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Article

Molecular Characterization of Isolates of the *Banana bunchy top virus* from the District of Chókwè, Mozambique

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Featured Application: The present work can contribute to understanding the origin of the Banana bunchy top virus isolates that occur in Mozambique, allowing the phytosanitary organs to trace the trajectory of the spread of the virus on the African continent and support control measures to avoid further damage.

Abstract: *Banana bunchy top virus* (BBTV) was recently detected in Mozambique and appears to be limited to the provinces of Gaza, Maputo and Zambezia, but it has great potential to spread to other provinces. Despite its importance, nothing is known about the BBTV isolates that occur in Mozambique. In this study, the sequences of the S and R genes of 40 isolates chosen as representatives of samples collected previously from eleven farms of the four administrative posts of the district of Chókwè, Gaza province, were sequenced and analyzed. The S-DNA nucleotide sequences of the analyzed isolates were highly conserved, with identity ranging from 97% to 100%. The same was observed for the R-DNA sequences, with most identities ranging between 98% and 100% among the isolates from Chókwè and above 90% when compared to the isolates from GenBank. The phylogenetic analysis showed that the Mozambican BBTV isolates belong to the Pacific-Indian Oceans (PIO) group, showing greater proximity to the isolate JQ820453 from Malawi than to the isolates from sub-Saharan countries, which were grouped in a distinct subclade. This is the first study conducted to determine the molecular characteristics of BBTV isolates present in Mozambique.

Keywords nanovirus; Pacific-Indian Oceans (PIO); South-east Asian (SEA); Phylogeny; DNA S; DNA R; Sub-Saharan African (SSA)

1. Introduction

Bananas and plantains are important food crops and daily sources of carbohydrates for at least 100 million people on the African continent [1–3]. The importance of bananas as a staple food is exemplified by Uganda, where per capita consumption reaches approximately 223 kg per person per year. Of the 20 million tons of bananas produced in East and Southern Africa, only 1% is exported, mainly by Zimbabwe, Uganda and South Africa. Approximately 4% of the 11 million tons produced in West and Central Africa are exported, mainly by Côte d'Ivoire and Cameroon [4,5].

In Africa, the production of bananas and plantains is often hampered by biological and environmental constraints. Many pests and diseases have been introduced to the continent through infected planting material. With the expansion of the banana industry in African countries, these

diseases and pests spread to several new areas. In many areas, disease-causing pathogens occur in epidemic proportions. The enormous costs involved in controlling some of these diseases make it almost impossible to continue banana production in severely affected areas [6].

In Mozambique, bananas (*Musa* spp.) are produced mainly on small farms; however, some large farms are found around the provinces of Maputo Province, Gaza, Nampula and Manica. These farms mainly cultivate Cavendish varieties and supply large cities such as Maputo and Beira and the markets of South Africa, Botswana and the Kingdom of eSwatini. Currently, banana plantations (farms) occupy 62,000 ha in Mozambique, with an estimated production of 470,000 thousand tons per year [7].

Production on small farms in southern Mozambique (Maputo Province and Gaza Province) averages 45 tons per ha for a production area of 3216 ha. In addition to the various pests (nematodes and weevils) and diseases (Sigatoka, Fusarium disease or Mal de Panamá - Foc TR4 and currently bunchy top da Bananeira) that can affect banana crops, the lack of production knowledge, low performance, low soil fertility and long drought periods (particularly in the south of the country) are factors that hinder crop management [7]. Among the diseases cited, Fusariosis or Panama disease (FocTR4) and *Banana bunchy top disease* (BBTD) have been the most important.

BBTD, whose etiological agent is the *Banana bunchy top virus* (BBTV), was introduced and reported for the first time in Sub-Saharan Africa (SSA) in the 1960s in the Democratic Republic of Congo [8]. Since then, the spread of BBTV has been confirmed in 16 countries in the Central, Southern and West African regions, and it was first recorded in South Africa in 2015 [9] and in Mozambique in June 2016, in the Chókwè irrigated perimeter, more specifically in the Primeira Zona locality, in Gaza province [7].

BBTV belongs to the domain *Monodnariria*, kingdom *Shotokuvirae*, phylum *Cresnaviricota*, class *Arfiviricetes*, order *Mulpavirales*, family *Nanoviridae* and genus *Babuvirus* [10]. BBTV affects only members of the genus *Musa* and is transmitted in a persistent circulatory manner by its only known vector, *Pentalonia nigronervosa* Coq. [11,12]. The BBTV genome has six circular single-stranded DNA (ssDNA) components (R-DNA, U3-DNA, S-DNA, M-DNA, C-DNA and N-DNA), each approximately 1 kilobase (kb) in size [13]. Each component potentially encodes a protein, except for R-DNA, which encodes two proteins linked to viral replication [14,15].

S-DNA encodes the capsid protein (CP), which forms isometric particles and aids in genome packaging [16]. C-, N- and M-DNA encode cell cycle binding proteins (Clink), nuclear transport proteins (NSPs) and movement proteins (MPs), respectively [17–19]. The function of U3-DNA remains unknown for BBTV. Rep and CPs are evolutionarily more conserved than the other proteins and therefore serve as useful markers for the identification and classification of ssDNA viruses [20].

