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Survey of banana bunchy top virus in southern Mozambique

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Summary. Banana bunchy top virus (BBTV) has been recently recorded in Mozambique and threatens economic viability of banana production in this country. Research on BBTV in the region is still initial, and collective effort is required to support efficient control measures. Surveys were made in four administrative areas of the Chókwè district, Gaza Province, to determine the incidence and distribution of the virus. Samples were collected from two banana cultivars in 23 production fields where the plants had characteristic virus symptoms. DNA was extracted from these samples and subjected to BBTV detection using polymerase chain reaction technology. BBTV was detected in 19 of the 23 sampled fields, with overall mean incidence of 54.3%, and minimum incidence of 20%. No infected plants were found in the fields of one farm. These results highlight the urgency to initiate strategies for control of BBTV in Mozambique.

Keywords. BBTV incidence, BBTV distribution, viral.

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INTRODUCTION

Bananas are of great socioeconomic importance to Mozambique, as this fruit generate income and jobs, and are an important food source. According to the Ministry of Agriculture and Rural Development (MADER), Mozambique produces approx. 500,000 tons of bananas per year, with production from two sectors: family production (74.5% of total production) and commercial production (25.5%) (Diário Econômico, 2021).

Among factors restricting banana production, banana bunch top disease (BBTD), caused by *Babuvirus musae* (syn. banana bunch top virus, BBTV), is one of the most economically important diseases. BBTV is one of the 100 most important invasive plant pathogens and is subject to strict quarantine measures within the International Plant Protection Convention (Kumar *et al.*, 2011; Global Invasive Species Database, 2025). BBTD was first reported in Fiji in 1879, in Egypt in 1900, and then in Sri Lanka and Australia in 1913

(Magee, 1927), and is currently present in Southeast Asia, the South Pacific, India and Africa (Bouwmeester *et al*., 2023; Ocimati *et al*., 2024).

From 1913 to 1920, banana plantations in Australia were destroyed by BBTD (Magee, 1927; Hooks *et al*., 2009). In the 1990s, the first serious BBTD epidemic in Africa caused drastic reductions in banana production in the Nkhatabay and Nkhotakota districts of Malawi, where planted areas reduced from 3,500 ha to approx. 800 ha (Soko *et al*., 2009; Kumar *et al*., 2011). In 2016, the disease was identified in Mozambique, in the Chókwè district, in the Primeira Zona area of an irrigated region. There are no precise estimates of yield losses due to BBTD in Mozambique, but reductions 90% have been reported in susceptible cultivars (e.g. 'AAA-Cavendish'), due to severe BBTD (IPPC, 2016).

BBTV belongs to *Nanoviridae* family, *Babuvirus* genus and has a circular and multipartite ssDNA genome in six components, each separately encapsidated within an isometric virion of diameter 17-19 nm. These components are: DNA-R (Master Rep); DNA-S (coat protein); DNA-C (cell cycle binding protein); DNA-M (movement protein); DNA-N (nuclear transport protein) and DNA-U3 which encodes a protein of unknown function (Thomas *et al*., 2021).

Symptoms caused by BBTV on banana plants include discontinuous stripes and dark green spots on leaf blades, central veins and petioles, chlorosis and drying of leaf margins. Other characteristic symptoms: plant stunting, due to short and narrow leaves at the top of plants ("rosette"), lack of fruit production and fruit bunches, which are not suitable for consumption (Kumar *et al*., 2015; Food and Agricultural Organization, 2018).

