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# Quantitation of Multipartite Banana Bunchy Top Virus Genomic Components and Their Transcripts in Infected Tissues of Banana (*Musa acuminata*)

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**Abstract:** Banana bunchy top disease (BBTD), caused by a Banana Bunchy Top Virus (BBTV), is the most devastating viral disease of bananas. The genome of multipartite BBTV is about 1 Kb in size and comprises six circular single-stranded (ss) DNA components, namely DNA-R, C, S, M, U3, and N. Gene expression in multipartite DNA viruses is highly flexible due to variations in the copy number and transcript levels of individual genomic components. This enables them to adapt to changing conditions and maintain optimal fitness. In the current study, the copy number of each genetic component of BBTV and its transcript level were determined in the leaf, midrib, and root tissue of the infected banana plant. The results of the study showed that DNA copies (copies/ng) were higher in midrib (11,105 ± 10,704.42), followed by leaf (6902.91 ± 1651.14), and were lowest in root (23.49 ± 9.21) tissues. In contrast, the transcript level was highest in the root (312.45 ± 106.69) and lowest in the midrib (0.72 ± 0.16). This suggests that BBTV independently regulates the transcriptional level of each DNA component. The distribution of BBTV in different tissues assists the identification of significantly higher viral load tissues for early and sensitive disease diagnosis, which will be beneficial for better disease management.

**Keywords:** multipartite DNA virus; Banana Bunchy Top Virus; qPCR assay; *Babuvirus*; *Nanoviridae*



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## 1. Introduction

Banana bunchy top disease (BBTD) is the most prevalent and destructive viral disease of banana in the Pacific and Asian regions. It is an economically important viral plant disease worldwide [1,2]. BBTD develops from Banana Bunchy top Virus (BBTV), which is a member of the genus *Babuvirus* in *Nanoviridae* family [3]. The aphid *Pentalonia nigronervosa* Coq is the most efficient vector for the persistent transmission of BBTV and infects only *Musa* members [4]. Banana Bunchy Top Virus (BBTV) is a multipartite circular single-stranded (CSS) DNA virus with six DNA components that are each about 1 Kb in size [5].

The complete BBTV genome consists of six ssDNA components, including DNA-R, S, C, N, and M, which encodes the master replication initiation protein (M-Rep), capsid protein, (CP) cell–cycle link protein (Clink), nuclear–shuttle protein (NSP), and movement protein (MP), respectively, while DNA-U3 encodes a protein for which no function has been found [6–9]. Each component may encode one large gene and contain an intergenic region with two homology regions, stem–loop common region (CR-SL) and the major common region (CR-M).

BBTV and other related multipartite viruses enclose their genomic components into distinct virions to preserve the integrity of the viral genome [10–12]. However, to date, there is no recognized mechanism involved in this biological process. The differential frequency of nano-viral components has been shown to altered the multipartite virus accumulation and symptom development in faba bean necrotic stunt virus (FBNSV), a member of the *Nanovirus* genus in *Nanoviridae* family. These frequencies were also host-specific [13], which suggests that the copy number regulation of individual components in multipartite viruses gives them additional flexibility of adaptation in different hosts. This differential transcriptional regulation of multipartite viruses in gene expression would enable them to maintain their optimal fitness in various in various cellular conditions [12,13].

For early disease detection, developing a sensitive and validated diagnostic method is imperative to manage BBTV. Therefore, knowledge of BBTV distribution in different tissues of banana will assist in choosing tissues with the highest viral loads. Many factors influence virus distribution within a plant, including viral strain, tissue type, environmental conditions, and host–virus interaction [14]. A virus enters its host through mechanical injury to epidermal cells or leaf trichomes, physical penetration of cell wall and by insect vectors. Viruses can move inside the plant via plasmodesmata (communication channels) [15,16]. Within an infected banana plant, information on the distribution and movement of BBTV is limited. Therefore, to better understand coordinated replication and transcription, this study includes a detailed analysis to quantify individual BBTV DNA components and their corresponding transcript levels in leaf, midrib, and root tissues via qPCR and RT-qPCR.

