Monitoring the Distribution of Banana Bunchy Top Virus in South Africa: A Country-Wide Survey

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Abstract

Banana bunchy top disease (BBTD) is the most devastating viral disease of bananas worldwide and is caused by the banana bunchy top virus (BBTV). The disease is spread by the banana aphid *Pentalonia nigronervosa* Coquerel (Hemiptera: *Aphididae*) and through infected propagation material. In 2015, the virus was detected in an isolated area in the South Coast region of the KwaZulu-Natal Province (KZN), South Africa. The aim of this study was to conduct surveys across three banana-producing regions in South Africa, *viz.* KwaZulu-Natal, Mpumalanga and Limpopo provinces. Over 1700 plant and aphid samples were collected from commercial farms and rural households in the three provinces and more intense sampling was done in the affected KZN region. A BBTV-specific polymerase chain reaction (PCR), targeting the putative replicase gene, was performed to detect virus-infected samples. The amplicons that yielded an expected band size of 349 bp, were sequenced. Comparative phylogenetic analyses showed that the South African BBTV isolates clustered within the Pacific Indian Ocean genomic group that included isolates from India and other regions in Africa with a bootstrap value of 94% . To date, the virus has been identified only in the South Coast region of the KwaZulu-Natal province. Intense management strategies, including scouting, removal of infected plants and control of aphids, have been implemented in areas where positive samples were identified to minimize the spread of the virus. Findings from this study emphasize the need to continue monitoring and containing the spread in the KZN South Coast region.

1. Introduction

Banana is one of the most important economic crops for developing countries in tropical and sub-tropical areas [12] and is cultivated in about 120 countries [33]. Available estimates indicate that average global banana production rose from 69 million tonnes in 2000-2002 to 116 million tonnes in 2017-2019, at an approximate value of 31 billion USD. These values are an estimate as the bulk of banana production is conducted informally thus making it difficult to obtain accurate figures [11]. Domesticated bananas are thought to have originated somewhere in New Guinea, Indonesia, the Philippines or the Southeast Asia Peninsula 7 000 to 10 000 years ago [10, 26]. Banana cultivation spread to other parts of the world reaching Africa possibly 3 000 years ago. During the period between 700 and 1 500 years ago, different banana varieties were repeatedly introduced to Africa and the south-west Indian Ocean Islands [21, 30].

Banana belongs to the genus *Musa* in the family *Musaceae* of the order Zingiberales. The genus *Musa* is divided into two sections: *Musa* and *Callimusa* [28] *Musa*, formerly known as Eumusa, is the larger section of the genus. The edible *Musa* species, their hybrids and polyploids originated from the two main wild species of banana, *viz*. *Musa acuminata* Colla and *M. balbisiana* Colla [25]. Banana is divided into two main groups namely dessert and cooking bananas. ‘Gros Michel’, which belongs to the AAA genomic group, was the first exported banana cultivar to be planted over thousands of hectares. Despite its susceptibility to *Fusarium* wilt, it is still grown in many countries because of its superior flavour. Another important cultivar is ‘Cavendish’, which may have originated in South China [16]. ‘Cavendish’ cultivars

yield the most common fruit and form the backbone of the domestic industries in countries like Australia, India, China and South Africa [32].

Banana bunchy top virus (BBTV), a multi-component, circular, single-stranded DNA virus that is 18-20 nm in diameter, is the type member of the genus *Babuvirus* and the family *Nanoviridae* [3]. The viral genome comprises at least six encapsidated components (DNA-R; DNA-S; DNA-M; DNA-N; DNA-C and DNA-U3) each approximately 1100 nucleotides in length [41]. Phylogenetic relationships, largely based on the DNA-R, -N and –S segments, grouped BBTV isolates worldwide as 'South Pacific' or 'Asian' origin[20]. This categorization was later modified into two different groups, *viz*. the Pacific Indian Ocean (PIO) and the South East Asian (SEA) groups based on their geographical delineation [4].

Banana bunchy top disease (BBTD), caused by BBTV is spread in a circulative manner by *Pentalonia nigronervosa* Coquerel (Hemiptera: *Aphididae*) commonly known as the banana aphid [22]. The first symptom of the disease is the appearance of dark green streaks on the undersurface of the leaf [22]. As the disease progresses, infected leaves become progressively stunted and malformed and have an upright bearing eventually resulting in a 'bunchy' display. Yield losses of up to 100% can be experienced when plants are infected with BBTV and fail to produce bunches [35].

BBTV was reported for the first time in the Fiji Islands in 1889 [22] thereafter, it has been identified in 44 countries in Africa, Australia, Asia and the South Pacific Islands [6, 22]. In Africa, BBTD was first reported in 1901 in Egypt. Currently it has been reported in 17 African countries including Cameroon, Zambia, Mozambique, Malawi and Nigeria [1, 13, 23]. The presence of BBTV in South Africa was confirmed in 2016 from the banana production area located in the South Coast region of the KwaZulu-Natal Province [18]. The source of infection in this region is currently unknown. BBTV is a quarantine virus included in the South African Phytosanitary Services list of pathogens that must be absent in imported *Musaceae* propagation material under the Agricultural Pests Act, 1983 (Act no. 36 of 1983). BBTV has inflicted a devastating impact on the banana industry in Australia in the 20th century, but due to strict quarantine regulations and enforcement, this disease has mostly been contained [9, 15].

Banana is amongst the most important commercial subtropical fruit grown in South Africa mostly for home consumption. Subsistence farming of banana also contributes to the food source of poorer communities and income is generated through informal trade at local markets. Only a small fraction of all the bananas produced is sold on the world market [7]. Approximately 415 000 tonnes were harvested during the 2018/19 marketing season valuing the industry at approximately 137 million USD [8]. Based on the recent detection of BBTV in South Africa, the extent of the spread of BBTV in South African banana production areas was investigated in order to establish effective management strategies in the affected regions. Prior to the identification and confirmation of BBTV in South Africa in 2015, the last survey provided no evidence for the presence of this virus in the country [27].