The R gene has been used to classify isolates from different countries located in distinct geographical regions [21]. There are two suggested groups, the Pacific-Indian Oceans (PIO) and South-east Asian (SEA) groups, characterized by an approximately 10% nucleotide difference, while the intragroup difference ranges from 1.9 to 10% [21].

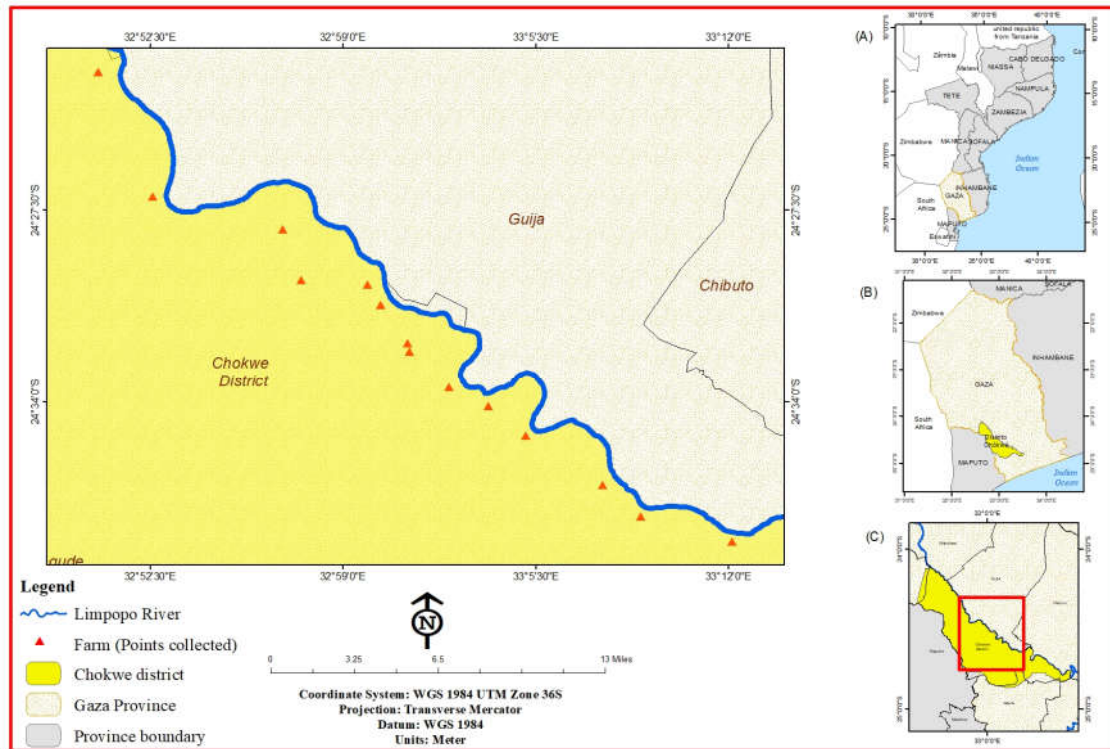
In Mozambique, the genetic characteristics of BBTV isolates occurring in producing fields located in the south of the country have not yet been reported. These data could allow inferences about the possible geographical origin of the viral isolates present and support preventive measures related to the control of viral spread in Africa. In this study, the R-DNA and S-DNA of 40 virus isolates, representing the 175 isolates previously collected in 23 locations of 11 farms located in the district of Chókwè, were sequenced and analyzed. The results obtained are presented and discussed here.

2. Materials and Methods

2.1. BBTV Isolates Analyzed

The 40 BBTV isolates used in this study were chosen proportionally to the number of isolates from 23 fields located on 11 farms in the 4 administrative posts of the Chókwè district (initial site of the outbreak), Gaza Province (Figure 1b) in Mozambique (Figure 1a), with the help of researchers

from the Institute of Agricultural Research of Mozambique - IIAM and commercial and family farmers involved in banana production. Located in southern Gaza Province, the district of Chókwè (Figure 1c) has the following geographical coordinates: 24°05' and 24°48' South latitude; 32°33' and 33°35' East Longitude [22].



2.2. Amplification and Analysis of the S and R Genes

Total plant DNA was extracted from leaf tissue in the laboratory of the Biotechnology Center of the Eduardo Mondlane University of Mozambique (UEM), following the protocol of Lodhi et al. [23]. For extraction, 0.35 g of fresh plant tissue was macerated in liquid nitrogen and then 1.5 mL of 2% CTAB buffer (100 mM Tris-HCl, pH 8.0; 20 mM EDTA; 1.4 M NaCl; 80 mM Na₂SO₃; 2% PVP-10 and 2% cetyl trimethyl ammonium bromide (CTAB) containing 0.2% β mercaptoethanol. After maceration, the tissue was transferred to a 1.5 mL sterile microcentrifuge tube. Then, 750 µl of extraction buffer with 2% PVP was added and incubated at 60°C for 30 minutes, and the tubes were mixed by inversion every ten minutes. A volume of 750 µl of chloroform:isoamyl alcohol solution (24:1) was added to the tubes and centrifuged at 12,000 rpm for 10 minutes at 23°C. The aqueous phase (supernatant) was transferred to new Eppendorf tubes, and the DNA was precipitated by adding 0.6 volumes of isopropanol and incubated at -20°C for one hour. After centrifugation at 12,000 rpm for 10 minutes, the supernatant was discarded, and the DNA was washed with 500 µl of 70% ethanol and resuspended in 100 µl of 1X TBE buffer. The tubes containing the extracted DNA were placed on dry ice and transported to Brazil to the Laboratory of Molecular Virology, Department of Plant Pathology, Federal University of Lavras (UFLA), for further studies.