## 2. Materials and Methods

### *2.1. Plant Material Acquisition*

Both BBTV-infected (P.TJ4 isolate described earlier by Bashir et al., 2022) and healthy plants with 3 replicates for each tissue were collected in June 2018 from the Tandojam area in the Sindh province and the experiment was repeated thrice. Plants were acclimatized in the growth chamber on an 11:13 h day/night life cycle at a temperature of 26 °C and relative humidity of 42%.

### *2.2. Primer Designing*

For the quantification of transcripts, BBTV and banana genome coding sequences were obtained from NCBI (National Center for Biotechnology Information), and primers were designed for all six BBTV genomic components (R, S, M, U3, C, and N) and banana (RPS2) genes using the IDT's PrimerQuest which incorporates Primer3 software (version 2.2.3) (http://www.idtdna.com/pages/scitools) accessed on 23 February 2016 (Table 1). For the quantification of genomic components, primers were designed based on the sequences from the Australian isolate that belongs to the Pacific Indian Ocean (PIO) group and have close phylogenetic relationships with TJ4 isolate (Table 1).

**Table 1.** Details of primers used to sequence BBTV genomic components from Pakistan.

| BBTV Genomic Component | Name of Primer | Sequence 5′-3′ Direction | Product Size (bp) |
|---|---|---|---|
| *Primers for qPCR and RT-qPCR* | | | |
| **DNA-R** | DNA-R F<br>DNA-R R | GGAAGATACAAGAATCGAAGG<br>GGAAGGAAGTTAGCCATTAC | 567 |
| **DNA-U3** | DNA-U3 F<br>DNA-U3 R | CCGGTCAACATTATTCTGG<br>CAGAAGAGAGAGAGCAATTATC | 140 |
| **DNA-S** | DNA-S F<br>DNA-S R | GACAACAAATGGCTAGGTATC<br>CCGTTACATTCTTCCTCAAC | 471 |
| **DNA-M** | DNA-M F<br>DNA-M R | ATGGCATTAACAACAGAGC<br>TTCCTTGCTCATCCCTTC | 325 |

**Table 1.** *Cont.*

| BBTV Genomic Component | Name of Primer | Sequence 5′-3′ Direction | Product Size (bp) |
|---|---|---|---|
| **DNA-C** | DNA-C F<br>DNA-C R | CGATGTGAAGAGAGAGATTAAG<br>GACAATCACTCCAAGAGAAC | 413 |
| **DNA-N** | DNA-N F<br>DNA-N R | GGATGCGATTGGAAGAAG<br>CATTACAGTTGTTGCTGTTAC | 373 |
| **RPS2** | RPS2-F<br>RPS2- R | AATTGGTCTACTACGGAAAC<br>CATCTCCACGGTACATAAG | 299 |
| *Primers for confirmation and sequencing* | | | |
| **DNA-R** | DNA-R F<br>DNA-R R | ATGGCGCGATATGTGGTATG<br>TCTGTCGTCGATGATGATCTTG | 1111 |
| **DNA-U3** | DNA-U3 F<br>DNA-U3 R | TTGTGCTGAGGCGGAAGAT<br>CCACCTTCACAGAAGAGAG | 1062 |
| **DNA-S** | DNA-S F<br>DNA-S R | GTATCCGAAGAAATCCATC<br>CTAGCCATTTGTTGTCTG | 1075 |
| **DNA-M** | DNA-M F<br>DNA-M R | ATGGCATTAACAACAGAGCG<br>TTAGCAGGGTCCTATTTATAGG | 1046 |
| **DNA-C** | DNA-C F<br>DNA-C R | GAATCGTCTGCTATGCCTG<br>CCAGAACTCCATTTCTCTTC | 1018 |
| **DNA-N** | DNA-N F<br>DNA-N R | GATGGATTGGGCGGAATCA<br>GCTTCTGCTTTGCTTTCGC | 1088 |