2. Materials And Methods

2.1. Surveys

Follow up surveys and delimiting surveys were conducted during the period of March 2017 to February 2021 to determine the occurrence of BBTV in commercial farms and rural households in the main banana-producing regions in the KwaZulu-Natal, Mpumalanga and Limpopo provinces of South Africa. Farms and rural households were selected based on information provided by government extension services situated at various locations in each province. The co-ordinates were recorded for each site where sampling took place using a Global Positioning System (Garmin Etrex 20x Ltd) (Table 1).

In each commercial field, or at individual households, plants were randomly selected and observed for typical BBTV symptoms. A section of the leaf from symptomatic and asymptomatic plants was collected and stored in a cooler box during transit from the field to the laboratory. Aphids were collected from symptomatic and non-symptomatic plants using a fine-tip brush followed by storage in a 2 ml microcentrifuge tube containing 99% ethanol. All samples were placed in carefully labeled plastic bags and transferred to the laboratory for further analysis. Yellow bucket traps, set up on metal stands, were filled halfway with water and a drop of Sunlight® liquid soap to trap aphids on commercial farms overnight (Fig. 1). The traps have an opening on one side to allow for the drainage of excess water in the event that it rains while the traps are out in the field. This opening is covered with a very fine mesh cloth so that samples are not washed out with the rain (Fig. 1). The contents of the traps were sifted using muslin cloth and a sieve to drain the water without losing the trap contents. The cloth was then placed in a honey jar containing 99% ethanol. The cloth was later examined under a microscope for the presence of banana aphids.

Delimiting surveys were extensively conducted at Marburg Farm (30°45'46.6"S 30°25'00.2"E), a commercial farm 42 km from the initial outbreak site. This was done according to a protocol developed by the International Institute of Tropical Agriculture (IITA) for conducting delimiting surveys. In each field, observations for symptoms were randomly recorded on 100 plants/mat by walking across a “W” shaped path inspecting 25 plants/mats at an equal distance from each other. In blocks where symptomatic plants were observed, five plants and aphids from these plants were collected for virus testing while in blocks with asymptomatic plants; the last 25 plants were sampled [5].

2.2. Nucleic acid extraction from plant and aphid samples

Nucleic acid was extracted from aphids using a non-destructive extraction method [31]. Four to six aphids from a sample stored in 99% ethanol were dried on tissue paper and placed in a 2 ml microcentrifuge tube containing 200 µl digestion buffer (10 mM of each of NaCl, Tris, EDTA) and Proteinase K (ThermoFisher Scientific, USA). Samples were incubated overnight in a shaking incubator at 55°C and transferred to a water bath at 72°C for 10 minutes (min). An aliquot of 180 µl of the lysate was transferred to a clean tube and the aphid specimens were stored. A one-tenth volume of sodium acetate (pH 5) was placed into each tube and samples were incubated for 30 min at -20°C. Samples were centrifuged at 13 500 rpm for 20 min and 160 µl of supernatant was transferred to a new tube. A volume of 224 µl of 98% (v/v) ethanol was added to the samples and they were incubated at -20°C for 3 hours

(h). The precipitated nucleic acid was collected by centrifugation at 13 500 rpm for 20 min and the supernatant was discarded. The pellet was washed twice with 70% (v/v) ethanol, once with 98% (v/v) ethanol, dried for 1 h and re-suspended in 20 µl of distilled water.

The modified cetyl trimethyl ammonium bromide (CTAB) protocol was used for nucleic acid extraction from symptomatic and asymptomatic banana tissue [39]. Thirty grams of banana leaves were cut into pieces and crushed using a mortar and pestle in 2 ml of CTAB Buffer (pH 8). The homogenate was kept on a shaker at 60°C for 30 min and then centrifuged at 3 000 rpm for 5 min. A volume of 900 µl of the supernatant was transferred to a new tube and an equal volume of chloroform:iso-amyl alcohol 24:1 (v/v) was added to give a 1:1 ratio. The tubes were centrifuged at 13 500 rpm for 12 min. Cold isopropanol was added to the aqueous phase of the supernatant in a new tube which was then kept overnight at 10°C for nucleic acid precipitation. Precipitated nucleic acid was collected by centrifugation at 13 500 rpm for 22 min and the supernatant was discarded without disturbing the pellet. The pellet was washed three times with 70% (v/v) ethanol, dried for 1 h and re-suspended in 100 µl of distilled water. The quality and quantity of each extraction was analyzed using a Nanodrop™ 1000 spectrophotometer (ThermoFisher Scientific, USA).

2.3. PCR detection of BBTV

Banana bunchy top virus detection was performed by polymerase chain reaction (PCR) using the primer pair, BBTV1: 5'-CTCGTCATGTGCAAGGTTATGTCG-3 and BBTV2: 5'-GAAGTTCTCCAGCTATTCATCGCC-3', designed to amplify a 349 bp product corresponding to a portion of the BBTV replicase gene [39]. PCR was performed in a 25 µl reaction containing 1x reaction buffer, 20 mM of each primer, 5 U MyTaq DNA Polymerase (Bioline, USA) and 1 µl of sample nucleic acid. The temperature profile was as follows: initial denaturation at 95°C for 1 min, 40 amplification cycles of denaturation at 95°C for 15 seconds (s), annealing at 60°C for 10 s, extension at 72°C for 10 s and a final extension for 5 min at 72°C. All PCR reactions were carried out using a Proflex PCR cycler (Applied Biosystems, USA). For electrophoretic analysis, 10 µl of the PCR product was run on a 1.5% (w/v) agarose gel in Tris-acetate EDTA (TAE) buffer, pre-stained with ethidium bromide. The amplified DNA bands were visualized using a UV transilluminator (Quantum CX 5, Vilber Lourmat, France).

