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RESEARCH PAPER

Biochemical changes in some banana cultivars infected by *Banana bunchy top virus*

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ABSTRACT

Bunchy top is one of the major diseases on bananas that caused economic losses. This study aimed to determine changes in protein content, the concentration of phenolic compounds, the amount of chlorophyll, and sugar content in several *Banana bunchy top virus* (BBTV)-infected banana cultivars. The research involved the artificial inoculation, extraction, and biochemical analysis of banana seedlings from the cultivars *Mas*, Cavendish, *Kepok*, and *Raja*. Inoculation was performed using the vector *Pentalonia* sp.. A factorial complete randomized design (CRD) was used as the experimental design. Each experimental unit consisted of 5 repetitions. Protein, phenol, chlorophyll, and sugar content measurements were performed using a UV-Vis spectrophotometer. Test samples were in the form of fresh leaves from BBTV-infected and non-BBTV-infected plants. Regression analysis and tests for significance were performed to determine the biochemical changes that occurred after testing. The results showed that the average levels of phenols, chlorophyll, protein, and total sugars in BBTV-infected plants were significantly altered. The chlorophyll content decreased from 82–87% in *Mas*, Cavendish, and *Raja* cultivars. Total plant phenol in all BBTV-infected cultivars increased by 69.2 to 348.3 ppm. Specificity was evident in the change in protein concentration, which increased by 95 ppm and 84.5 ppm in the *Mas* and Cavendish cultivars, respectively. This contrasts with the *Kepok* and *Raja* cultivars, which decreased by 660.5 ppm and 113.6 ppm, respectively. Sugar levels increased from 3133.9 to 3298.6 ppm in all BBTV-infected breeds. According to the data, BBTV infection has been shown to cause physiological and biochemical changes in *Mas*, Cavendish, *Kepok*, and *Raja*.

Key words: BBTV, chlorophyll, phenols, proteins, sugars

INTRODUCTION

Banana bunchy top virus (BBTV) is a member of the genus Babuvirus, family Nanoviridae (Bressan & Watanabe, 2011). Banana bunchy top virus consists of six ssDNA components, each 1018 to 1111 bp in size. They are DNA-R, DNA-S, DNA-M, DNA-N, DNA-C, and DNA-U3 (Wickramaarachchi et al., 2016). Each DNA molecule, known as deoxyribonucleic acid, serves a specific purpose within the cell. DNA-S encodes the coat protein (CP). DNA-R is responsible for encoding an important replication-associated protein called M-Rep. These findings were supported by studies conducted by Wickramaarachchi et al. (2016) and Arumugam et al. (2017). In addition, DNA-N is responsible for encoding the nuclear shuttle protein (NSP), as shown by Baldodiya et al. (2019) and Ji et al. (2019). Finally, DNA-M functions as a motility protein (Debbarma et al., 2019). BBTV is transmitted by the banana aphid, *Pentalonia nigronervosa* Coquerel, and is commonly found during the transport of banana seedlings (Furuya et al., 2006). It has been hypothesized that virus-plant interactions affect plant production. Plant viruses not only interfere with the production of secondary metabolites, but also increase their numbers (Bahar et al., 2020).

Some signs of BBTV were observed, but the stunting symptoms were typical of banana (Rahayuniati & Subandiyah, 2022). Qazi (2016) explained that banana bunch top virus infects most banana cultivars and causes stunted growth and chlorosis/necrosis symptoms, resulting in huge economic losses to plantations. The virus is believed to be the cause of the most economically devastating plant diseases, resulting in losses of up to 100% of the banana crop. Banana plants infected with BBTV have significantly smaller, erect, yellowish leaves, and leaf development is disrupted. Later symptoms include young leaves that develop straighter, shorter, and narrower, and stalks that are shorter than normal and yellow at the edges (Kumar et al., 2015).

The effect of BBTV on banana plants can

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be evaluated physiologically by measuring the chlorophyll concentration (Mahfouze et al., 2020; Rahayuniati et al., 2021; Rahayuniati & Subandiyah, 2022). Most pathogen invasions lead to a decline in the photosynthesis rate of the host (Yang & Luo, 2021). Photosynthesis produces carbohydrates that are used by the plant for growth and development. A decrease in photosynthesis due to a decrease in chlorophyll content can lead to a reduction in carbohydrate production, which can limit the plant's growth and yield (Dong et al., 2014). Hooks et al. (2008) and Rahayuniati & Subandiyah (2022) found a substantial difference in chlorophyll levels between infected and not-infected banana plants. The chlorophyll content of infected banana plants decreases. Because of the loss in chlorophyll, banana plants infected with BBTV were slightly pale, and the leaf margins underwent chlorosis. Chlorophyll depletion also affects the development of enzymes in banana plants, which are essential in the photosynthetic process. This causes banana plant stunting (Zhao et al., 2016).

