species of BSV. This conclusion is further strengthening upon putting the low amino acid similarity between the four accessions into account (Table 2). Harper *et al.* (2005) upon comparing several sequences of the Ugandan BSV mentioned that identity of less than 85% in the RT/RNase H nucleotide sequence could be used to describe new species. Hence, at least 15 species of BSV were found in Ugandan banana (Harper *et al.*, 2005).

The four Egyptian accession of BSV varied in nucleotide sequence identities when compared with similar BSV from Cuba, Uganda, Kenya and China. In fact this was not surprising since several investigators had considered BSV as a generic name of several species showing up to 30% nucleotidic divergence but provoking the same disease in banana plants (Geering *et al*., 2005b; Harper *et al* , 2004, 2005; Jaufeerally-Fakim *et al.* 2006; Gayral and Iskra-Caruana, 2009; Gayral *et al*., 2010; Iskra-Caruana *et al*., 2010).

BSEVes and SCBEVes clustered in one clade apart from other foreign badnaviruses indicating that BSEVes and SCBEVes are distinctive genomically. Similarly, Ugandan BSVes clustered separately from the clade circumventing the other BSVes (Iskra-Caruana *et al*., 2014). Such results are similar to other phylogenetic studies which indicated that BSV and SCBV isolates clustered together due to their close genetic relationship (Geijskes *et al*., 2002; Harper *et al*., 2005; Jaufeerally-Fakim *et al*., 2005, Muller *et al*., 2011; Iskra-Caruana *et al*., 2014).

The present study detected and identified on the DNA sequencing level four Egyptian BSV obtained from Williams' (AAA, Cavendish subgroup) banana (*Musa acuminate*) variety. The current work also indicated the presence of an episomal BSV in the interspecific hybrid Pradica (AAB) banana variety through PCR analysis. However no DNA sequence analysis was made on the produced amplicons. It is known that banana with AAB genome can harbor integrated complete DNA sequences of eBSV as *Banana streak Imove virus* (BSImV), BSGFV, BSOLV, and BSMyV (Geering *et al*., 2000; Gayral *et al*., 2008; Iskra-Caruana, *et al.,* 2010; 2014). Such eBSV can be reactivated into episomal viruses under stress conditions and function as sources for infection through vertical and/or horizontal virus transmission. Therefore, future studies on BSV in Egypt should be focused on the presence of such viruses through DNA

sequencing or at least using specific primers for these viruses.

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