2.4. Sequencing and Phylogenetic studies

Fourteen PCR-positive representative isolates from the KZN South Coast region were sequenced at Inqaba Biotechnical Industries (Pretoria, South Africa). The nucleotide sequences were aligned using MAFFT and BioEdit [14, 19] software. For genetic analyses, nucleotide sequences from DNA-R components were aligned with closely related replicase gene BBTV sequences downloaded from the National Center for Biotechnology Information (NCBI) database including two South African isolates (Genbank accession numbers KY770984 and KY770985) from a previous study [31]. Construction of the phylogenetic tree was performed using MEGA 11 [38] and Abaca bunchy top virus (ABTV, genus *Babuvirus*, Genbank accession No. EF546813) was used as an outgroup. The bestfit model was determined using MEGA 11. The evolutionary history was inferred by using the Maximum Likelihood

method based on the Tamura 3-parameter model with a Gamma distribution [37]. The bootstrap consensus tree was inferred from 1 000 replicates. Branches corresponding to partitions reproduced in less than 70% bootstrap values were collapsed.

3. Results

3.1. Surveys

Surveys were initially carried out in the KZN South Coast region where the BBTV outbreak was confirmed, including a vast region comprising of rural households that cultivate banana for home consumption and subsistence farming. The surveys were then extended to the North Coast of KwaZulu-Natal as well as the Mpumalanga and Limpopo provinces of South Africa covering over 5 000 ha in total (Table 1 and Online Resource 1). A total of 1 704 plant and aphid samples were collected in the three provinces (Fig. 2). BBTV symptom expression was only observed in samples collected from the South Coast region in the KwaZulu-Natal province (Fig. 3). The level of infection within a field ranged from just a few diseased plants to complete infection of all plants. Symptom severity ranged from mild to severe in the field and was determined by visual inspection of plant parts. Mild symptoms included the dot-dash symptoms visualized on banana leaves and the streaky symptom seen on the pseudostem (Fig. 4a). Subsequently, the leaves become yellow and curl with a leathery feel (Fig. 4b and 4c). The most distinctive symptom detected in severely affected plants is the upright growth of an infected plant with severe stunting on smaller plants (Fig. 4d).

3.2. Nucleic acid extraction and PCR detection of BBTV

Regardless of symptom expression, all samples collected during the field surveys were screened with the PCR protocol and positive samples yielded amplicons of ~349 bp in size, which corresponded to a partial DNA-R sequence of the replicase gene. There was no amplification found in non-symptomatic plant samples with the BBTV-specific primers. From 379 plants collected in the KZN South Coast region (Table 1), 76 (20%) tested positive for BBTV. A total of 236 aphids were collected and 50 (21%) of these tested positive for BBTV. Rural households accounted for a larger portion of positive samples while BBTV infections were confirmed at only two commercial farms in the KZN South Coast region. Upon detection of the virus at the farm (30°45’46.6” S, 30°25’00.2” E), approximately 42 km from the initial outbreak site, there was a need to conduct delimiting surveys at the said farm. It was interesting to note that infections on this farm were detected in areas with higher altitude. From the delimiting surveys, it was found that infections had spread to more sections of the farm.

3.3. Sequencing and Phylogenetic studies

Fourteen randomly selected PCR positive amplicons were submitted to Inqaba Biotech (Pretoria, South Africa) for Sanger sequencing and the sequences were deposited into the NCBI Genbank. The accession numbers are MT023045, MT023046, MT023047, MT023048, MT023049, MT023050, MT023051, MT023052, MT023053, MT023054, MT023055, MT023056, MT023057 and MT023058. The

phylogenetic results showed that all BBTV isolates from the South Coast region in KZN grouped within the Pacific Indian Ocean (PIO) group along with isolates from India, Pakistan, Fiji and Australia with 94% bootstrap value amongst the isolates (Fig. 5). The tree topology and branch lengths indicate that the South African isolates are closely related to the other isolates within the PIO group.

Table 1

Number of samples collected from various banana-growing regions in South Africa[a]

Location	GPS-coordinates	Number of samples		BBTV positives	
		Aphids	Plants	Aphids	Plants
South Coast (KwaZulu-Natal)	-30.500933, 30.467350	236	379	50 (21%)	76 (20%)
North Coast (KwaZulu-Natal)	-29.639439, 31.067040	94	140	0	0
Mpumalanga	-25.452267, 31.968867	271	304	0	0
Limpopo	-23.811533, 30.181833	140	140	0	0

[a]Complete breakdown of all samples collected available in Online Resource 1

4. Discussion

Banana bunchy top virus poses a major threat to banana cultivation in South Africa, especially in the KZN region currently affected. In this study, plant and aphid samples were collected in the main banana producing provinces of South Africa for detection of BBTV (Table 1 and Fig. 2). BBTV-1 and BBTV-2 primers designed from the DNA-R gene were able to detect the virus in the PCR analysis on positive plant and aphid samples. Multiple studies showed the reliability of using PCR as a detection tool for BBTV (Xie and Hu 1995; Su et al 2003; Selvarajan et al 2010)[34, 36, 42]. Infected aphids were detected on the majority of plants displaying clear BBTV symptoms and 22% of aphids collected from these plants tested negative for the virus. A plausible explanation is that the aphids may have just landed on the plants that tested positive for BBTV and had not acquired the virus at the time of sample collection. A study was conducted to determine the minimum amount of time required for aphids to acquire the virus once gaining access to an infected plant [2]. The study confirmed that it took 6 hours for aphids to acquire the virus [2]. In a previous survey study from the KZN South Coast region, approximately 78% of the aphids collected from symptomatic plants in infected plantations tested positive for BBTV [31]. *Pentalonia nigronervosa* Coquerel (*Hemiptera*: *Aphididae*), the known vector that transmits BBTV was found in all surveyed banana fields across the country [22, 29]. In all surveyed fields, the occurrence of aphids was higher where no vector control was implemented. Interrupting the virus transmission chain is effective as the virus is transmitted by aphids and through infected plant material. Poor maintenance of the banana crop and its dense canopy might also help in increasing the aphid vector population [43]. The dense

canopy partially prevents rainfall from reaching the leaves and pseudostem and thereby favouring aphid multiplication [43]. The importance to scout for aphids in banana plantations for early BBTV detection was demonstrated.