In the DNA amplification reaction, primers specially designed to flank the S gene region and the R gene region were employed, as specified in Table 1.

Table 1. Primers used for amplification of the R and S Genes with the respective annealing temperatures and amplified fragment.

Primer	Sequence (5'-3')	Gene	Temp (°C)	Size (bp)
BBTV 67SF	CGTTTAGATGGGTTTGGG	DNA S	49	718
BBTV 784SR	CGTTTAGATGGGTTTGGG			
BBTV 32RF	CGGGACGGGACATTTGC	DNA R	52	1007
BBTV 1038SR	CGTTAGGATAGCACAATC			

DNA amplification was performed in a 25 µl reaction consisting of 2.5 µl of 10X PCR buffer containing 15 mM MgCl₂, 2.0 µl of 10 mM dNTPs, 1.0 µl of forward primer and 1.0 µl of reverse primer at a concentration of 10 µM, 0.25 µl of Taq DNA polymerase (Sigma, USA), 1.0 µl of DNA and enough water for a total reaction volume of 25 µl. Amplification was performed in a Master Cycler Thermocycler (Eppendorf, Germany) with the following program: 94°C for 2 minutes followed by 35 cycles of 94°C for 30 seconds, 49°C (DNA S) or 52°C (DNA R) for 20 seconds and 72°C for 60 seconds, with a final extension of 72°C for 5 minutes. The amplified products were analyzed by electrophoresis in a 1% agarose gel and counterstained with Gel Red (Biotium). The bands were visualized and documented using an Alpha Imager (Alpha Innotech Corp., USA).

2.3. Sequencing and Analysis of S and R DNA Sequences

The PCR products for each of the R-DNA and S-DNA (CP) fragments were purified and sent for sequencing at the Brazilian company FIOCRUZ. The sequence data were analyzed using the BioEdit software program (version 7.0.90) and NCBI (National Center for Biotechnology Information). The multiple alignments of the nucleotide and amino acid sequences of the studied isolates with other viral isolates available in GenBank (Table 2), as well determination of the identity between them, were performed using the program CRUSTAL W2 (V.2.0). Isolates from the PIO group, including some isolates from sub-Saharan Africa, and from the SEA group were chosen for comparison. An isolate of pineapple bunchy top virus (ABTV). The phylogenetic trees were constructed by the neighbor-joining (NJ) method in the MEGA7.02 program [24] using 2,000 bootstrap replicates.

Table 2. BBTV isolates of R DNA and S DNA and the number of accessions available in GenBank, used for comparison with the Mozambican isolates.

BBTV Group *	DNA R			DNA S		
	Origin	GenBank	Reference	Origin	GenBank	Reference
PIO	Egypt	HQ259074	Islam et al. [25]	Egypt	KM607471	Stainton et al. [28]
PIO	Egypt	AF416465	Horser et al. [15]	Egypt	KM607470	Stainton et al. [28]
PIO	Fiji	AF416466	Horser et al. [15]	Tonga	KM607584	Stainton et al. [28]
PIO	Malawi	JQ820453	James et al. [26]	Malawi	JF755980	Kumar et al. [8]
PIO	Rwanda	JQ820465	James et al. [26]	Rwanda	JQ820467	James et al. [31]
PIO	Tonga	AF416467	Horser et al. [15]	Tonga	KM607550	Stainton et al. [28]
PIO	Pakistan	AM418538	Amin et al. [27]	Pakistan	MK140621	Bashir et al. [32]
PIO	DRC	KM607635	Stainton et al. [28]	DRC	KM607505	Stainton et al. [28]
PIO	Australia	KM607623	Stainton et al. [28]	Australia	KM607489	Stainton et al. [28]
PIO	Burundi	KM607598	Stainton et al. [28]	Burundi	KM607456	Stainton et al. [28]
SEA	China	HQ616074	Yu et al. [20]	China	KM607536	Stainton et al. [28]
SEA	China	HQ378190	Yu et al. [20]	China	AF238876	He et al. [33]
SEA	Japan	AB108458	Furuya, Kawano and Natsuaki [29]	Japan	AB078023	Kawabe and Onuki [34]
SEA	Indonesia	AB186924	Furuya, Kawano and Natsuaki [29]	Indonesia	MT433375	Rahayuniati et al. [35]

SEA	Vietnam	AF416477	Bell et al. [11]	Myanmar	AB252644	Furuya and Natsuaki [36]
SEA	Vietnam	AF416472	Bell et al. [11]	Myanmar	AB252642	Furuya, Kawano and Natsuaki [29]
SEA	Japan	AB108455	Furuya, Kawano and Natsuaki [29]	Malawi	JQ820455	James et al. [31]
SEA	Taiwan	AF416468	Horser et al. [15]	Taiwan	KM607468	Stainton et al. [28]
SEA	Filipinas	AF416469	Horser et al. [15]	Filipinas	KM607469	Stainton et al. [28]
SEA	Indonesia	AB186925	Furuya, Kawano and Natsuaki [29]	Indonesia	MT433376	Rahayuniati et al. [35]
OuGR	Malaysia	EF546813	Sharman et al. [30]	Malaysia	NC_010316	Sharman et al. [30]

*PIO Group according to R DNA analysis, SSA countries in bold.