### *2.3. DNA Extraction and PCR Amplification*

Total genomic DNA was extracted from a BBTV-infected leaf, midrib, and root of a banana (*Musa acuminata* cv. Dwarf Cavendish) using a CTAB method [17]. The PCR amplification was carried out using an Eppendorf® Mastercycler using Taq DNA Polymerase (recombinant) (Fermentas UAB, Vilnius, Lithuania). In a typical reaction, there was about 50 ng DNA template, 1xTaq buffer, 1.5 mM $MgCl_2$, 200 µM of each dNTPs, 5 units of Taq DNA polymerase (Thermo Fisher Scientific, Waltham, MA, USA) and 25 pmol (picomoles) of each primer. The thermal profile consisted of pre-PCR denaturation (96 °C for 3 min) followed by 35 cycles of denaturing at 96 °C for 20 s, annealing at 55 °C for 20 s, extension at 72 °C for 40 s, and a final extension at 72 °C for 20 min. After PCR, the products were visualized by electrophoresis using 1% (*w*/*v*) agarose gel in TAE buffer (40 mM Tris-acetate, 1 mM EDTA pH 8.1). Amplified full-length DNA components were directly sequenced via commercial DNA Sequencing Facility of Macrogen (Macrogen, Inc., Seoul, South Korea). The consensus sequence of each component sequenced from TJ4 isolate was submitted to GenBank. The accession numbers were MK140626 for DNA-R, MK140629 for DNA-U3, MK140620 for DNA-S, MK140617 for DNA-M, MK140614 for DNA-C, and MK140623 for DNA-N, respectively.

### *2.4. Standard Curve Establishment*

Already cloned DNA-R [18] was used to establish a standard curve. The recombinant plasmid was identified with PCR which was then validated by sequencing. Recombinant plasmid concentration was determined using a NanoDrop spectrophotometer (Colibri Microvolume Spectrometer, Pforzheim, Germany). The copy number of the BBTV component plasmid was calculated by the following formula: Copy number/µL = [Concentration of plasmid (ng/µL) × 1 µL] × $10^{-9}$/[(Length $_{plasmid}$ + Length $_{insert}$) × 660] × 6.02 × $10^{23}$. An initial template was diluted to $10^1$, $10^2$, $10^3$, $10^4$, $10^5$, $10^6$, and $10^7$ with PCR water to establish the standard curves.

### *2.5. Absolute Quantitative Analysis of BBTV Components*

By using qPCR (Applied Biosystems-StepOnePlus™, Carlsbad, CA, USA), the copy number and transcript of each component of BBTV in ng and mg were determined in triplicate in leaf, midrib, and root tissues.

### *2.6. RNA Extraction and First Strand cDNA Synthesis*

Total RNA from leaf, midrib, and root tissue of BBTV infected plants was isolated using GeneJET Plant RNA Purification Mini Kit (Thermo Scientific, Waltham, MA, USA). Extracted RNA was quantified via a NanoDrop spectrophotometer (Colibri Microvolume Spectrometer, Pforzheim, Germany). RNA samples displaying a ratio of 260/280 ranging from 1.9–2.0 were selected further for RNA quality inspection and cDNA synthesis. RNA samples were treated with DNase I (RNase free) (NEB 2U) to minimize the risk of genomic DNA contamination.

For first-strand cDNA Synthesis, 5 µg of DNase I treated total RNA was used via RevertAid first-strand cDNA Synthesis Kit (Thermo Scientific, Waltham, MA, USA) following the manufacturer's instructions. In brief, RNA samples were heated at 65 °C for 5 min in the presence of 100 µM Oligo (dT)18 primer and nuclease-free water and then quickly chilled on ice. After that, 5X Reaction buffer, RiboLock RNase inhibitor (20 U/µL), RevertAid M-MuLV RT (200 U/µL) and 10 mM dNTP mix were added to the mixture, followed by incubation for 60 min at 42 °C. The reaction was inactivated by heating at 70 °C for 5 min.

### *2.7. Absolute Quantification of BBTV Components*

RT-qPCR reactions were set up in triplicates containing 12 µL volume in total for each BBTV component from leaf, midrib, and root in the Applied Biosystems-StepOnePlus™ system. A typical reaction mixture with 12 µL volume contained 2 µL template, 2 µL evagreen (5x HOT FIREPol® EvaGreen® qPCR Supermix (Solis Biodyne, Tartu, Estonia), 2 µL primer mix (10 pmol), and 6 µL dd$H_2O$. The reaction was normalized for each gene using the housekeeping RPS2 banana gene as an internal control. The thermal profile includes pre-PCR denaturation at 95 °C for 10 min followed by 35 cycles of denaturing at 96 °C for 20 s, annealing at 56 °C for 20 s, extension at 72 °C for 20 s, and a final extension at 72 °C for 10 min. Absolute quantities were obtained after generating amplification curves for BBTV components.