Different scenarios can be proposed for the introduction of BBTV into South Africa. Firstly, the virus may have spread by aphids from Mozambique into South Africa with which it shares a border. Although this may seem like the most plausible explanation, one key factor may argue this possibility. All samples collected from areas directly adjacent to the South African/Mozambican border tested negative for BBTV. Secondly, the virus may have been introduced unknowingly into the KZN South Coast region in South Africa through infected planting material and this resulted in its widespread dissemination. Since cultivated bananas are propagated vegetatively, the potential for human-meditated spread is high. Symptom expression can take between 25-85 days in a BBTV-infected plant so when an infected, but asymptomatic banana propagule, is introduced to a region where *P.nigronervosa* is present, an outbreak can occur if transmission between virus and vector takes place [35]. Spread of BBTV from the first reported KZN outbreak site in a southerly direction to a farm approximately 42km away led to many speculations with the most probable being that aphids were carried with wind currents to this farm and may have transmitted the virus.

Phylogenetic analysis of the DNA-R partial sequence was carried out to determine the relationship of the South African isolates with the other BBTV isolates detected worldwide (Fig. 5). The use of the replicase gene and the coat protein gene sequences to construct phylogenies has been the prime approach to explain the evolutionary history of BBTV and other *Nanoviruses* [41]. Phylogenetic analysis showed two broad clades/groups of BBTV, namely, the South East Asian and Pacific Indian Ocean groups, with high bootstrap support values of 96% and 94%, respectively. The KwaZulu-Natal South Coast isolates from this study, clustered under the Pacific Indian Ocean group along with isolates from other African countries, Australia, Hawaii, Fiji, Pakistan and India. The branch lengths indicated minimal genetic difference between the South African BBTV isolates from this study, the two South African reference isolates with (Genbank accession numbers KY770984 and KY770985) and the other isolates within this group. Even though the tree topology does not show the probable origin of the South Africa isolates, it does however confirm its grouping within the Pacific Indian Ocean group. The monophyletic origin of the isolates within the Pacific Indian Ocean group is also confirmed by a 94% bootstrap value. Two Malawian isolates (Genbank accession number: JF55994 and JF55993) grouped within a separate clade, away from Malawian isolate with Genbank accession number JF755995.

Socio-economic factors such as lack of funds needed to purchase chemicals for pest control and resistance to proper/adequate removal of infected plant material in the rural community is a contributing factor responsible for the spread of the aphid vector. Commercial farmers in the region follow repeated management strategies such as stringent scouting, use of chemicals as part of aphid control for orchard management, and therefore are accustomed to chemical control methods while this is not the case for rural households. In this region, the use of chemicals for aphid control is not a sustainable option due to economic constraints. A study showing the effectiveness of consistent rouging in managing the disease

concluded that it is possible for smallholder farms to recover banana productivity if such a practice is carried out accurately [24].

In the affected region, some field-workers have lost their jobs in the mentioned commercial farms as infected plants in various plots have been uprooted. Some households that were visited mentioned that they had incurred loss of income generated from the sale of bananas (personal communication). This has a negative impact on food security and sustainability of banana production for the region. To reduce the impact of BBTV in the region, awareness campaigns, in conjunction with the Department of Agriculture, Land Reform and Rural Development (DALRRD) and Department of Agriculture and Rural Development (DARD) have been launched in the KZN South Coast region and efforts are ongoing to contain the spread of BBTV. Informative pamphlets have been handed out in the local language to educate growers about BBTV as well as identification and control strategies. Several stakeholder meetings were organized in each ward of the affected region and practical excursions to smallholder plantations were held. This has resulted in a more positive engagement from the rural community as they are able to identify infected plants and inform their local extension officers. The officers in turn notify the Agricultural Research Council (ARC) and send the samples for testing. ARC is collaborating with DALRRD on action plans, awareness campaigns and advice on how to prevent its spread. Although efforts are now involving more stakeholders, more financial input is needed to prevent the further spread of BBTV in plantations the rural communities.

Conclusion

Once established, BBTV has never been completely eradicated in any country, it is however possible to manage it [17]. The spread of BBTV in the KZN South Coast region and monitoring thereof in the rest of the banana producing regions in South Africa was discussed here. Banana plants and aphids collected from other regions in the country tested negative for BBTV therefore the importance of task teams that will perform continuous scouting to monitor any outbreaks cannot be highlighted enough, especially in the regions neighboring Mozambique, which have positive BBTV sites. Integrated control strategies are the key to contain the spread of BBTV in a region. Awareness needs to be raised amongst stakeholders at all levels (policymakers, extension services, commercial and small-holder farmers) by promoting regular scouting for symptoms and aphids as well as removal of infected mats to reduce inoculum pressure. Applying strict quarantine measures to avoid movement of propagation material and the use of certified tissue culture material of the disease is another recommendation.