Spread of BBTV over long distances, within and among countries and continents, occurs through infected planting material (suckers or tissue cultured plants). Dissemination of the virus over short distances can occur through infected planting material and the banana aphid *Pentalonia nigronervosa* Coquerel (Hemiptera: Aphididae). This aphid transmits BBTV in a circulative, non-propagative manner, while there is no evidence for mechanical virus transmission (Bressan and Watanabe, 2011; Watanabe and Bressan, 2013; Qazi, 2016; Thomas *et al*., 2021). Although *P. nigronervosa* was initially reported in associations with hosts belonging to *Zingiberales* and *Arecales*, morphological and morphometric studies have confirmed that the aphids found on these plants are of different species (Bhadra and Agarwala, 2010; Thomas *et al*., 2021). *P. nigronervosa* has high specificity for *Musa* spp. hosts and has been found in almost all banana-producing countries (Kumar *et al*., 2011). Transmission of BBTV by aphids to other hosts, such as *Heliconia* sp., *Alpinia galanga* and *Curcuma longa*, has been recently reported (Ngatat *et al*., 2022; Mendoza *et al*., 2024).

Information on BBTD in Mozambique has not been previously reported, so a survey was carried out to identify and determine the epidemiology of this virus in the region, aiming to acquire information for development of effective management strategies, particularly for the small-scale agriculture system in Mozambique. The study used polymerase chain reaction (PCR) tests for detection of BBTV, and to determine incidence and distribution of BBTD in Chókwè district of Gaza Province.

MATERIALS AND METHODS

Sample collection

Sample collections were carried out from 11 farms the Chókwè district, Gaza province, Mozambique (Figure 1), in January 2020. The Chókwè district is in the south of the Gaza province, close to the middle course of the Limpopo River (24°05' to 24°48' south latitude, 32°33' to 33°35' east longitude). Gaza province is bordered to the north by the Limpopo River, which separates it from the districts of Massingir, Mabalane and Guijá; to the south by the Bilene district and the Mazimuchope River; to the east by the Chibuto district; and to the west by the districts of Magude and Massingir.

The present study was carried out on farms that produced bananas, regardless of size (including large and small producers). For each collection site, termed a field, the geographic coordinates, average temperature, altitude, crop size, banana cultivar and presence of the *P. nigronervosa* vector were recorded. Banana leaves were collected at each site (sample size calculated as described below). Leaf samples were placed in zip lock plastic bags containing silica gel, and were transported to the laboratory of the Biotechnology Center of the Eduardo Mondlane University of Mozambique (UEM) for DNA extraction.

Sampling

To create the representative samples, BBTV foci were chosen in the four administrative areas (Macarretane, Chókwé-Sede, Lionde and Xilembene) of the Chókwè district, Gaza province, according to data from the MADER of Mozambique.

The estimated variable for disease incidence of Cooke (1998) was used to calculate the minimum num-