A serially diluted plasmid of the known copy number was used to construct standard curves by plotting cycle threshold (Ct) values using the StepOne software, California, USA. Each sample was analyzed using three technical replicates, and the viral copy number was calculated with the mean Ct value. Regression analyses were adjusted to the standard curves using Excel software (Microsoft, Washington, DC, USA).

### *2.8. Statistical Analysis*

The differences between the tissues of different genomic components of BBTV data were presented as mean ± standard errors of means (SEM). The significance of the obtained results was determined using Tukey's HSD test with SPSS v. 23 (IBM, Chicago, IL, USA) at a probability level of $p < 0.05$.

## 3. Results

### *3.1. Quantitative Analysis by qPCR Showed Differential Levels of BBTV Genomic Components*

The complete genomic components of BBTV (of about 1 kb) and RPS2 banana gene (299 bp) were amplified from infected banana leaf, midrib, and root tissue using specific primers (Figure 1) for confirmation and sequencing. The standard curve for the BBTV DNA-R component was constructed using qPCR primers mentioned earlier in Table 1. The optimized qPCR system efficiently amplified BBTV genomic components with a correlation coefficient of 0.99.

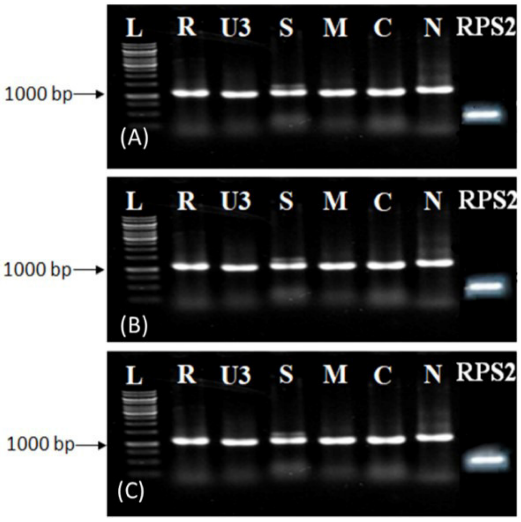


**Figure 1.** PCR amplification of BBTV genomic components and RPS2 banana gene. L = GeneRuler 1 kb DNA ladder (Thermo Scientific, Vilnius, Lithuania), PCR amplified fragment of DNA-R, -U3, -S, -M, -C, -N, and RPS2 banana gene from Leaf (**A**), Midrib (**B**), and Root tissues (**C**).

The qPCR analysis of BBTV DNA in virus-infected banana leaves revealed that DNA-U3, DNA-N, and DNA-C components were more abundant than other essential BBTV components (Figure 2A). DNA copy numbers in DNA-U3, DNA-N, and DNA-C were 6902 copies/ng, 5512 copies/ng, and 3186 copies/ng, respectively (Table S1). In other components, DNA-R, DNA-M, and DNA-S were present at lower levels of 799 copies/ng, 529 copies/ng and 192 copies/ng, and in RPS2 banana gene, it was at 2877 copies/ng. In midrib, the DNA copy number was higher in DNA-U3, DNA-N and DNA-C, with copy numbers 11,105 copies/ng, 3549 copies/ng, and 1814 copies/ng, respectively. Meanwhile, in DNA-M, DNA-R, DNA-S and RPS2 banana gene copy numbers were at a low level, 1638 copies/ng, 702 copies/ng, 376 copies/ng, and 1400 copies/ng, respectively. In root tissues, the copy number was highest in DNA-U3 (252 copies/ng) and least in DNA-R (23 copies/ng) (Table S1). DNA copies per mg (Figure S1A; Table S2) were also estimated, which were highest for DNA-U3 (1,355,318 copies/mg, 2,593,184 copies/mg) in leaf and midrib, whereas for root, it was the highest in RPS2 banana gene (42,468 copies/mg). RNA/DNA ratio for copies per ng was highest in the root of DNA-C while leaf (1.8%) and midrib of DNA-S (0.58%) also showed increased values (Figure 2C; Table S1).