Clustered regularly interspaced short palindromic repeats commonly known as CRISPR is a developed genome editing tool applied in crop improvement for desired traits such as disease resistance [40]. CRISPR can be applied in banana to develop resistance to BBTV by targeting genes responsible for susceptibility in the BBTV genome or the host plant [40]. To date, this tool is applied to control Banana streak virus, another economically important virus infecting banana. This approach can be a major breakthrough as a control strategy against the spread of BBTV. The effectiveness of consistent rouging in managing the disease is worthwhile to investigate [24]. They found that it is possible for smallholder

farms to recover banana productivity if such a practice is carried out accurately. Such an approach can be explored in the KZN South Coast region as it is a practical control strategy that needs little to no financial implementation costs. Our findings therefore strengthen the need to continue monitoring and containing the spread in the KZN South Coast region in order to prevent any further spread beyond this region.

Declarations

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References

1. Adegbola, R. O., Ayodeji, O., Awosusi, O. O., Atiri, G. I., Kumar, P. L. (2013). First Report of banana bunchy top disease caused by Banana bunchy top virus in Banana and Plantain (Musa spp.) in Nigeria. *Plant Disease*, **97**: 290
2. Anhalt, M.D., Almeida, R.P.P. (2008). Effect of Temperature, Vector Life Stage, and Plant Access Period on Transmission of Banana bunchy top virus to Banana. *Virology*, **98**: 743-748 http://dx.doi.org/10.1094/PHYTO-98-6-0743
3. Amin, I., Qazi, J., Mansoor, S., Ilyas, M., Briddon R.B. (2008). Molecular characterisation of Banana bunchy top virus (BBTV) from Pakistan. *Virus Genes* **36** : 191-198 http://dx.doi.org/10.1007/s11262-007-0168-y

4. Baldodiya, G.M., Baruah, G., Borah, B.K., Modi, M.K., Nath, P.D. (2019). Molecular characterization and sequence analyses of Banana bunchy top virus infecting banana cultivar Jahaji (Dwarf Cavendish) in Assam, India. *3 Biotech*, **9**: pp.1-10. http://dx.doi.org/10.1007/s13205-019-1636-5
5. BBTV Survey Protocol. (2018). Protocols of the BBTD containment and recovery in Africa: Building capacity and piloting field recovery approaches through a learning alliance, IITA, Ibadan, Nigeria. 21pp.
6. CABI (2020). Banana bunchy top virus (bunchy top of banana). https://www.cabi.org/isc/datasheet/8161#tosummaryOfInvasiveness Accessed 4 June 2021 11h48am
7. DAFF. (2018). Abstract of Agricultural Statistics 2018. Published by the Department of Agriculture: Agriculture, Forestries and Fisheries.
8. DALRRD. (2020). Abstract of Agricultural Statistics. Directorate: Statistics and Economic Analysis, Pretoria.
9. Dale, J.L. (1987). Banana bunchy top: an economically important tropical plant disease. *Advances in Virus Research* **33**: 310–325 http://dx.doi.org/10.1016/S0065-3527(08)60321-8
10. Denham, T., Haberle, S., Lentfer, C.J. (2004). New evidence and revised interpretations of early agriculture in Highland New Guinea. *Antiquity* **78**: 839-857 http://dx.doi.org/10.1017/S0003598X00113481
11. FAO. (2019) Food Outlook - Biannual Report on Global Food Markets – November 2019. Rome. http://www.fao.org/3/ca6911en/CA6911EN.pdf Accessed 22 June 2021 at 13h06
12. Ghag, S.B. Ganapathi, T.R. (2018). Banana and Plantains: Improvement, Nutrition, and Health. In: Mérillon JM, Ramawat K (eds) Bioactive Molecules in Food. Reference Series in Phytochemistry. Springer, Cham, pp 1–20
13. Gondwe, W.T., Mwenebanda, B.M.L., Natha, E., Mutale, P. (2007). Banana bunchy top disease in Mozambique and Zambia. InfoMusa **16**: 38-39.
14. Hall, T.A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**:95-98
15. Hort Innovation. (2018). Banana Bunchy Top Virus Control Data. Horticulture Innovation Australia. Sydney, New South Wales.
16. Jones, D.R. (2000). Introduction to banana, abaca and enset. In *Diseases of Banana, Abaca and Enset*, pp. 1-27. Ed D.R. Jones. Wallingford, UK: CABI Publishing.
17. Jones, D.R. (2009). Disease and pest constraints to banana production. *Acta Horticulturae* ISHS **828**: 21-36 http://dx.doi.org/10.17660/ActaHortic.2009.828.1
18. Jooste, A.E.C., Wessels, N., van der Merwe M. (2016). First Report of Banana bunchy top virus in Banana (*Musa* spp.) from South Africa. *Plant Disease* **100**: 1251 http://dx.doi.org/10.1094/PDIS-12-15-1422-PDN