Phenolic compounds are part of inducible resistance in plants. In plant defense, phenolic compounds can act as physical or chemical barriers against pathogenic invasion. Phenolic compounds are also signalling molecules in the defense mechanism of plants and they mediate the transport of auxin (Kumar et al., 2020; Wallis & Galarneau, 2020). In response to the BBTV infection, the total phenolic of BBTV-infected banana cultivars increased significantly. Furthermore, total phenolic fluctuate among sick banana cultivars naturally (Anuradha et al., 2015). Tanuja et al. (2019) discovered that an increase in the number of phenols can be linked to plant defence mechanisms in which plant polyphenols operate as secondary metabolites. The biochemical mechanisms involved in plant disease resistance are complex phenomena.

Sugars influence all phases of the plant life cycle, interact with other signalling molecules, including phytohormones, and control plant growth and development due to their position as energy and carbon sources and regulatory activities (Jeandet et al., 2022). Total sugars have long been considered important compounds related to plant defence. According to previous studies, total sugars were significantly higher in BBTV-infected plants compared to healthy plants in banana cultivars. The total of sugar that increases during infection can control the photosynthetic inhibition process and produce plant disease symptoms (Pandey & Singh, 2019).

Plant protein content fluctuates in response to viral infections. Protein alterations can occur in virus-infected cultivars in response to the activation of a plant's defense mechanisms against pathogens. Plant protein production protects against fungal, bacterial, viral, and viroid infections, as well as other plant-damaging toxins. Protein content increased in plants infected with BBTV, but not significantly when compared to banana plants that were not infected with BBTV (Kaur et al., 2022; Madhumitha et al., 2020). Plant proteins that are produced naturally can act as virus suppressors. Proteins of several sorts mediate plant viral defence responses. Responses to viral infections typically include the production of various plant proteins that prevent the further spread of the virus. Pathogenesis-related (PR) proteins are activated in response to pathogen infections or stress, such as pathogen attack, damage, or exposure to certain plant hormones, such as salt, cold, or drought. Production of PR proteins in uninfected plant parts can prevent the further spread of viral infection. Antimicrobial peptides (AMPs) possess antiviral activity in vitro and are considered potential antiviral agents with biotechnological applications. Other plant proteins have also shown antiviral properties in vitro. Such activity is characteristic of ribosome-inactivating proteins (RIPs), RNA-binding proteins (RBPs), and some defense-related proteins. Another way to overcome viral attacks is through RNA silencing, which acts as the basic antiviral immune system of plants. Proteins that play central roles in antiviral RNA silencing pathways include members of the argonaute (AGO), RNA-dependent RNA polymerase (RDR), and plant Dicer-like (DCL) protein families. All these proteins are thought to be involved in plant defense responses to viral infections or have been shown to have antiviral effects in vitro and have potential for use in agricultural economics. However, the mechanism of antiviral effects of plant proteins is still not completely understood. They are known to directly occupy the virus infection site and interact with the virus by inhibiting protein synthesis and the viral genome or indirectly activating plant defense systems, thereby increasing virus resistance (Musidlak et al., 2017).

As a result, it's worthwhile to assess physiological and biochemical alterations in the banana cultivars, as well as to quantify biochemical changes caused by BBTV infection. This study focused on changes in chlorophyll content and the biochemical changes (total phenols, total proteins, and sugars) in four banana cultivar (*Mas*, Cavendish, *Kepok*, and *Raja*) that are widely cultivated and have high economic value in Indonesia.

MATERIALS AND METHODS

Research Site. The research was carried out in the Laboratory of Plant Protection and Screen House of Agriculture Faculty of Universitas Jenderal Soedirman, Purwokerto, Central Java, Indonesia.

Banana Plant Collection. Banana plants were grown from cultured tissues. In total, 10 tissue culture seedlings (1 month after acclimatization) of each cultivar (cv.) were collected from the horticulture nursery in Magelang district for cv. *Mas* (AA), cv. Cavendish (AAA), cv. *Kepok* (ABB), and cv. *Raja* (AAB), where the genome is given in parentheses.