3. Results

3.1. Amplification of the S and R Genes

The 40 BBTv isolates from eleven farms of the four administrative posts of the district of Chóckwè, Gaza province, Mozambique, were sequenced and analyzed. The bands amplified from the S gene can be seen in Figure 2 and those from the R gene in Figure 3.

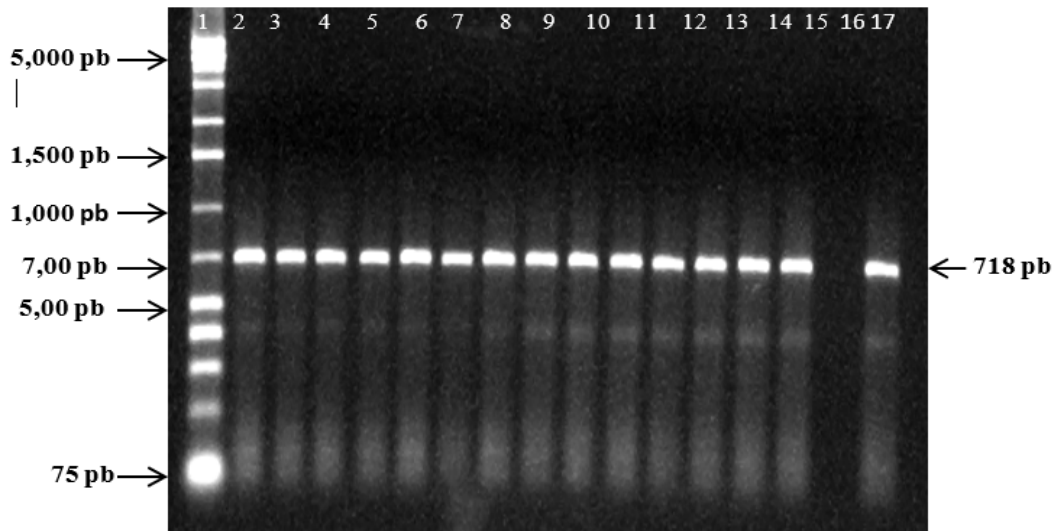


Figure 2. Electrophoretic analysis of PCR products generated by the DNA S gene of BBTv isolates in fourteen different samples: 1: 1Kb marker; line of 2 to 15 samples that tested positive among those analyzed; 16: negative control; 17: positive control.

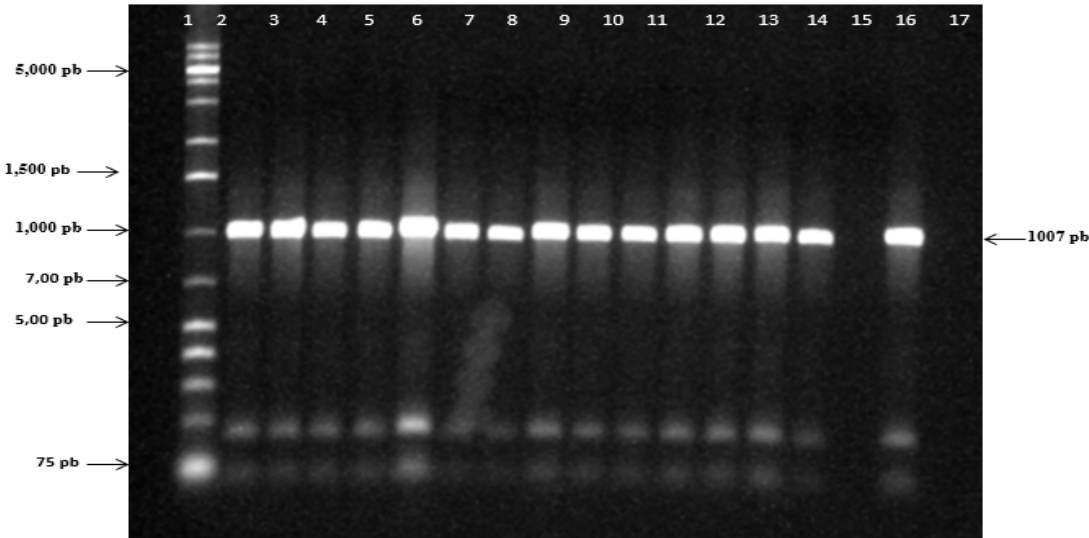


Figure 3. Electrophoretic analysis of PCR products amplified from the R DNA of BBTV isolates in fourteen different samples. 1: 1Kb marker; 2 to 15: band pattern of the amplified isolates; 16: negative control; 17: positive control.