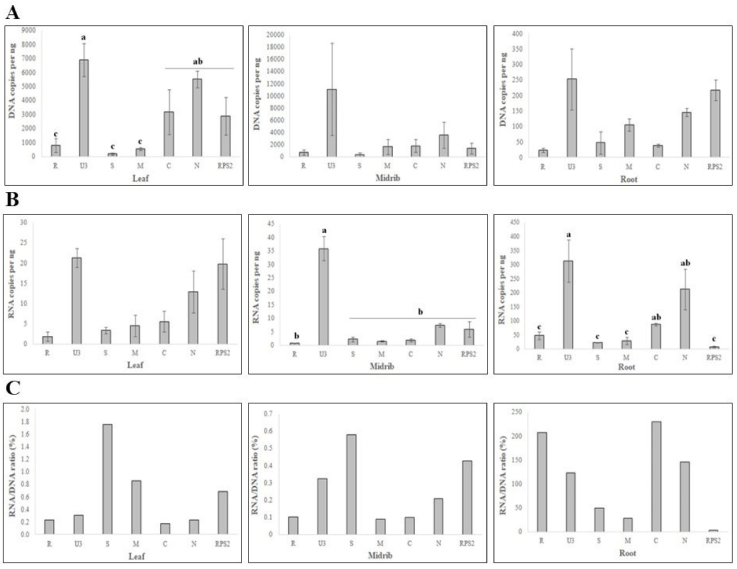


**Figure 2.** Quantification of DNA and RNA copies per ng of each genomic component of BBTV. DNA

copies per ng in leaf, midrib, and root (**A**). RNA copies per ng in leaf, midrib, and root (**B**). RNA/DNA ratio of each BBTV component (**C**). Values are presented as mean ± SEM ($n$ = 3). Bar values with different letters are significantly different from each other at $p < 0.05$.

### *3.2. Quantitative Analysis by RT-qPCR Confirmed Differential Levels of BBTV Transcripts*

Total RNA extracted from leaf, midrib, and root were processed for cDNA synthesis. The transcripts of BBTV DNA-R (567 bp), -U3 (140 bp), -S (471), -M (325), -C (413), -N (373), and RPS2 banana gene (299 bp) were amplified from infected banana leaf, midrib, and root tissue using gene-specific primers (Figure 3).

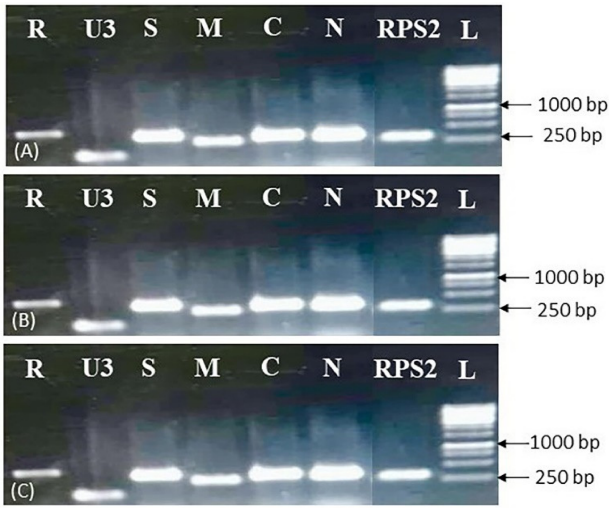


**Figure 3.** RT-qPCR amplification of BBTV transcripts. L = GeneRuler 1 kb DNA ladder (Thermo Scientific, Vilnius, Lithuania), RT-qPCR amplified fragment of DNA-R, -U3, -S, -M, -C, -N, and RPS2 banana gene from Leaf (**A**), Midrib (**B**), and Root tissues (**C**).

