Original Research Article

Differential biochemical response among Banana (*Musa* spp.) genotypes against Banana Bunchy Top Virus (BBTV)

.

ABSTRACT

The present study was carried out to explore the adaptive mechanism and biochemical responses in two banana cultivars viz., Rasthali and Grand Naine against the banana bunchy top virus (BBTV). In the leaf samples of BBTV infected Rasthali and Grand Naine, estimated the total chlorophyll, carbohydrates, phenols and enzyme activities such as peroxidase (POX), polyphenol oxidase (PPO), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and superoxide dismutase (SOD). The infected samples of both the cultivars revealed a significant increase in the defense enzymes such as PPO, POX, APX, GPX and CAT over the healthy sample. Higher total phenols in Rasthali healthy plants which further significantly increased after BBTV infection was observed in comparison to Grand Naine. In contrast to Grand Naine, Rasthali showed higher polyphenol oxidase (PPO) activity contributing to increased polyphenol formation. The higher superoxide dismutase (SOD) activity in virus infected Rasthali was observed in comparison to Grand Naine. The increased amount of total phenols, polyphenols and SOD activity in Rasthali might have contributed to less susceptibility to bunchy top virus. However, total protein and chlorophyll content were reduced after BBTV infection in both the banana cultivars.

Keywords: [Banana Bunchy top virus, Rasthali, Grand Naine, Biochemical changes and Defense enzymes]

1. INTRODUCTION

Plants are frequently exposed to infection by a wide array of pathogens that show different responses in the host plant. During compatible plant-pathogen interaction, along with the development of visible symptoms, the pathogen also adversely affects the growth and

development, physiological status and yield of a plant [35]. Plants have evolved various pre-existing physical and chemical barriers, as well as inducible defense responses that restrict pathogen colonization [18,37,38]. Globally, bananas and plantains are the fourth most important agricultural produce which is attacked by various pests and pathogens causing major production problems. Grand Naine (AAA subgroup Cavendish) is one of the most commonly cultivated commercial Cavendish cultivar in the world. Rasthali (AAB subgroup Silk) is cultivated mostly in India and is popular in the local and world market as a premium dessert variety similar to Cavendish bananas. Plant viral diseases cause significant losses by reducing plant growth and yield. Among the banana viral diseases, Banana bunchy top disease (BBTD) is one of the most destructive viral diseases affecting various banana cultivars. There is no resistance germplasm available in bananas and plantains; however, the level of susceptibility varies among the banana cultivars.

Banana bunchy top disease affects the fruit and foliage and is caused by a single-strand DNA virus, the banana bunchy top virus (BBTV; Genus *Babusvirus*; Family *Nanoviridae*). The BBTV virus colonizes in the phloem tissue, which is damaging the host cells. The name of the disease comes from the symptom which occurs in banana plants, in which the emerging new leaves are narrower than usual, yellow and flat, which causes a "bunchy" appearance at the top. In addition, few distinctive symptoms are 'morse code streaking,' 'green J hooks' and 'keikis'. Viruses depend on the host for their replication and other metabolic processes, which instigate significant changes in their usual physiological processes such as loss of pigment contents, increasing respiration rates, soluble sugar, and starch accumulation and production level of enzymatic antioxidants. Due to the viral infection, various changes occur in the host plants at the molecular level, thereby leading to various biological and physiological changes. Hence, it is necessary to estimate the physiological and biochemical changes in banana cultivars Rasthali and Grand Naine to known the biochemical changes occurring due to BBTV infection. The present investigation will lead to better understanding of the defense mechanism in two banana cultivars, which will be useful for adopting suitable control strategy against bunchy top disease in banana.

2. MATERIAL AND METHODS

2.1 Plant material and source of infection

Banana cultivars Rasthali and Grand Naine were used in the present investigation. Leaf samples were collected from BBTV infected plants in Orchard of Tamil Nadu Agricultural University, Coimbatore district, Tamil Nadu, India. The leaf samples from the healthy plants of each cultivar were taken as control.

2.2 PCR confirmation of BBTV presence in the infected banana samples

The plant genomic DNA was extracted from healthy and infected (showing characteristic symptoms of BBTV) samples of Rasthali and Grand Naine by cetyl trimethyl ammonium bromide (CTAB) method with some modification as described by [10] from 100 mg leaf samples and subjected to PCR using the BBTV specific primer designed for Replicase gene F-**5'** ACGACAGAATGGCGCGA**3'** and R- **5'**TCAGCAAGAAACCAACTTTATTC**3'.** The PCR products were resolved on 1 % Agarose gel, electrophoresed at 70 V for one h and the amplicons were assessed with 1.0 kb DNA ladder.