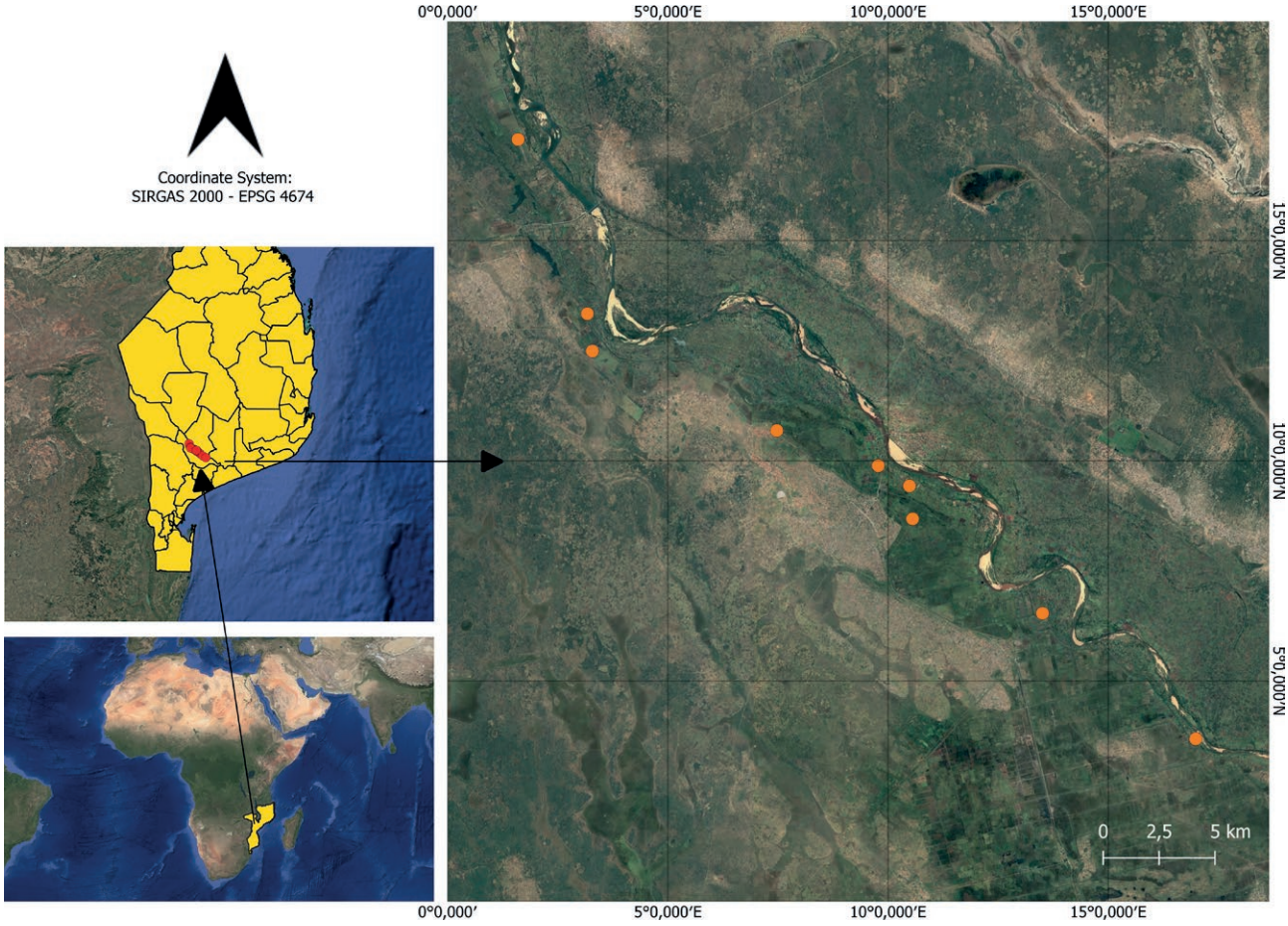

Figure 1. Maps of Africa and Mozambique, and a satellite image indicating sample collection sites for the 11 farms in the Chókwè district, Gaza province, Mozambique analyzed in this study. The orange dots and latitude and longitude co-ordinates for the satellite image show the geographic where the samples were collected.

ber of samples per location in each administrative area. This equation is:

$$n = \frac{Z^2.p.q.N}{d^2.(N-1)} + Z^2.p\,q$$

where:
n = the number of samples to be collected
Z = the tabulated value, referring to the abscissa of the standard normal curve, with a 90% confidence level (in this case, 1.65)
p = 0.6, an estimate of the probability of finding BBTV in the sample, as determined by the virology laboratory at the Federal University of Lavras
q = equal to 1-p
N = the population size or total number of plants of each banana producer's field, in the administrative area of the Chóckwè district, according to MADER data; and
d = the sampling error (the error attributed to the probability of finding or not finding a BBTV in the collected sample).