To confirm the differential transcript levels of each BBTV genomic component, RT-qPCR was used to quantify BBTV transcripts using the qPCR primer pairs. DNA-U3 transcribed the most abundant mRNA in leaf tissues with 21 copies/ng (Figure 2B; Table S1). DNA-R made the least abundant mRNA copies (1.8 copies/ng), while for other components, the mRNA copies ranged from 3.3–19.7 copies/ng. In midrib, DNA-U3 transcribed the most abundant mRNA copies (35 copies/ng), and DNA-R transcribed the least abundant copies (0.7 copies/ng), while for other components, it ranged from 1.4–7.4 copies/ng. The transcript level in root tissues showed the highest transcripts for DNA-U3 (312 copies/ng), and the least number of transcripts for the RPS2 banana gene (8 copies/ng), while for other components, the transcript level ranged from 23 to 212 copies/ng. After DNA-U3, the most highly transcribed BBTV component was DNA-N. DNA-N intergenic region architecture may favor replication complex accessibility. In contrast, a low level of DNA-R was observed, suggesting that Rep is required at relatively low levels during all stages of replication [19]. mRNA copies per mg were also calculated (Figure S1B; Table S2), which showed the highest number of copies for DNA-U3 in leaf, midrib, and root (5330 copies/mg, 8742 copies/mg, and 39,994 copies/mg, respectively).

### *3.3. Independent Modulation of Transcript Levels in Each BBTV Genomic Component*

To assess the transcriptional activity of each DNA component, the ratio of transcript copies to DNA copies was calculated. These ratios ranged from 0.2–1.8% in leaf, 0.09–0.58% in midrib, and 3.71–229.48% in root tissues (Figure 2C; Table S1). BBTV components, DNA-R, -C, and -N were transcribed at a relatively lower level with an RNA/DNA ratio of 0.2% in the leaf, while DNA-S (0.58%) in midrib and DNA-C (229.48%) in root tissues were transcribed at a relatively high rate (Table S1). Nanoviruses' components have intergenic

regions which include promotor region and conserved major common region (CR-M) and stem–loop common (CR-SL) regions that regulate replication and transcription. The nucleotide sequences of CR-M and CR-SL in Pakistani isolates were highly conserved, sharing 99% sequence identity between DNA-S and DNA-M [2], but the RNA/DNA ratios showed wide variations. Therefore, we can speculate that each component promoter sequence in the intergenic region might be responsible for the transcriptional activity. In addition, DNA-N was the actively transcribed BBTV component after DNA-U3. RNA transcripts per DNA copy from DNA-N were 0.2% in leaf and midrib, while they were 145.98% in root tissues (Table S1). RNA/DNA ratios in copies/mg were also calculated in infected BBTV plant, which exhibited a higher level of RPS2 banana gene (0.65%) in the leaf. In contrast, in the midrib, DNA-S (0.60%), and in the root, DNA-C (150.72%) had higher RNA transcripts per DNA copies ratio (Figure S1; Table S2).

## 4. Discussion

For many decades, extensive research on the biology of BBTV transmission has been performed. Since then, various studies have investigated epidemiological factors, such as host age, plant access period, temperature, and vector life stage [20–23]. Information on the distribution of BBTV after initial viral infection remained very sparse. This information could be useful in designing and implementing detection strategies for BBTV. The detection and quantitation of different genomic segments can be used to study the possible differential accumulation of genomic segments with respect to different aspects of virus biology (e.g., in plant hosts versus vectors) [24] which could provide valuable insights for the epidemiology of virus and disease control strategies based on restricting the dispersion of viruses by vectors. Viral quantification can also be used to evaluate the resistance level to virus accumulation in plant breeding programs. Therefore, this study aimed to examine the DNA concentration and their transcripts of individual components in BBTV-infected banana leaf, midrib, and root tissues.

The study revealed variations in DNA and RNA contents (Figure 2). DNA-U3 showed most abundant transcripts, followed by DNA-N and DNA-C in leaf, midrib, and root tissues. DNA-U3 encodes a small protein whose function is not known, its induced transcript levels point towards an important function yet to be elucidated. In contrast, DNA-N performs numerous roles during virus infection. Apart from nuclei transport and stress response, the protein encoded by DNA-N interacts with and redistributes the coat protein (CP) of BBTV inside plant cells [22]. Due to the presence of the FNGSF motif, this protein might have some additional functions, which can bind to the banana Ras-GAP SH3 domain binding protein and prevent the formation of stress granules in the host [23]. DNA-N is required in all stages of virus infection, which ultimately necessitates its high transcript level [25]. DNA-C encodes a cell cycle link protein during early stages of viral infection, which modulates the host cell into S phase and enables viral DNA replication [26,27]. Furthermore, the Clink protein contains an LXCXE motif, which has been linked to retinoblastoma (Rb) binding-like proteins. A site-specific mutation in either the C or E residues of the LXCXE motif can reduce or abolish this interaction. To facilitate viral DNA replication, Rb-binding proteins are assumed to alter the host cellular environment [28], a function that would require the protein to be expressed early in the virus replication process. The BBTV genome comprises of easily identifiable intergenic regions due to considerable conservation in genome organization for viral replication and gene regulation.