Experimental Design. A factorial complete randomized design was used as the experimental design. Plant cultivar (A1= cv. *Mas*, A2= cv. Cavendish, A3= cv. *Kepok*, and A4= cv. *Raja*) used as the first factor, and inoculation treatments (P1= inoculated with viruliferous aphid and P0= inoculated with non-viruliferous aphid) as the second factor. Each experimental unit consisted of 5 replications. Regression analysis and tests for significance were performed to determine biochemical changes that occurred after testing. Statistical analyses were performed using two-way analysis of variance using SPSS 24 software (IBM, Armonk, NY, USA). Mean comparisons were performed using Duncan's multiple range test. Tests were performed with $p< 0.05$.

Aphid Mass Rearing. In this study, we used aphids from the Rahayuniati collection, which was identified molecularly and deposited in GenBank under accession OL96646 *Pentalonia nigronervosa* voucher JOG 001 (Rahayuniati & Subandiyah, 2022). Aphids were mass-reared on caladium (*Caladium bicolor*). Ten non-viruliferous, wingless adult aphids were transferred to caladium that had been kept in screen boxes. After nymphs reproduced on caladium, colonies were re-tested for the presence of BBTV before being used as vectors.

BBTV Inoculation. Virus transmission was carried out according to Rahayuniati et al. (2021), with slight modifications. Non-viruliferous aphids were transferred from caladium to infected bananas as the source of BBTV inoculum. After 48 h of acquisition, 20 aphids were transferred to each banana (cv. *Mas*, cv. Cavendish, cv. *Kepok*, and cv. *Raja*) using a soft wet brush. The aphids were placed on the underside of a coiled banana leaf, then placed in a room covered with organza fabric in the greenhouse at 25–28 ºC. After 48 h of incubation, all plants were sprayed with 5% imidacloprid insecticides. As a control, banana plants infected with non-viruliferous aphids. The observed variabels were incubation period and plant height. Early symptoms observed included chlorosis of new leaves that occurred after inoculation. In addition, the following characteristics were also monitored: yellowing of leaf margins, presence of dark green lines on petioles or pseudostemes, changes in the size of new leaves, rosette symptoms, and plant death. Leaf samples for the next analysis were collected from the infected and un-infected plants after the fastest incubation period.

Chlorophyll Content. Chlorophyll content analysis was carried out based on Fadhlullah et al. (2020) and Rahayuniati & Subandiyah (2022). A total of 100 mg of fresh leaf sample was extracted in 10 mL of 80% acetone (Merck) and stirred to dissolve the chlorophyll, followed by filtering the extract using Whatman paper filter number 93. Subsequently, the samples were placed into the 3 mL quartz cuvette and measured using an ultraviolet-visible Shimadzu 1800 UV-Vis spectrometer at wavelengths of 663 nm and 645 nm.

The calculating chlorophyll content was determined based on these equations. Amount of the chlorophyll was measured in mg/L (Rahayuniati & Subandiyah, 2022).

Chlorophyll a	=	(12.7A)	–	(2.69B)
Chlorophyll b	=	(22.9B)	–	(4.68A)
Total Chlorophyll	=	(8.02A)	+	(20.2B)

A= Absorbance at 663 nm; B = Absorbance at 645 nm.

Total Phenol Analysis. Total phenol analysis was performed according to Anuradha & Selvarajan (2021) with slight modification. A total of 1 g of fresh banana leaf from each cultivar was macerated in 5 mL 80% ethanol (Merck), and filtered using Whatman paper filter number 93. Exactly 0.5 mL Folin-Ciocalteau reagent was added followed by aquadest to reach a volume 6 mL. Extract was vortexed for 5 min, before adding 2 mL 20% Na_2CO_3 (Sodium Carbonate) and 30 min incubation period. We used UV-Vis Shimadzu 1800 spectrometer at 660 nm absorbance to gain the quantity of total phenolic compound of each leaf sample.