2.3. Leaf samples for biochemical analyses

The most recent fully expanded leaves of BBTV infected samples were collected for various analyses. All biochemical parameters were measured using a spectrophotometer (Jasco V-730 BIO spectrophotometer, USA).

2.4 Estimation of photosynthetic pigments:

The photosynthetic pigments such as total chlorophyll, chlorophyll 'a' and chlorophyll 'b' contents of healthy and infected leaves were estimated according to the non-destructive DMSO method [16]. The absorbance was recorded at 663 and 645 nm, respectively in a spectrophotometer, taking full concentration of the DMSO has blank. Chlorophyll a, b and total chlorophyll were calculated by the following formulas:

$$\text{Chlorophyll a (mg g}^{-1}\text{ tissue)} = \frac{[12.7(OD663) - 2.69(OD645)]/ x\ V}{1000} \times W$$

$$\text{Chlorophyll b (mg g}^{-1}\text{ tissue)} = \frac{[22.9(OD645) - 4.68(OD663)]/ x\ V}{1000} \times W$$

$$\text{Total Chlorophyll (mg g}^{-1}\text{ tissue)} = \frac{[8.02(OD663) + 20.20(OD645)]/ x\ V}{1000} \times W$$

Where OD, Optical density at respective nm, V, Final volume of chlorophyll extract, W, Fresh weight of the tissue extracted.

2.5 Total sugars and starch content:

Total reducing sugars were calculated according to the method described by [11] and the total starch content method explained by [26]. About 0.5 gm of healthy and infected leaves were taken and homogenised with 80 % ethanol and centrifuged at 5000 rpm for 15 min. The supernatants were pooled and heated in a water bath at 85 °C until the ethanol was evaporated entirely from the samples.

For total reducing sugar determination, healthy and virus-infected pooled supernatants were taken and cold anthrone reagent was rapidly added to each tube and incubated for 10 min

on ice and cooled at room temperature. The absorbance of the samples was recorded at 625 nm in a spectrophotometer along with the blank sample. The amount of total sugars was estimated by using a standard curve prepared for D-glucose. The content of reducing sugar was expressed as mg g^{-1} fresh weight.

For starch determination, the extract for total sugar was solubilized in five ml of 52 % perchloric acid (PCA) and boiled at 80 °C for 10 min in a water bath. Three ml of distilled water and five ml of anthrone reagent was added, incubated for 10 min on ice. The absorbance of the samples was recorded at 625 nm in a spectrophotometer. The amount of starch was determined by using D-glucose standard curve. The content of total starch was expressed as mg g^{-1} fresh weight.

2.6 Phenolic content:

Phenol content was measured using the Folin-Ciocalteau reagent. One-gram of fresh plant material was homogenized using 80 % ethanol. The extract was subjected to centrifugation at 3000 rpm for 15 min and the supernatant was separated. Then, 0.1 ml of ethanol extract was evaporated on a water bath, to which six ml water was added and shaken well before the addition of 0.5 ml Folin-Ciocalteau reagent. Two ml of 20 % sodium carbonate solution was added to each test tube and after 30-45 min of incubation, the absorbance was recorded at wavelength 660 nm against a reagent blank. Using pyrocatechol as standard, a standard curve was generated to determine the concentration of total phenols in the leaf extract [12].

2.7 Measurement of total protein content

Total protein was estimated by using the Bradford method [3] and absorbance was recorded at 595 nm. Bovine serum albumin was used as a standard. Protein contents in leaf samples were recorded as µg of protein per gram of leaf tissue.

2.8 Preparation of enzyme extract:

To obtain the total enzyme extract, a one-gram leaf sample was homogenized at 4°C in 1 ml of extraction buffer [50 mM potassium phosphate buffer (pH 7.0), 1 % Triton X-100 and 7 mM 2-mercaptoethanol]. The obtained homogenate was then centrifuged at 12000 rpm for 20 min at 4°C. The resulting supernatant was used for analysis of enzymes.

2.8.1 Peroxidase activity

POX activity was assessed following the oxidation of pyrogallol [23]. For the assay, 3.5 ml of phosphate buffer (pH-6.5) was taken in a clean and dry cuvette to it, 0.2 ml of enzyme

extract and 0.1 ml of freshly prepared pyrogallol solution were added. Then 0.2 ml of 0.2 M H_2O_2 was added and instantly the absorbance of the reaction mixture was recorded at 430 nm at every 30-sec intervals up to 3 min. The specific activity of the enzyme was expressed as micromoles pyrogallol oxidized per minute per milligram protein.