3.2. Analysis of the BBTV S-DNA Sequences

The nucleotide identities between the 40 isolates collected in Mozambique (Table 3) and the isolates available in GenBank (Table 2) showed that this gene is highly conserved, with more than 50% of the isolates having 100% identity, and the lowest identity was 98%. When compared to the GenBank isolates, the lowest identities observed (92-93%) occurred with isolates MT433376 and MT433375 from Indonesia, KM607468 from Taiwan, KM607469 from the Philippines, KM607536 and AF238876 from China and AB078023 from Japan. The highest identities occurred with isolates JQ820467 from Rwanda, KM607470 from Egypt, KM607505 from the Democratic Republic of Congo (DRC), and JF755980 and JQ820455 from Malawi, all from the African continent, although from different geographical regions. Only Malawi is found in the surrounding region, bordering western Mozambique. These isolates present in the African continent probably had a common geographical origin.

Table 3. Names of the BSV isolates collected Chokwe district, Gaza Province, Mozambique, with their respective accession number in GEnBank.

BSV Isolate Name	GenBank Accession Number	
	DNA R	DNA S
BBTV 252 F1	PP097007	PP096947
BBTV 260 F1	PP097009	PP096949
BBTV 261 F1	PP097010	PP096950
BBTV 262 F1	PP097011	PP096951
BBTV 271 F1	PP097012	PP096952
BBTV 273 F1	PP097013	PP096953
BBTV 280 F2	PP096991	PP096931
BBTV 281 F2	PP096992	PP096932
BBTV 285 F2	PP096995	PP096935
BBTV 286 F2	PP096996	PP096936
BBTV 291 F2	PP096999	PP096939
BBTV 292 F2	PP097000	PP096940
BBTV 299 F2	PP097001	PP096941

BBTV 301 F2	PP097003	PP096943
BBTV 308 F2	PP097005	PP096945
BBTV 232 F3	PP096986	PP096926
BBTV 241 F3	PP096988	PP096928
BBTV 243 F3	PP096989	PP096929
BBTV 340 F4	PP096983	PP096923
BBTV 344 F4	PP096984	PP096924
BBTV 329 F5	PP096980	PP096920
BBTV 335 F5	PP096982	PP096922
BBTV 311 F7	PP096979	PP096919
BBTV 201 F8	PP096977	PP096917
BBTV 204 F8	PP096978	PP096918
BBTV 205 F9	PP096975	PP096915
BBTV 210 F9	PP096976	PP096916
BBTV 217 F10	PP096971	PP096911
BBTV 222 F10	PP096972	PP096912
BBTV 224 F10	PP096973	PP096913
BBTV 225 F10	PP096974	PP096914
BBTV 171 F11	PP096954	PP096894
BBTV 172 F11	PP096955	PP096895
BBTV 177 F11	PP096959	PP096899
BBTV 179 F11	PP096961	PP096901
BBTV 180 F11	PP096962	PP096902
BBTV 183 F11	PP096963	PP096903
BBTV 187 F11	PP096967	PP096907
BBTV 191 F11	PP096968	PP096908
BBTV 193 F11	PP096969	PP096909

The amino acid identities observed among the Mozambican isolates were similar, with only seven isolates varying between 96 and 99% and the others showing 100% identity. When compared to isolates from *GenBank*, in addition to the higher identities already specified above, they also showed high identities with isolates from South and Southeast Asia and South Pacific: MK140621 from Pakistan, AB252642 from Miamar and KM607584 from Tonga. Notably, isolates BBTV_172_F11 from Mozambique and AB078023 from Japan showed 98% and 93% nucleotide identity, respectively, when compared to the other isolates. The amino acid identity of the Mozambican isolate was between 95 and 97%, indicating that the nucleotide substitutions were of the synonymous type, while the Japanese isolate presented amino acid identities between 95 and 98%, indicating that the substitutions were of the synonymous type.

Figures 4 and 5 illustrate the phylogenetic trees constructed based on the nucleotide and amino acid sequences, respectively, of the S-DNA. There was a clear subdivision of isolates into two clades, one with isolates from China, Japan, the Philippines, Taiwan and Indonesia and one with Mozambican isolates and the remaining isolates from *GenBank*.