Protein Analysis. Protein analysis of banana leaves was performed for each treatment using the Lowry method according to Hasan (2022); Waterborg & Matthews (1984). A 0.1-g fresh banana leaf sample was ground until smooth and dissolved in 5 mL sterile distilled water. The extract was filtered through Whatman No. 93 filter paper. Measurement of dissolved protein levels

was carried out by adding 1 mL of extract and 1 mL of distilled water; then, 8 mL of Lowry B reagent was added and incubated for 10 min. After incubation, 1 mL of Lowry A reagent was added and homogenized using a vortex. Sample absorbance was measured using a Shimadzu 1800 UV-Vis spectrophotometer at 600 nm. As a standard, protein measurements were performed using bovine serum albumin (BSA) at concentrations of several levels, namely 0, 30, 60, 90, 120, 150, 180, 210, 240, and 300 ppm.

Total Sugar. The dinitrosalicylic acid (DNS) method was used to perform total sugar analysis for each treatment (Teixeira et al., 2012). Each 0.1 g fresh banana sample was ground until smooth and then dissolved in 5 mL of sterile distilled water. Extract was filtered using Whatman No. 93 filter paper and 2 mL of distilled water was added. The total sugar test was done by adding 1 mL of HCL to the filtrate and then heating it in a water bath for 30 min at 90 ºC. The sample was then cooled to room temperature, stored in a freezer at -7 ºC to be neutralized with 40% NaOH to pH ±7, and adjusted to 25 mL. Next, 8 mL of the sample was placed in each test tube and 1 mL of DNS reagent was added. All the filtrate and reagent mixture were then heated in a water bath for 10 min at 70 ºC to allow the reaction between glucose and DNS to occur. The sample was cooled to room temperature, and 0.5 mL of 40% Potassium sodium tartrate tetrahydrate ($KNaC_4H_4O_6 \cdot 4H_2O$) was added. The absorbance of each solution was measured at 540 nm using a Shimadzu 1800 UV-Vis spectrophotometer. A glucose standard was used for the measurement of total sugar, i.e., 0, 200, 400, 600, and 800 ppm.

RESULTS AND DISCUSSION

In this study, infected banana will show some symptom including yellowing of the leaves, narrow leaf size, sign like a morse on the midrib and leaf, and dwarf stem. In this research, we used that sign to justify the incubation period and infected plant. The results showed that cv. *Mas* had the shortest incubation period of 30 days after infection (DAI), while cv. *Kepok* had the greatest incubation period of 43 DAI. Cv. *Kepok* showed evidence of delayed development as compared to other cultivars such as *Mas*, *Raja*, and Cavendish (Table 1). According to Rahayuniati & Subandiyah (2022), the *Raja* cultivar's incubation period is equivalent to that of the *Mas*, Cavendish, and *Kepok* cultivars. In this investigation, the incubation period for the dwarf symptom did not differ between cultivars. According to Leiwakabessy (2017), the incubation period for banana stunting ranges from 25 to 85 days after infective aphid infestation. The disease severity of each cultivar was not significantly different. However, *Kepok* tended to show the lowest, with a severity of 1.19%. Although the incubation period of *Raja* was faster than Cavendish, but the disease severity was lower.

Biochemical pathway components are critical in defending plants against infections. Infections with viruses in plants usually result in reactions that are generally connected to genetic changes caused by viral activity in plant cells. Chlorosis is a common symptom of viral infection, characterized by yellow mosaic symptoms on the leaves. Virus-induced changes in the number or size of chloroplasts, as well as structural changes: chloroplast membrane invaginations, tubular

Table 1. Incubation period and disease severity of Banana cultivar infected with *Banana bunchy top virus*

Banana cultivar	Symptom description	Incubation period (days)	Disease severity (%)
P0A1	No chlorosis, no morse sign	0	0.00
P0A2	No chlorosis, no morse sign	0	0.00
P0A3	No chlorosis, no morse sign	0	0.00
P0A4	No chlorosis, no morse sign	0	0.00
P1A1	Chlorosis, morse sign, smaller emerge leaf size, short stalk	30	1.98 ± 0.021 a
P1A2	Chlorosis, morse sign, smaller emerge leaf size, short stalk	40	1.98 ± 0.026 a
P1A3	Chlorosis, morse sign, smaller emerge leaf size, short stalk	43	1.19 ± 0.013 a
P1A4	Chlorosis, morse sign, smaller emerge leaf size, short stalk	37	1.59 ± 0.027 a

P0= Control/infected with non-viruliferous aphids; P1= Infected with viruliferous aphid; A1= cv. *Mas*; A2= cv. Cavendish; A3= cv. *Kepok*; A4= cv. *Raja*.

stromal development, and changes in the number or appearance of grana or starch grains have all been linked to chlorotic symptoms (Hooks et al., 2008; Paudel & Sanfaçon, 2018). Rahayuniati & Subandiyah (2022) discovered that genetic features, physiological alterations, and environmental factors may all impact leaf colour variances induced by BBTV infection. Furthermore, a research study by Anuradha et al. (2015) also indicates that carbohydrates may accumulate in the leaves following viral infection.