Regulatory processes that are in play during DNA replication and transcription have been well documented in the past. Recently, it has been recognized that the copy number of genes also has a fundamental role in DNA replication. In most organisms, gene expression is enhanced considerably if a cell has several copies of a gene [29,30]. For instance, many genetic disorders stem from the difference in the copy number of genes. Mutations in gene copy numbers are common among protozoans and bacteria as they have multiple mechanisms that help them cope with stress conditions [27,31]. Our study showed that BBTV, which belongs to the genus *Babuvirus*, also displays copy number variations in its

genomic constituents (Figure 2). DNA quantities of DNA-R and S components in BBTV are comparatively lower, which is in accordance with previous studies [12,13]. DNA-N is more prevalent in plants than DNA-S, indicating that the genetic components of BBTV are highly variable. These findings advocate that copy number variations prevalent in genomic constituents of viruses might be customary systems by which single-stranded multipartite DNA viruses regulate gene expression.

Promoter activity of BBTV DNA-N in transgenic banana plants revealed that in vascular-associated tissues, the promoter (BT6.1) was primarily active, and BT6.1 directs tissue-specific expression [19,31]. A virus replicates in an intricate setting where both virus and host compete for available resources. In the case of a multipartite virus, the host only becomes infected when one copy of each segment is present. The considerable disparity in the quantity of genomic elements of BBTV possibly indicates an unpredicted advantage for multipartite viruses. For instance, the nominal copy number as well as the transcript quantity of DNA-R, responsible for encoding a protein that triggers replication, adequately fulfill the requirement of maintaining the activity of enzymes involved in initiating the replication and circularization of ssDNA [12]; however, if DNA-R is present in ample amount, then these activities are hampered. It was interesting to note that higher DNA concentrations were found in the leaf tissues. This relates to aphids that prefer to clustered around the unfolded leaf and base of petiole that covers the leaf, which are ideal places for feeding and protection as well as acquiring BBTV virions. Meanwhile, in the root, the RNA transcript level was high, suggesting that BBTV replicated at the site of infection for a brief time before moving down the pseudostem to the basal meristematic region and then into the roots. A low RNA/DNA ratio in midrib might suggest that the virus has moved into this tissue but may not have replicated or accumulated to a significant level.

Conclusively, our data suggest that BBTV could autonomously regulate the replication and transcription of its respective components. A discrepancy exists in the transcripts of BBTV DNA components; some are transcribed at a higher rate while others are transcribed sparingly. DNA-N was the most over-represented of six BBTV components in the current study, implying that small nucleotide changes in essential replication characteristics of DNA-N may better bind the Rep protein complex, sequestering Rep from other DNA components. Additionally, the possible advantage behind the differential regulation of the gene copy number in multipartite segmented viruses is that this directly enforces restraints on the amount of each genome segment. Furthermore, information on the virus load in different tissues will enable the informed selection of suitable plant parts with relatively higher concentrations of the pathogen for indexing (testing) of mother plants in virus-free planting material in tissue culture. DNA-N is also one of the most conserved BBTV genomic components and can be targeted for virus diagnosis.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12122990/s1. Figure S1: Quantification of DNA and RNA copies per mg of each genomic component of BBTV; Table S1: Absolute quantification of DNA and RNA copies per ng of each genomic component of BBTV; Table S2: Absolute quantification of DNA and RNA copies per mg of each genomic component of BBTV.

**Author Contributions:** M.Z.H. Conceived, supervised, and administered the research; F.M.-P. set the methodology; S.B. wrote the original draft and performed formal analysis and data curation; A.M. provided plant resources; H.M., T.Y., and T.B., reviewed and edited the final draft; S.F., S.B., and S.I., and F.M.-P. performed software analysis and validation; A.B. was editing, writing, supervision, and funding. All authors have read and agreed to the published version of the manuscript.

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