BBTV detection

DNA extractions

To extract DNA in the UEM laboratory, the protocol described by Lodhi *et al.* (1994) was used, with minor modifications. Fresh plant tissue (0.35 g) was macerated in liquid nitrogen, and then 1.5 mL of cetyl trimethylammonium bromide (CTAB) buffer (100 mM Tris-HCl, pH 8.0; 20 mM EDTA; 1.4 M NaCl; 80 mM Na_2SO_3; 2% polivinilpirrolidone and 2% CTAB) containing 0.2% β mercaptoethanol was added to macerated plant tissue mixture. This was then transferred to a 1.5 mL capacity sterilized microcentrifuge tube, and 750 µL of extraction buffer was added. The tubes were then incubated

at 60°C for 30 min., and 750 µL of chloroform: isoamyl alcohol solution (24:1) was added to each tube, and the tubes were centrifuged at 12,000 rpm for 10 min. at 4°C. The aqueous phase (supernatant) in each tube was transferred to new microcentrifuge tube, and the DNA was precipitated by adding 0.6 volumes of isopropanol and incubating at -20°C for 1 h. After centrifugation at 12,000 rpm for 10 min, the supernatant was discarded, and DNA was washed with 500 µL of 70% ethanol and the resuspended in 100 µL of 1× Tris-EDTA buffer. The tubes containing the extracted DNA were placed in dry ice and transported to the Laboratory of Molecular Virology, Department of Phytopathology, Federal University of Lavras (UFLA), Brazil for further analyses.

Polymerase chain reaction (PCR)

PCR was used to confirm the identity of the BBTV with primers flanking the region of the S gene encoding the capsid protein: BBTV 67SF and BBTV 784SR (Barros *et al*., 2024). Each DNA amplification reaction was carried out with 2.5 µL of 10× PCR buffer, containing 15 mM $MgCl_2$, 2.0 µL of 10 mM dNTPs, 1.0 µL of the forward primer and 1.0 µL of the reverse primer at a concentration of 10 µM, 0.25 µL of Taq DNA polymerase (Cellco, BRA), 1.0 µL of DNA (30 ng μL^{-1}), plus water for a total reaction volume of 25 µL. The amplification was carried out with the following program: 94°C for 3 min, followed by 35 cycles each of 94°C for 30 sec, 49°C for 30 sec, and 72°C for 60 sec. with a final extension of 72°C for 5 min. The amplified products were analyzed by electrophoresis in a 0.7% agarose gel and contrasted with Gel Red (Biotium®). The gel bands were visualized and documented using MiniBis Pro photodocumenter (DNR Bio-Imaging Systems®).

Data analysis

Microsoft Excel® software was used to organize the data, create tables, and calculate the mean incidence of BBTD with the following equation:

$$ID = \frac{UI}{UO}.100\%$$

where:
ID = incidence of disease (%)
IU = number of units affected by BBTD
UO = total number of plants assessed.

The assumptions of the analysis of variance were evaluated from the residuals of the three assays carried out. The residuals did not present normal distributions according to the Shapiro–Wilk test ($P < 0.05$), and the homogeneity of variances was violated. Nonhomogeneous residuals ($P < 0.05$) were identified by the ncvTest function and the Durbin-Watson independence test, where the residuals were correlated and there was no independence ($P < 0.05$). The analysis was carried out using the dwtest function of the lmtest package for the R statistical program.

The nonparametric Kruskal–Wallis test was used with the Kruskal test function in the statistical program R (R Core Team, 2021). This test allows comparison of three or more groups of independent samples and is used in cases where ANOVA requirements are not met (Kruskal and Wallis, 1952). The Dunn test was then applied with *P* value adjustment by Bonferroni (Dunn, 1961) with the rstatix package, through the Dunn test function in the R statistical program.

Histograms and maps of the 11 farms in the Chókwè district were created with the ggplot2 package for the R statistical program. For multiple comparisons (compare_means) between farms, the ggpubr package for R was used.

RESULTS

Detection of BBTV in collected samples

Presence of BBTV was confirmed in the Chókwè district of southern Mozambique. This region has farmers who practice subsistence agriculture, harvesting bananas for their personal needs, and commercial producers. A total of 175 plant samples were collected from 11 farms. Regardless of the presence of disease symptoms, all the collected samples were analyzed by PCR. Infected (positive) samples produced amplification bands each of 718 bp, as expected for the BBTV.