The results showed that the symptoms of chlorosis in the infected plants appeared more quickly in the *Mas* cultivar than in the other cultivars. This research indicated that chlorophyll b on the infected plant come down up to 82% on cv. *Mas*. Otherwise, there were no chlorophyll changes on cultivar Cavendish, Kepok and Raja. The chlorophyll a content also decreased by 82% in the cv. *Mas* infected with BBTV, whereas in the infected Cavendish, *Kepok*, and *Raja* cultivars the chlorophyll a did not change compared to the not infected plants (Figure 1). Changes in chlorophyll a and b also lead to changes in the total chlorophyll content of banana leaves. The average total chlorophyll content of not infected banana leaves was 2.85–9.28 mg/L, while that of infected plants was 1.41–6.63 mg/L. A decrease of 87% in total chlorophyll occurred in the *Mas* cultivar. However, in the Cavendish, *Kepok*, and *Raja* cultivars, these changes did not occur. Based on the data, the lowest total chlorophyll content was found in the *Mas* cultivar infected with BBTV, whereas the highest total chlorophyll content was found in the not infected *Mas* cultivar. This is in line with the research of Anuradha et al. (2015), who stated that reduced chlorophyll content due to virus infection can be associated with the stimulation of enzymes in cells, such as chlorophyllase, which degrades chlorophyll. In addition, the virus has been shown to interfere with physiological processes, including photosynthesis and the use of viral proteins and precursors in viral protein synthesis. Pigment alterations are also frequently regarded as a secondary effect on host plants, which can occur because of the enormous number of viruses that accumulate in the cell cytoplasm (Zhao et al., 2016). Even if this is a secondary effect, it affects disease incidence or resistance and decrease the plant production.

The total phenolic content of numerous banana cultivars differed significantly from the control, particularly in the *Mas* and Cavendish cultivar. Infected with BBTV, the total phenolic content increased by 71% in the *Mas* cultivar and 78% in the Cavendish cultivar (Figure 2). According to Anuradha & Selvarajan's (2021) investigation, the phenolic content in infected plants was much higher than in control plants in banana plants. Elevated phenolic levels may be attributed to the acceleration of the phenol production pathway during viral infection, as well as induced resistance to restrict virus invasion. Phenolic buildup might be interpreted as an indication of infection or virus development prevention. The rise in total phenol compounds is also linked to plant defence. Because phenol influences plant resistance to pathogen-caused diseases, an increase in the amount of phenolic in diseased banana plants can contribute to plant resistance to pathogenic virus infections. A rise in phenolic levels also implies that the phenol production pathway is accelerating following pathogen infection (Anuradha et al., 2015).

Plant viruses can induce changes in the subcellular localization of some host proteins, facilitating or inhibiting viral accumulation and spread (Rodriguez-peña et al., 2021). Viral infection causes dramatic changes in protein accumulation throughout the plant, including leaves (Souza et al., 2019). Protein content is