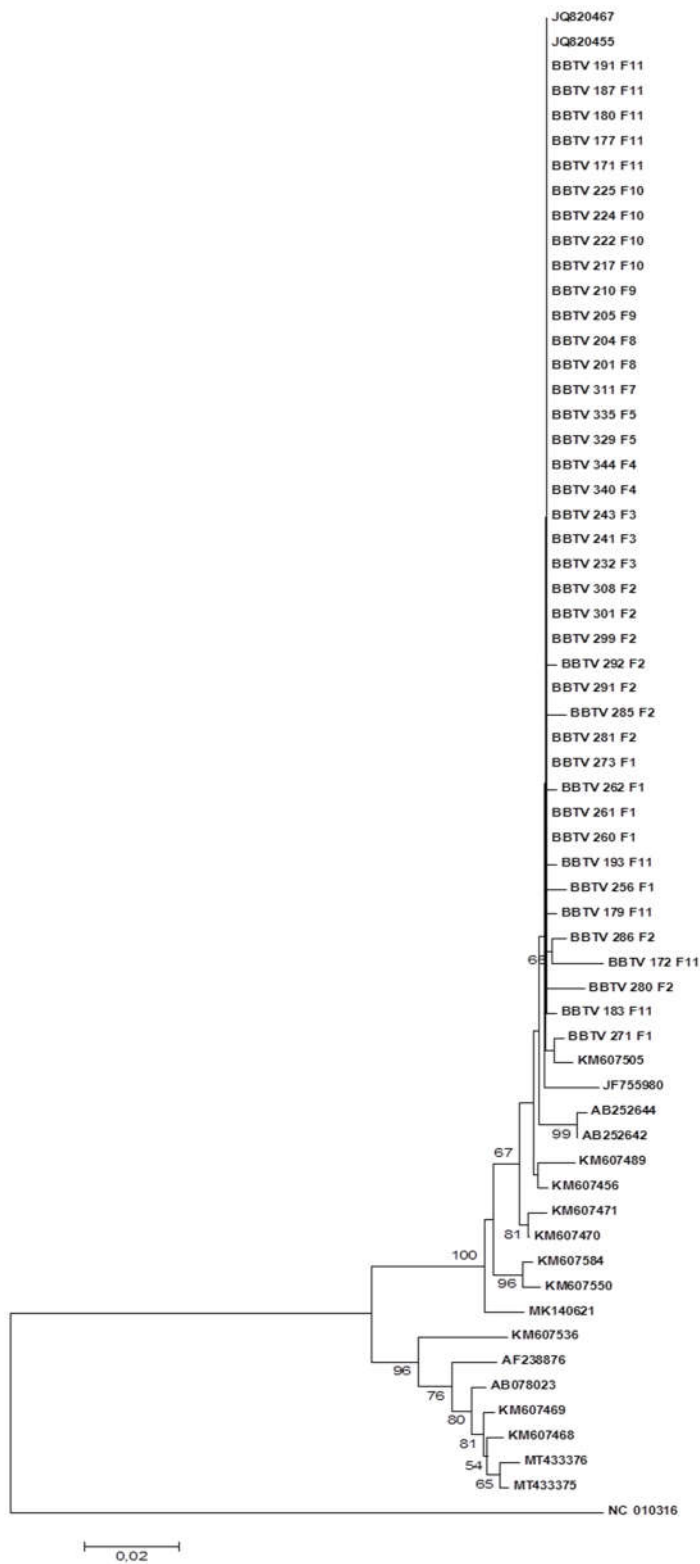


Figure 4. Phylogenetic tree constructed based on the nucleotide sequence of the S DNA of BBTv isolates collected in the district of Chókwè – Mozambique and isolates from *GenBank*. Bootstrap values were obtained using the MEGA 7.0 and Neighbor Joining program with 2,000 repetitions.