Incidence of BBTV in the collected samples ranged from zero (*i.e*., no infection) to 100%. The obtained PCR bands are illustrated in Figure 2, and incidence of BBTV are outlined in Table 1.

Incidence and distribution of BBTV

The greatest incidence of positive BBTV samples was recorded from Farm 2 (100%), followed by Farm 1 (84%), Farm 11 (83%), Farm 5 (80%) and Farm 3 (78%). The other farms had BBTV incidence levels lower than 70%, with the lowest incidence recorded on Farm 9 (20%) (Figure 3). BBTV was detected in most of the farms sampled, with the small family farms hav-

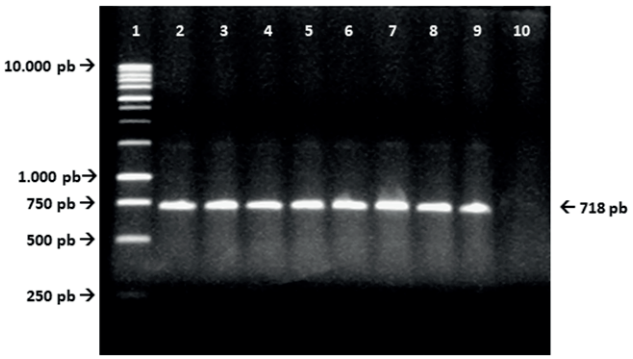

Figure 2. Electrophoretic analysis of PCR products obtained from BBTV diagnostic tests on DNA samples extracted from banana trees suspected to be infected by the virus. Lane 1:1 kb marker; lanes 2 to 9: BBTV positive samples; lane 10: negative control.

ing greatest proportions of BBTV positive samples (102 infected out of 116 samples). However, BBTV was not detected in Farm 6 and in two fields (Field 2 of Farm 4; Field 3 of Farm 10) (Table 1). The sampled farms had average temperature of 24.2°C, average monthly precipitation of 833.5 mm, and altitudes from 20 to 37 m a.s.l. Banana production in the region is predominantly of cv. 'Dwarf Cavendish' and 'Williams' (AAA genome). *Pentalonia nigronervosa* (the aphid vector of BBTV) was found only at one farm (Farm 5).

Based on the results of Kruskal-Wallis test, incidence of the BBTV was significantly different between most of the surveyed farms ($P < 0.05$). Dunn tests indicated significant differences for 28 combinations, while 23 were not significant ($P > 0.05$), with similar incidence levels (Table 2) The greatest differences in incidence ($P < 0.0001$) were observed between farms: 1 and 6, 2 and 4, 2 and 6, 2 and 8, 2 and 9, 2 and 10, and 6 and 11. The Dunn tests also showed that when Farm 6 was compared to Farms 4, 8, 9 10, there were no statistically significant differences ($P > 0.05$).

It is newsworthy to point out that Farm 6, where BBTV was not detected, was a commercial area with high productivity, in which intense control methods were used, (including roguing and establishing planted areas with suckers indexed as virus-free). On the other hand, the sampled Farms that showed highest incidences

Table 1. BBTV detection results from banana samples collected in the Chókwè district, Gaza province, Mozambique.