19. Katoh, K., Misawa, K., Kuma, K. Miyata, T. (2002). MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* **30**: 3059-3066 http://dx.doi.org/10.1093/nar/gkf436
20. Kumar, P.L., Hanna, R., Alabi, O.J., Soko, M.M., Oben, T.T., Vangu, G.H.P., Naidu, R.A. (2011). Banana bunchy top virus in sub-Saharan Africa: Investigations on virus distribution and diversity. *Virus Research* **159**: 171-182 http://dx.doi.org/10.1016/j.virusres.2011.04.021
21. Lejju, B. J., Robertshaw, P., Taylor, D. (2006). 'Africa's Earliest Bananas?' *Journal of Archaeological Science* **33**: 102–113.
22. Magee, C.J.P. (1927). Investigation on the bunchy top disease of the banana. Council for Scientific and Industrial Research Melbourne page 86
23. Niyongere, C., Losenge, T., Ateka, E.M., 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**:102-107
24. Omondi, B.A., Soko, M.M., Nduwimana, I. (2020). The effectiveness of consistent roguing in managing banana bunchy top disease in smallholder production in Africa. *Plant Pathology*, **69:**1754-1766 http://dx.doi.org/10.1111/ppa.13253
25. Opara, U.L., Jacobson, D., Al-Saady, N.A. (2010). Analysis of genetic diversity in banana cultivars (Musa cvs.) from the south of Oman using AFLP markers and classification by phylogenetic, hierarchical clustering and principal component analyses. *Journal of Zhejiang University Science B* **11**:332–41. http://dx.doi.org/10.1631/jzus.B0900310
26. Perrier, X. (2011). Multidisciplinary Perspectives on Banana (*Musa* spp.) Domestication', Proceedings of the National Academy of Sciences of the United States of America *108*, 11311–11318. http://dx.doi.org/10.1073/pnas.1102001108
27. Pietersen, G., Staples, M.S., Sward, G., Korsten, L. (1996). Non-confirmation of banana bunchy top virus in South Africa. *African Plant Protection* **2**:1-8
28. ProMusa (2020). *Musa sections*. Page last modified on Wednesday, 22 July 2020 16:39:12 SAST from https://www.promusa.org/Musa+sections Accessed 9 August 2021 at 15h02
29. Qazi, J. (2016) Banana bunchy top virus and the bunchy top disease. *Journal of General Plant Pathology*, **82**: 2–11 http://dx.doi.org/10.1007/s10327-015-0642-7
30. Randrianja, S., Ellis, S. (2009). Madagascar: A Short History. Chicago, IL: University of Chicago Press.
31. Robbertse, N., Omondi, B.A., Millar, I.M., Kruger, K., Jooste, A.E.C. (2019). Non-destructive DNA extraction from aphids: the application in virus - vector studies of Banana Bunchy Top Virus (BBTV). *European Journal of Plant Pathology* **153**: 571-582. http://dx.doi.org/10.1007/s10658-018-1552-2
32. Robinson, J.C. (1996). Bananas and Plantains. Crop Production Science in Horticulture 5.CAB International, Wallingford, UK page 238
33. Rustagi, A., Jain, S., Kumar, D., Shekhar, S., Jain, M., Bhat, V., Sarin, N.B. (2015). High efficiency transformation of banana [*Musa acuminata* L. cv. Matti (AA)] for enhanced tolerance to salt and

drought stress through overexpression of a peanut Salinity-Induced Pathogenesis-Related Class 10 protein. *Molecular Biotechnology* **57**: 27–35 http://dx.doi.org/10.1007/s12033-014-9798-1

34. Selvarajan, R., Sheeba, M.M., Balasubramanian, V., Rajmohan, R., Dhevi, N.L., Sasireka, T. (2010). Molecular characterization of geographically different banana bunchy top virus isolates in India. *Indian Journal of Virology* **2**: 110-116 http://dx.doi.org/10.1007/s13337-010-0020-1
35. Stainton, D., Martin, D.P., Muhire, B.M. (2015). The global distribution of banana bunchy top virus reveals little evidence for frequent recent, human-mediated long distance dispersal events. *Virus Evolution* **1**: 1-15
36. Su, H.J., Tsao, L.Y., Wu, M.L., Hung, T.H. (2003). Biological and Molecular categorization of strains of banana bunchy top virus. *Journal of Phytopathology* **151**: 290-296 http://dx.doi.org/10.1046/j.1439-0434.2003.00721.x
37. Tamura K. (**1992**). Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G + C-content biases. *Molecular Biology and Evolution* **9**:678-687.
38. Tamura, K., Stecher, G., and Kumar, S. (2021). MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution*, 38: 3022-3027
39. Thomas, D., Dietzgen R.G. (1995). Detection of DNA and RNA plant viruses by PCR and RT-PCR using a rapid release protocol without tissue homogenization. *Journal of Virological Methods* **54**: 85-95
40. Tripathi, I., Ntul, V.O., Tripathi, J.N., Kumar, P.L (2021). Application of CRISPR/Cas for diagnosis and management of viral diseases of Banana. *Frontiers of Microbiology* **11**: 609784
41. Wickramaarachchi, W. A., Shankarappa, K. S., Rangaswamy, K. T., Maruthi, M. N., Rajapakse, R. G., & Ghosh, S. (2016). Molecular characterization of banana bunchy top virus isolate from Sri Lanka and its genetic relationship with other isolates. *Virus Disease*, **27**: 154–160 http://dx.doi.org/10.1007/s13337-016-0311-2
42. Xie, W.S., Hu, J.S. (1995). Molecular cloning, sequence analysis and detection of Banana bunchy top virus in Hawaii. *Phytopathology* **85**: 339-347 http://dx.doi.org/10.1094/Phyto-85-339
43. Young, C.L., Wright, M.G. (2005). Seasonal and spatial distribution of banana aphid, *Pentalonia nigronervosa* (Hemiptera: Aphididae) in banana plantations in Oahu. *Proceedings of the Hawaiian Entomological Society* **37**: 73-80.