2.8.2 Polyphenol oxidase activity

PPO activity was determined according to the method described by [28]. For the assay, 0.2 ml of the enzyme extract was taken, to which 1 ml of catechol and 3.5 ml phosphate buffer was added. The extract was incubated at 30 °C for 30 min. The activity was measured by monitoring the increase in absorbance for 3 min at 410 nm. The specific activity of the enzyme was expressed as micromoles catechol oxidized per minute per milligram protein.

2.8.3 Catalase activity

Catalase activity was calculated by measuring the rate of disappearance of H_2O_2 using the method followed by [22]. The reaction mixture containing 2.5 ml of 50 mM phosphate buffer (pH 7.4), 0.1 ml of 1 % H_2O_2 and 50 µl of enzyme extract was diluted to keep measurements within the linear range of the analysis. The decrease in H_2O_2 was followed as a decline in absorbance at 240 nm. Catalase activity was expressed as micromoles of H_2O_2 oxidized per minute per milligram protein.

2.8.4 Ascorbate peroxidase activity

APX activity was determined using the method described by [6]. The one ml reaction mixture consists of 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA, 0.5 mM ascorbate, 1.54 mM H_2O_2 and 50 µl of enzyme extract. The oxidation of ascorbate was followed by a decrease in the absorbance at 240 nm. The enzyme-specific activity is expressed as micromoles ascorbate oxidized per minute per milligram protein.

2.8.5 Guaiacol peroxidase activity

GPX activity was calculated using the method described by Upadhyaya et al. [36]. The reaction mixture contained 2.5 ml of 50 mM phosphate buffer (pH 6.1), one ml of 1 % H_2O_2, one ml of 1 % guaiacol and 20 µl of enzyme extract. The increase in absorbance at 420 nm was recorded for 1 min. The enzyme-specific activity is expressed as micromoles guaiacol oxidized per minute per milligram protein.

2.8.6 Superoxide dismutase activity

SOD was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) using the method described by [8]. The reaction mixture consists of 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 M NBT, 2 M riboflavin, 0.1 mM EDTA and 50 µL of enzyme extract. Riboflavin was added last and the tubes were subjected to intermittent shaking. The absorbance of the reaction mixture was recorded spectrophotometrically at 560 nm.

2.9 Statistical analysis:

All the experiments were performed in two duplicates (n=4). The significance of differences between healthy and infected samples was determined by using one-way analysis of variance (*ANOVA)* and means and standard errors were calculated. Differences in means were considered significant when the *P*-value was <0.05.

3. RESULTS AND DISCUSSION

3.1 PCR based confirmation of BBTV

The presence of BBTV in symptomatic leaves of Rasthali and Grand Naine was confirmed by PCR amplification of 870 bp BBTV Rep gene using designed gene specific primers (Fig.1.)

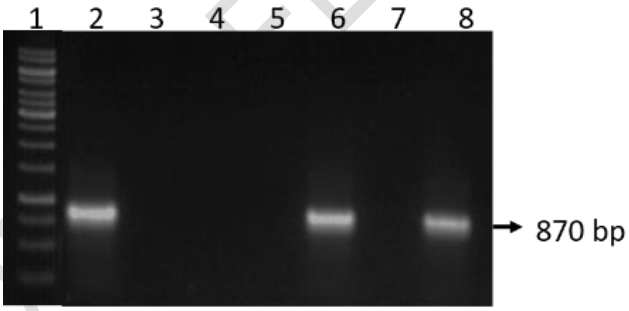


Fig. 1. PCR amplification of BBTV Rep gene in symptomatic Rasthali and Grand Naine plants

Lane 1, 1 kb ladder; 2, positive control; 3, negative control; 4, water control; 5-6, Rasthali healthy and infected sample; 7-8, Grand Naine healthy and infected sample.

3.2 Effect of BBTV incidence on the photosynthetic pigment

The BBTV infected cultivars exhibited a two- fold significant reduction in photosynthetic pigment contents (chlorophyll a, chlorophyll b and total chlorophyll) compared to healthy cultivars (Fig. 2). The reduction in the chlorophyll content in the infected plant reduces the photosynthetic capacity and plant growth, leaving the plant stunted and chlorotic resulting in the symptom expression. This difference in the chlorophyll content was attributed to the

stimulation of cell enzymes like chlorophyllase that degrades chlorophyll [14], or it may be the effect of the virus on pigment synthesis [2, 33] which disturbs the physiological processes like photosynthesis. A recent study suggests the possibility of BBTV utilizing the chloroplast for the synthesis of viral proteins [39]. They found during BBTV infection, outer membranes of chloroplasts are disputed and crystalline aggregation of virus- like particles accumulates in it.