Farms	Field	N° of collected samples	BBTV infected samples		Altitude (m a.s.l.)	Area of banana crop (ha)	Banana cultivar
			N°	%			
Farm 1	1	10	7	70	37	<1	'Dwarf Cavendish'
	2	10	10	100	37	<1	Dwarf Cavendish
	3	6	5	83	37	<1	Dwarf Cavendish
Farm 2	1	10	10	100	37	<1	Dwarf Cavendish
	2	10	10	100	29	<1	Dwarf Cavendish
	3	6	6	100	29	<1	Dwarf Cavendish
	4	10	10	100	29	<1	Dwarf Cavendish
Farm 3	1	9	5	56	31	<1	Dwarf Cavendish
	2	10	10	100	31	<1	Dwarf Cavendish
Farm 4	1	8	4	50	37	55	Dwarf Cavendish
	2	4	0	0	37	35	Williams
Farm 5	1	10	8	80	33	2	Dwarf Cavendish
Farm 6	1	7	0	0	29	30	Williams
	2	3	0	0	29	20	Williams
Farm 7	1	3	2	67	35	2	Dwarf Cavendish
Farm 8	1	10	3	30	33	<1	Dwarf Cavendish
Farm 9	1	10	2	20	26	<1	Williams
Farm 10	1	10	4	40	26	<1	Dwarf Cavendish
	2	2	1	50	34	<1	Williams
	3	3	0	0	34	<1	Williams
Farm 11	1	10	8	80	34	<1	Dwarf Cavendish
	2	10	7	70	31	<1	Dwarf Cavendish
	3	4	4	100	20	<1	Dwarf Cavendish
Total	23	175	116	54.3			

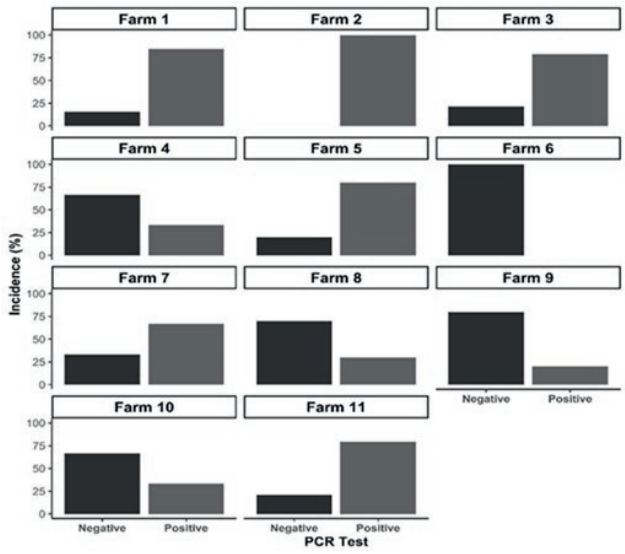

Figure 3. Average percentage of BBTV infected (positive) and healthy (negative) plants from banana trees as indicated by PCR assays for the virus in 11 farms (fields) in the Chóckwè district.

of BBTV belonged to farmers with small areas of bananas, who do not use the technologies indicated for the management of viral diseases.

DISCUSSION

Presence of BBTV in Mozambique, as shown in this study, represents a significant threat to banana cultivation in Mozambique, especially in the currently affected south region. BBTV presence was detected by PCR, using primers designed to flank the S gene region, in every sampled area, except for one commercial farm (Farm 6). On this farm, practices have been adopted to control BBTD, including appropriate roguing of affected plants and use of suckers with certified health.

Although no statistically significant differences (Dunn tests) were found when the results from Farm 6 were compared to those for Farms 4, 8, 9, and 10, BBTV was not present at Farm 6, but was detected on 4 other farms, at incidences from 20% to 30%. Therefore, the epidemiological significance of these incidences cannot be considered as equal. At Farm 6, no BBTV spread would be likely as long as the virus was not introduced. As aphids transmit and transport BBTV, incidences equal to or greater than 20% are likely to act as sources of inoculum for dissemination through whole farms in short periods. These sources would provide imminent risks for banana producers (Almeida *et al*., 2009; Dato *et al*., 2021; Qazi, 2016).

Soil and climate conditions in the Chókwè district promote favorable environments for the propagation of the vector *P. nigronervosa* and BBTV. Other studies have analyzed the climatic conditions that favor the dissemination of BBTV in banana plantations in different regions of the African continent (Ocimati *et al*., 2024; Bouwmeester *et al*., 2023), and the present study data corroborate the reported virus incidences. Greater numbers of fields infested by the aphid vector were expected, but the data were collected during January, which is the rainy season in the region. Presence of the aphid vector in only one farm probably demonstrates the negative correlation between vector and precipitation, as observed by Niyongere *et al.* (2013).