Figures

Fig. 1

Figure 1

Yellow bucket trap used for the trapping of P. nigronervosa aphids

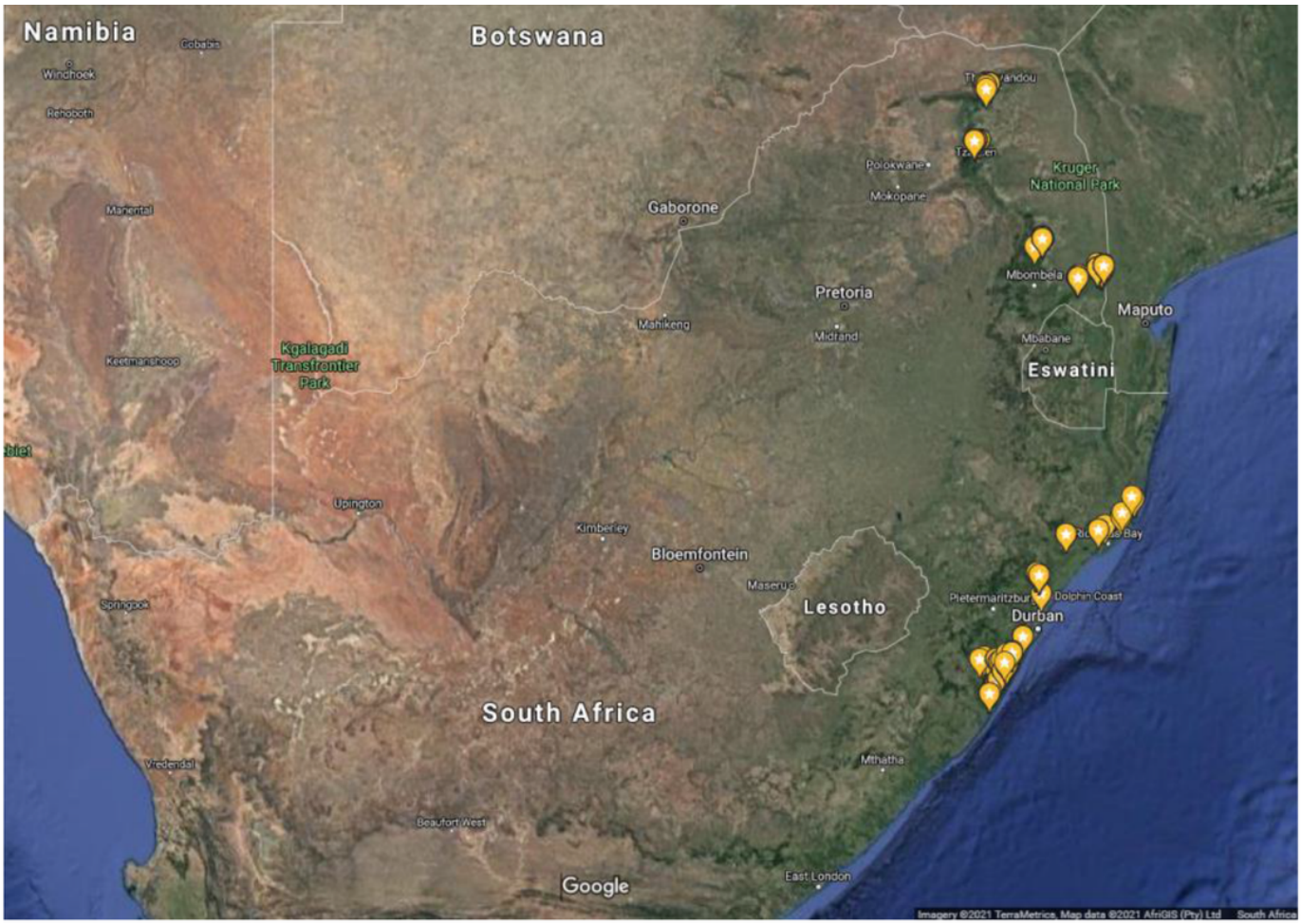

Fig. 2

Figure 2

Map of surveyed locations indicated by yellow tear-drop points. Samples were collected from KwaZulu-Natal, Mpumalanga and Limpopo province