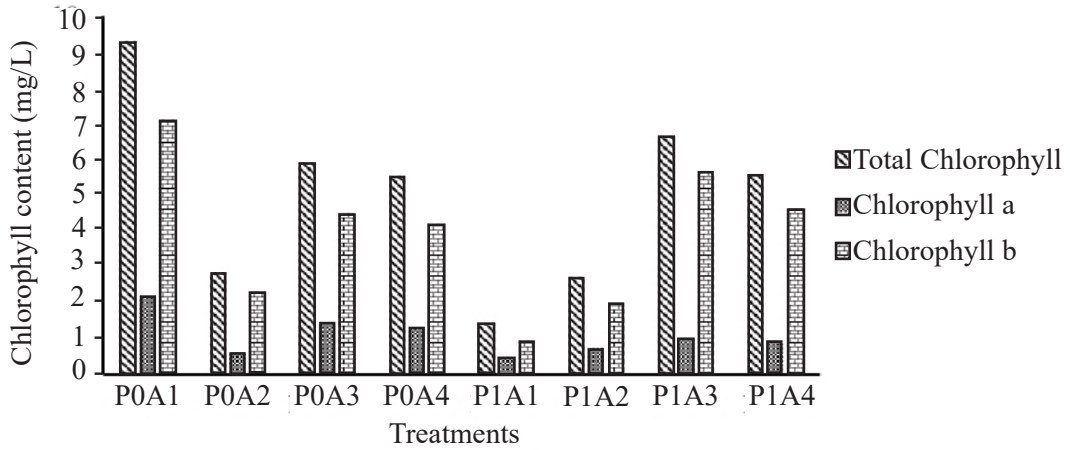

Figure 1. Chlorophyll content of banana plant test. P0= Infected with non viruliferaous aphid; P1= Infected with viruliferous aphid; A1= cv. *Mas*; A2= cv. Cavendish; A3= cv. *Kepok*; A4= cv. *Raja*.

also altered when plants are infected with BBTV. Protein concentrations in not infected plants ranged from 670.8 to 1167 ppm, whereas those in infected plants ranged from 506.5 to 824.8 ppm (Figure 3). The reduction in protein was showed in the cultivar of *Kepok* (57%) and *Raja* (15%) but increased in *Mas* (14%) and Cavendish (11%) cultivar. A decrease in protein content may be due to BBTV infection, which causes denaturation or degradation of proteins, polypeptide chains and bound amino acids (Siddique et al., 2014). The increase in the total protein in *Mas* and Cavendish could be due to the BBTV activities in the cytoplasm. In the presence of plant viruses, host cytoplasmic proteins can reach the viral replication site through interactions with viral proteins, which contribute to essential viral processes. The replication process activated the host defense mechanisms and the synthesis of virus-specific proteins during pathogenesis and accumulation of virus particles. However, few studies have documented changes in the subcellular localization of proteins with antiviral activity (Rodriguez-peña et al., 2021). Viral infections usually cause symptoms that lead to morphological and physiological changes in the infected plant host, always resulting in performance degradation such as reduced host biomass and crop yield loss (Zhao et al., 2016).

Sugars play a key role in plant defense responses to various abiotic and biotic stress factors (Jeandet et al., 2022). Sugars are not only the main substrates utilized in respiration processes, supplying energy for cellular defense responses against pathogens, but also provide the carbon skeleton for the synthesis of defense compounds, including secondary metabolites such as flavonoids, stilbenes, and lignins (Jeandet et al., 2021). In this study, it was found that the average total sugar content of non-infected plants was 1049.6–1161.8 ppm, whereas in plants infected with BBTV the sugar content was 4254.1–4348.2 ppm (Figure 4). The average total sugar content increased by 314% in cv. *Mas*, 274% in Cavendish, 275% in *Kepok*, and 270% in *Raja*. In previous studies, an increase in total sugar when plants were infected with BBTV could control the photosynthetic inhibition process and produce a higher symptomatic carbohydrate content. Based on Anuradha et al. (2015), viruses that infect plants can change the

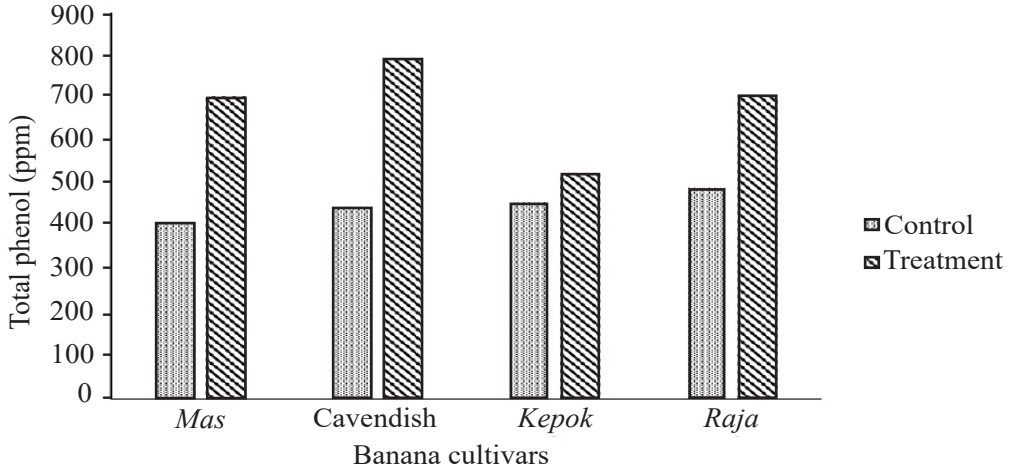

Figure 2. The changes of total phenolic compound of banana cultivars after BBTV infection.