High BBTV incidence at Farms 1, 2, 3, 5, 7 and 11 could have been because these farms are in areas where

Table 2. Probabilities(indicated by Dunn tests) for positive incidence of BBTV in banana plant samples from 11 farms.

Farm	1	2	3	4	5	6	7	8	9	10	11
1	-	**0.0165***	0.6395^{ns}	**0.0019****	0.7636 ^{ns}	**<0.0001****	0.4771 ^{ns}	**0.0018****	**0.0003****	**0.0011****	0.6302 ^{ns}
2		-	**0.0049****	**<0.0001****	**0.0075****	**<0.0001****	**0.0008****	**<0.0001****	**<0.0001****	**<0.0001****	**0.0048****
3			-	**0.0134***	0.9739 ^{ns}	**<0.0001****	0.6928 ^{ns}	**0.0121***	**0.0029****	**0.0087****	1,0000 ^{ns}
4				-	**0.0357***	0.0545 ^{ns}	0.3506 ^{ns}	0.9025 ^{ns}	0.5219 ^{ns}	1.0000 ^{ns}	**0.0083****
5					-	**0.0004****	0.7290 ^{ns}	**0.0318***	**0.0101***	**0.0271***	0.9785^{ns}
6						-	**0.0104***	0.0767^{ns}	0.1675 ^{ns}	**0.0500***	**<0.0001****
7							-	0.3173 ^{ns}	0.1706 ^{ns}	0.3268 ^{ns}	0.6685 ^{ns}
8								-	0.6506 ^{ns}	0.8909 ^{ns}	**0.0076****
9									-	0.4984 ^{ns}	**0.0016****
10										-	**0.0050****
11											-

This table outlines *P* values from two-by-two comparisons of PCR test for presences of BBTV on 11 farms in Chókwè district, Gaza province, Mozambique. Statistically significant probabilities are indicated (at, respectively, $P < 0.05$ (*) or $P > 0.01$ (**).

semi-commercial fields predominate, and where only tall banana varieties are produced rather than shorter varieties (e.g. 'William'). Robson *et al.* (2006) believe that *P. nigronervosa* prefers small varieties, where it is mainly found. The rise of monoculture in these places could also favor the insect. Regardless of plant size, all banana varieties are susceptible to BBTV, although the banana genotypes with genome A are more susceptible than genotypes with genome B (Niyongere *et al*., 2011).

Low incidences of BBTV in other farms (4, 8, 9, and 10) could be because these fields contained at least two banana varieties ('William' and 'Cavendish'), and were also surrounded by other crops, especially leafy vegetables. In addition to the use of tall banana trees which are unfavorable to the vector, the aphids are important pests of vegetables grown in that region. According to Zawadneak *et al.* (2015), aphids are major pests of vegetables. However, when vegetable producers control these insects using chemical pesticides, it is likely that some sprays will drift into banana plantations in neighboring areas.

Different scenarios can be proposed for the introduction of BBTV into Mozambique. The virus may have been disseminated by aphids from countries neighboring Mozambique (Kenyon *et al*., 1997; Jooste *et al*., 2016; Shimwela *et al*., 2022). A second scenario is that vegetative banana propagation enhances virus dissemination via infected suckers. Therefore, virus incidence in the Chókwè district could be associated with some local growers acquiring virus-infected banana suckers from neighboring countries that have previously reported BBTV occurrence (EPPO, 2021).