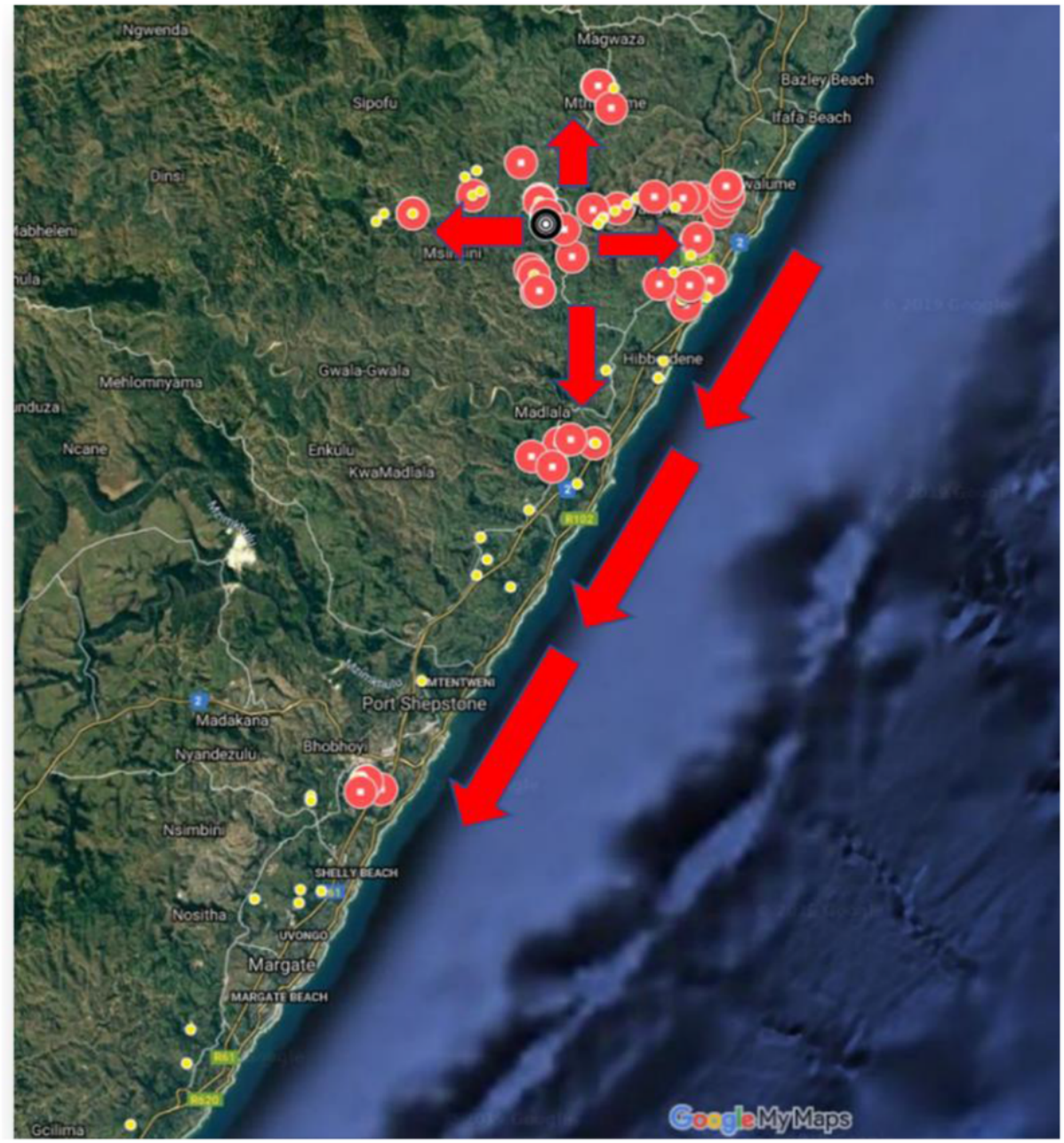

Fig. 3

Figure 3

A close-up view of the South Coast region in KwaZulu-Natal where BBTV was discovered. The black circle represents the initial outbreak site and the arrows demonstrate how the virus has spread across the different areas of the region from the initial detection site. The yellow dots represent the sites sampled from

Fig. 4

Figure 4

Various symptoms visible on BBTV-infected plants. A: Arrow indicating dark green lines (streaky/dot dash symptoms) on the stem. B-C: Yellowing and curling of leaf margins on infected plants. D: A banana plant exhibiting the most characteristic BBTV symptom, a bunchy appearance

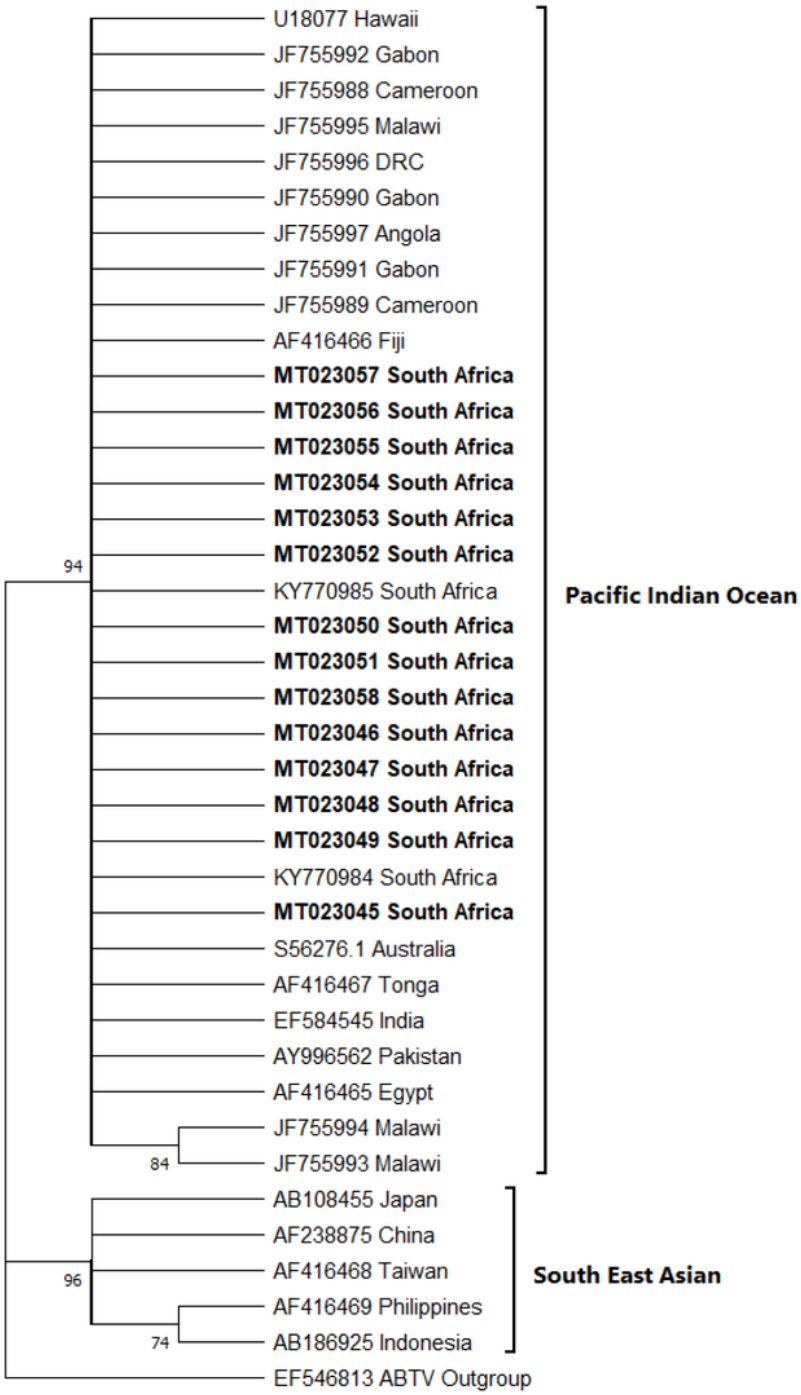

Fig. 5

Figure 5

Phylogenetic tree showing the genetic relationships between sixteen randomly selected BBTV isolates from the South Coast region of KwaZulu-Natal, South Africa, other isolates of BBTV and an outgroup [Abaca bunchy top virus (ABTV)]. The fourteen random samples from this current study are marked in bold

Supplementary Files

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