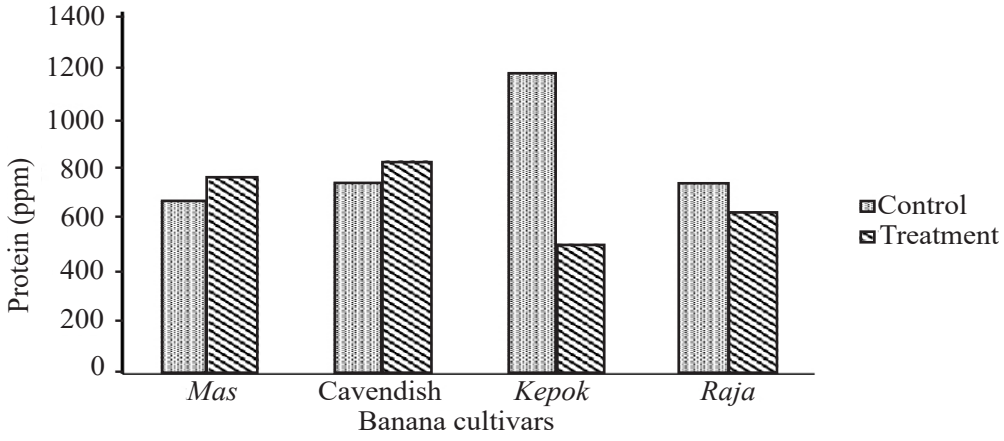

Figure 3. Total protein changes of banana cultivars infected by BBTV.

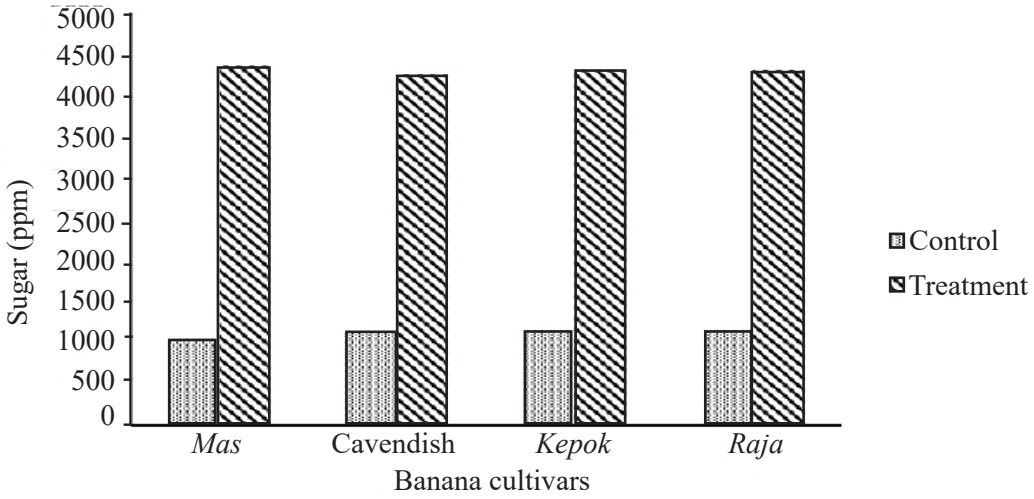

Figure 4. Total sugar changes of banana cultivars infected by BBTV.

rate of photosynthesis and the rate of translocation. Thus, affecting the amount of carbohydrates contained in plants and greatly affecting overall plant growth.

CONCLUSIONS

BBTV infection has been shown to induce physiological and biochemical changes in Mas, Cavendish, Kepok and Raja banana cultivars. Symptoms of chlorosis, reduced leaf area and dwarfism are the result of a decrease in chlorophyll, changes in leaf protein concentration and an increase in total sugars. An increase in phenolic content indicates a plant resistance response.

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AUTHORS' CONTRIBUTIONS

RFR and N considered and planned the experiment and carried out the test including aphid mass rearing. RFR performed biochemical work analysis. REKK performs statistical analysis and interprets the data. RFR prepared the manuscript. All the authors have read and approved the final manuscript.

COMPETING INTEREST

No competing interest in this manuscript.

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