Exchange of banana propagation material, generally common among local producers, could have caused the high BBTV incidence levels observed in Chókwè, and increased by *P. nigronervosa* transmitting the virus. This movement of infected material from one area to another may have contributed to the rise in the BBTD incidence in many places where BBTV was detected (Kumar *et al*., 2015; Djailo *et al*., 2016; Kolombia *et al*., 2021; Magee, 1927). The aphid vector is the primary source of inoculum for the dissemination over long distances, favoring spread of BBTD (Wall, 2016).

The major socio-economic factors that contribute to the dissemination of BBTD include: lack of necessary finance to buy chemical products for the control of *P. nigronervosa*; resistance from local family producers to removal of infected plant material; high workforce costs and lack of resources; time required to eradicate large numbers of plants; and the scarcity of workers to answer the seasonal plant culture requirements. These factors do not generally affect large quantity of commercial vegetable producers, as they are likely to adopt effective management strategies involving chemical control of vectors and roguing of virus-infected plants. For low-scale producers to recover productive banana yields, competent authorities are required for educating growers of the efficacy of consistent roguing and virus disease management (Omondi *et al*., 2020).

Niyongere *et al.* (2013) investigated the dissemination of BBTV from the banana crops of small farmers in Burundi, that were established using sucker from mother plants and seedlings produced in tissue cultures laboratories from different cultivars, and from areas localized at different distances from inoculum sources. They observed that at 9 months after planting, in the crops established inside areas affected with BBTD, incidence of the virus varied between 22% and 56% and was most prominent with diminishing inoculum distance. These authors observed that the BBTD incidence increased during 2007 to 2009, having varied in intensity according to banana cultivar.

Safari Murhububa *et al.* (2021), working in the Democratic Republic of the Congo, showed that banana plants infected with BBTV, independent of genotype, were attractive to wingless and winged *P. nigronervosa* that could carry a significant virus loads. Plantains, which are a triploid (AAB) *Musa paradisiaca* hybrids derived from mixing *M. acuminada* × *M. balbisiana*, have also been more attractive to aphids than dessert bananas which are strict *Musa acuminata* triploids (AAA), independent from host infection stage. Increased attraction of aphids by different varieties of bananas could be due to increased quantities of volatile organic compounds expelled by some genotypes, independent from plant growth stage. In the same study, *P. nigronervosa* preferred to settle in infected banana trees (apparently, less susceptible to BBTV) than on 'Cavendish' dessert banana (Su *et al*., 1993; Hooks *et al*., 2009). Dessert bananas are susceptible to many diseases due to their narrow genetic bases (Safari Murhububa *et al*., 2021).

Once present in banana crops, dissemination of BBTV is difficult to prevent, even with intense roguing practices, and once established, BBTD was never been eradicated from any country (Jones, 2009). However, if appropriate measures are not taken, banana cultivation is impracticable when this disease becomes established in a particular region. For improved control of BBTD, roguing must be accompanied by other practices, including monitoring of plantings and use of planting material that is free from viruses.

The present study has shown that there was wide distribution of BBTV across farms in Chókwè, highlighting the potential for BBTD to spread across all banana-producing regions in Mozambique. BBTD is circum-

scribed to the provinces of Maputo, Gaza, and Zambezia. Therefore, in addition to eliminating infected plants from the Chókwè region, development and enforcement of educational programs by extension practitioners, as well as local farmer use of indexed virus-free plants of acceptable banana varieties, are all essential for avoiding the rapid dissemination of BBTV. Data provided by this study give information on occurrence and distribution of BBTV in banana crops in Mozambique, and is background knowledge for developing BBTD management strategies actions that must be taken implemented through extension practitioners, technicians, and production activities of local banana farmers.

CONCLUSIONS

BBTV was found in 116 samples from ten farms in the Chókwè district, confirming presence this virus in more than 82% of the sampled fields. The average proportion of infection was over 54% (ranging from 20% to 100%). BBTV was absent only in the banana plantations of one farm, indicating that when BBTD control measures are carried out rigorously the disease can be controlled among other infected areas. This knowledge highlights the importance of banana plantation management for effective control of BBTV.

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