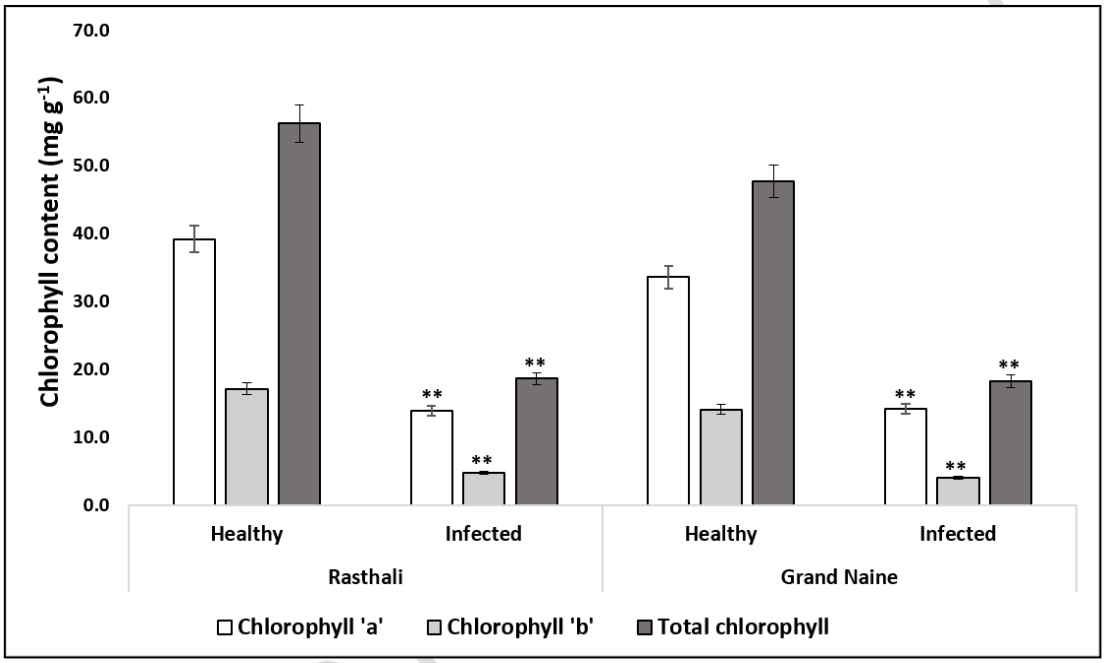
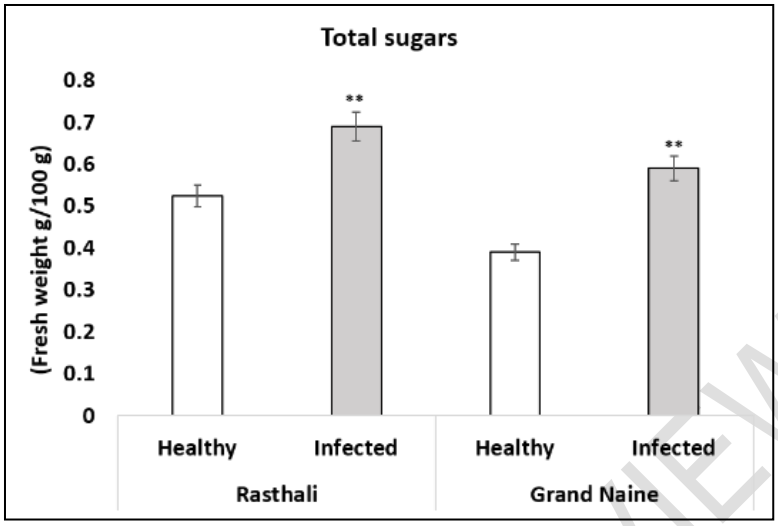


Fig. 2. Chlorophyll content of healthy and infected Rasthali and Grand Naine plants.