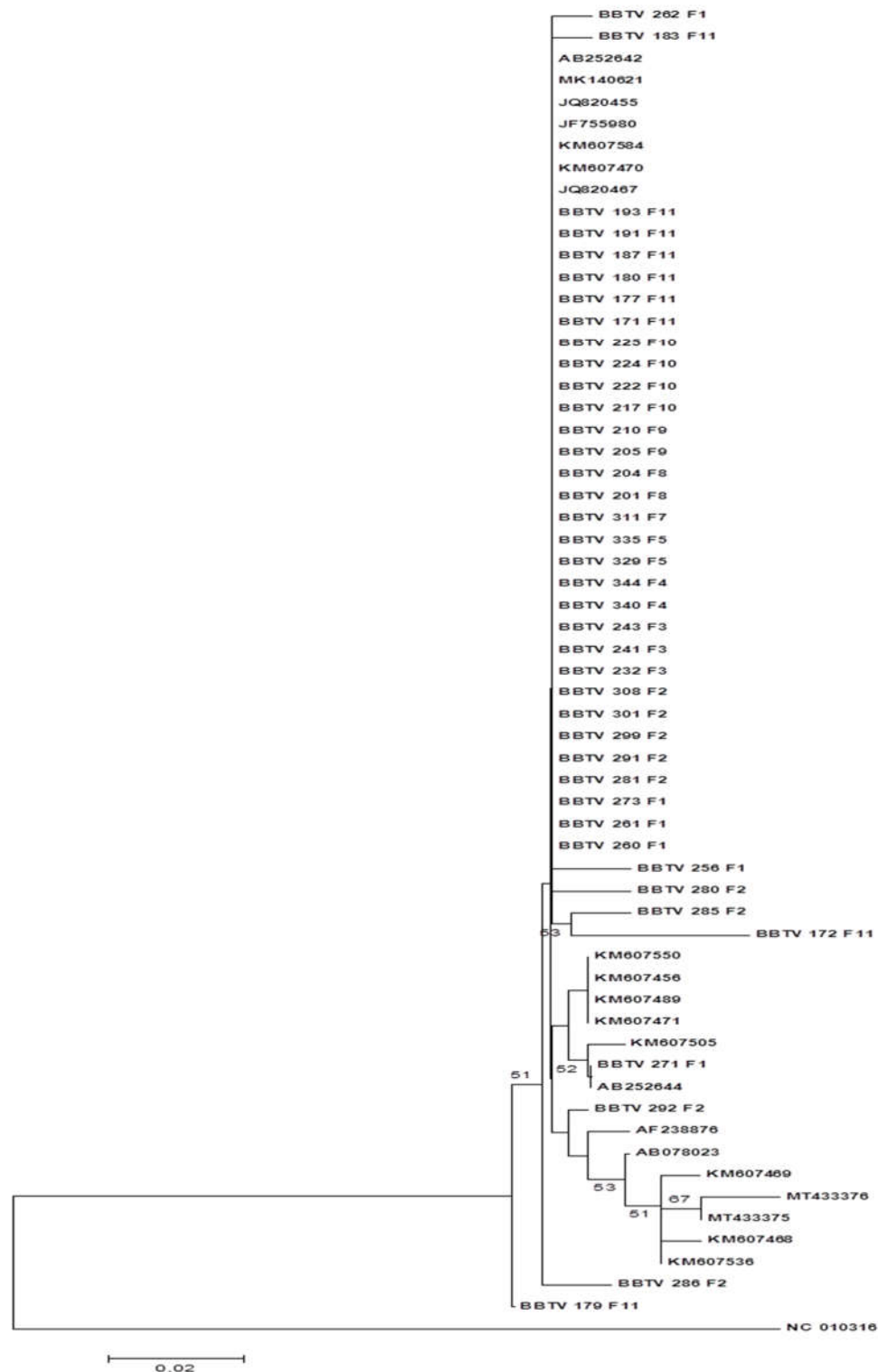


Figure 5. Phylogenetic tree constructed based on the amino acid sequence of the S DNA of BBTV isolates collected in the district of Chókwe – Mozambique and isolates from *GenBank*. Bootstrap values were obtained using the MEGA 7.0 and Neighbor Joining program with 2,000 repetitions.

In the tree constructed based on amino acid sequences, in addition to the isolates from Malawi and Rwanda, the isolates from Myanmar, Pakistan, Tonga and Egypt were also grouped with the Mozambican isolates. This must have occurred because the nucleotide substitutions are synonymous. In this tree, some isolates moved and occupied other subclades. Isolate BBTV_271_F1 occupied a subclade together with isolate AB252644 from Miami and isolate KM607505 from the DRC; isolate BBTV_292_F2 was placed in a subclade close to isolates from China, Japan, the Philippines, Indonesia and Taiwan, while isolates BBTV_286_F2 and BBTV_179_F11 were placed in different subclades and clades.

3.3. Analysis of the BBTV R DNA Sequences

Like the S-DNA, the R-DNA gene was also highly conserved. Except isolates BBTV_286_F2 and BBTV_273_F1, which presented identities between 93 and 96% when compared to each other, all the other isolates presented identities between 98% and 100%. When compared with the PIO isolates from GenBank, the identity of these two isolates ranged from 94% to 96%, while all other Mozambican isolates exhibited identities between 96% and 99%. On the other hand, when compared with the SEA isolates, the two isolates presented identities between 87 and 89%, while the others presented identities between 90 and 93%.

When comparing the identities between the PIO isolates from *GenBank*, it was observed that they have 97% to 99% identity among themselves and between 90 and 93% identities with the SEA isolates. These results allowed us to infer that the isolates that occur in Mozambique are of the PIO type, i.e., they belong to the Indian Pacific Ocean group.

The amino acid identities between the two isolates cited above (BBTV_273_F1 and BBTV_286_F2) and the other Mozambican isolates were lower than that of the nucleotides, between 91 and 93%, indicating that the nucleotide substitutions were nonsynonymous.

The same was observed with isolate 3, whose nucleotide identity ranged from 94% to 99% and amino acid identity ranged from 91% to 97%. Most isolates presented identities of 100% when compared to each other and from 97% to 100% when compared to the isolates from the PIO group and 91% to 95% when compared to the isolates from the SEA group.

The phylogenetic tree based on the nucleotide sequences is illustrated in Figure 6. The isolates were grouped into two distinct clades, one with the isolates of the PIO group and the other with the isolates of the SEA group. All Mozambican isolates were grouped into a subclade with the Malawi isolate JQ820453. The other isolates of the PIO group were grouped in the same clade but in a distinct subclade.



Figure 6. Phylogenetic tree constructed based on the nucleotide sequence of the R DNA of BBTV isolates collected in the district of Chókwè – Mozambique and isolates from *GenBank*. Bootstrap values were obtained using the MEGA 7.0 and Neighbor Joining program with 2,000 repetitions.

In the phylogenetic tree based on amino acid sequences (Figure 7), the distribution was slightly different. There was also a separation of the isolates into two clades, one with the isolates of the PIO group and the other with the isolates of the SEA group. However, in addition to isolate JQ820453 from Malawi, isolates KM607635 from DRC, JQ820465 from Rwanda and, a little further away, AF416467 from Tonga also grouped together with most of the Mozambican isolates. Another eight

isolates from Mozambique were mixed with isolates from different parts of the world in different combinations. Both the identity and phylogenetic results support the classification of the studied isolates within the PIO group.



Figure 7. Phylogenetic tree constructed based on the amino acid sequence of the R DNA of BBTv isolates collected in the district of Chókwe – Mozambique and isolates from *GenBank*. Bootstrap values were obtained using the MEGA 7.0 and Neighbor Joining program with 2,000 repetitions.

4. Discussion

In this study, the R-DNA and S-DNA genes of Mozambican BBTV isolates from the Chókwè district of Gaza were sequenced and analyzed for the first time, revealing new information about their classification and possible geographical origin.

The nucleotide identities of the R-DNA among the 40 analyzed isolates revealed that 38 of them presented a difference of at most 2%, showing the high conservation of this gene, which has already been observed by authors from other regions of the world. Adegbola [37] in Nigeria also found 100% identities among the local isolates. Another study conducted in Cameroon by Oben et al. [38] showed a similar result, that is, 100% homology between nucleotide sequences of the analyzed isolates.

In phylogenetic studies performed by Karan et al. [39] and reviewed by Yu et al. [20], the classification of BBTV isolates, based on nucleotide sequences, into two groups, South Pacific group and the Asian group, was proposed. The Mozambican isolates showed higher identities with isolates from the PIO group, between 97% and 99%, and the lowest identities with the isolates from the SEA group, between 90% and 92%, indicating that the Mozambican isolates belong to the first group. These results were corroborated by the distribution of the isolates in the phylogenetic tree based on nucleotide sequences, which showed a clear subdivision of the isolates into two distinct clades, with all the Mozambican isolates grouped together with the isolates of the PIO group. Other authors studying isolates from different African countries also reported that the isolates belonged to the PIO group [21,37,38,40–43].

Interestingly, the Mozambican isolates were very similar to isolate JQ820453 from Malawi, which borders Mozambique to the south and west. This demonstrates that there is a high probability that the Mozambican isolates came from this country. Kumar et al. [8] had already considered Malawi as an area for the dissemination of BBTV through the exchange of planting material of preferred cultivars among farmers in bordering countries. However, it is necessary to consider that South Africa also has the presence of BBTV and shares a border with southern Mozambique. As there is no information on the complete gene sequences of the R-DNA in GenBank, it could not be included in this study. However, South Africa cannot be ruled out as the origin of the inoculants that reached Malawi and Mozambique, as it is geographically closer to southern Mozambique than Malawi.

Research on the introduction of BBTV in sub-Saharan Africa (SSA) indicated that the arrival of BBTV in Africa may have occurred in two ways: in the DRC in 1950, it was probably through infected propagules brought from southern Asia or the South Pacific [44–46]; in Equatorial Guinea and Gabon, it may have been introduced by infected aphids (*Pentalonia nigronervosa*) brought by migrant workers from the Philippines. Then, it is assumed that it has spread to other African countries (Rwanda, Burundi and Central African Republic) [46,47].

This virus is known to be widely prevalent in Central African countries and in Malawi, Southern Africa [8]. To date, the occurrence of the disease has been reported in 16 countries of Central and Southern Africa (Angola, Benin, Egypt, Nigeria, Gabon, Burundi, Cameroon, Central African Republic, DRC, Congo, Equatorial Guinea, Malawi, Rwanda, Zambia, South Africa and Mozambique) [48].

In the nucleotide sequence analysis of the S gene of the isolates from Mozambique, it was observed that it is also highly conserved, with more than 50% of the isolates being 100% identical, and the lowest identity was 98%. Additionally, in the case of the S gene, the greatest nucleotide identities were observed with the isolates of the PIO group, especially the African isolates JQ820467 from Rwanda, KM607470 from Egypt, KM607505 from the DRC, and JF755980 and JQ820455 from Malawi. The phylogenetic tree constructed based on nucleotide sequences, similar to that observed for the R gene, also indicated similarity to the isolates of the PIO group, especially isolates RJQ820467 from Rwanda and JQ820455 from Malawi.

Considering the danger that this disease poses to the banana industry and to food safety, urgent measures are needed to combat it, such as the installation of indexing laboratories and the availability of positive controls to increase confidence in the diagnosis based on PCR. It is also necessary to train the local team involved in teaching and extension activities and the farmers in the recognition and control of the virus. In this context, there is an urgent need to make farmers and government officials

aware of the disease and the need to implement control measures, including (i) large-scale production and supply of virus free planting materials of the varieties preferred by farmers to rehabilitate banana production in the affected regions, preventing the use of infected planting material; (ii) raising awareness among farmers of the need to destroy infected material; (iii) greater phytosanitary surveillance and implementation of measures including restrictions on the movement of planting materials from regions affected by the disease, especially those bordering areas affected by BBTD; (iv) implementation of awareness programs among farmers, extensionists and regulatory agencies; (v) training to improve disease monitoring and diagnosis capacity; (vi) application of quarantine standards and integrated farming practices (ICP) aimed at reducing the spread of the disease and the aphid vector *Pentalonia nigronervosa*; and (vii) promoting and developing robust research programs to identify resistant varieties, different strains of the virus and its origins as well as the genetic diversity existing in each banana production region in Mozambique. Such measures are also recommended by several researchers, such as James et al. [31], Oben et al. [38], Adegbola et al. [37] and Ximba et al. [49].

Considering that the presence of this virus in Mozambique and in neighboring countries constitutes a major threat to commercial crops and family banana producers, whose culture is considered subsistence for this segment of the population in Mozambique, these actions are essential and need to be implemented urgently and then supported by government agencies.

5. Conclusions

The results of this study support the classification of Mozambican isolates within the PIO group, as observed in Malawi. These and other results indicate that the introduction of BBTV in Mozambique from Malawi through the spread of infected material for planting is highly probable.

These results emphasize the need for intensive research to assess the extent of the geographical spread and severity of BBTV in Mozambique and for the implementation of quarantine and phytosanitary measures to prevent the internal and transboundary spread of this virus.

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