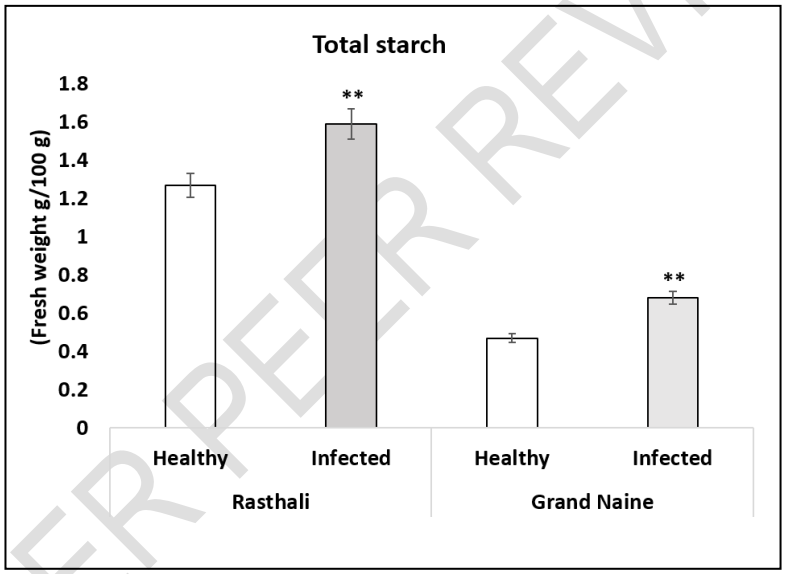
*Data represent the mean ± standard error of mean of four independent replications. Significant differences in healthy and infected from each cultivar analysed by Student's t test (*P<0.05, **P<0.01) are shown.*

3.3 Carbohydrates

The total sugars and starch were significantly high in infected plants compared to healthy in both the cultivars of banana (Fig. 3 a-b). Our study suggests that sugar increases during BBTV infection may control photo inhibitory processes and produce symptoms. The carbohydrate content reported by [1] was similar to the findings of our study where changes in the sugar and starch content were the same in all the banana cultivars viz., Virupakshi, Grand naine and Rasthali. Some viruses appear to have little effect on carbohydrates in the leaves, while others may alter both their rate of synthesis and rate of translocation which affects the overall growth of the plant [13].



(a)



(b)

Fig. 3. Carbohydrate content (a) Total sugars and (b) Starch of healthy and infected Rasthali and Grand Naine plants.

*Data represents the mean ± standard error of mean of four independent replications. Significant differences in healthy and infected from each cultivar analysed by Student's t test (*P<0.05, **P<0.01) are shown.*

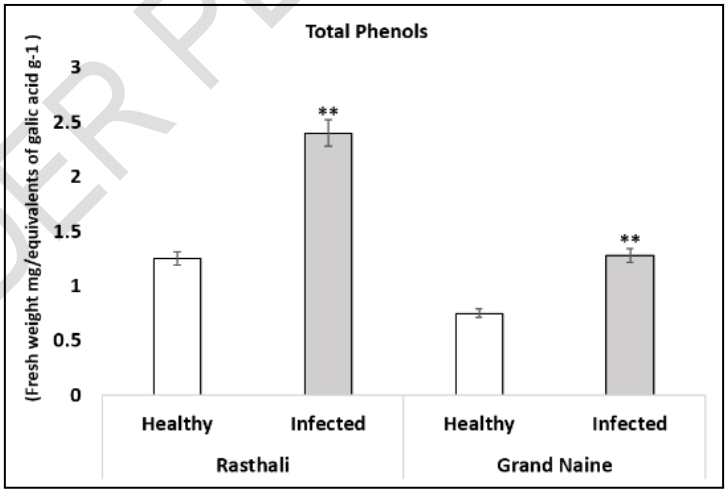
3.4 Total Phenol

In the present investigation showed a significant variation in the total phenolic compound of banana cultivars in response to infection with BBTV (Fig. 4a). The amount of total phenol was significantly higher in virus-infected leaves in both the cultivars of banana and the increased quantity of phenols might be attributed to a defense mechanism where plant polyphenols act as secondary metabolites. It was reported that the resistance to disease caused by pathogen was attributed to the presence of a high amount of phenol

[17,21,23,29,33]. It has been reported that the Rasthali takes viral infection later than the Grand Naine cultivar because of the difference in the genome [27]. Although no Musa genotype is known to be resistant to BBTV, cultivars in the AA and AAA genomic groups are highly susceptible, whereas cultivars containing the B genome are regarded as less susceptible. The less BBTV susceptible Rasthali had higher total phenol content in healthy plants which further increased >2 fold after BBTV infection. This is in contrast to Grand Naine displaying lower total phenol content in healthy plants which increased to lower level after BBTV infection. Hence, the increased quantity of phenolics in the infected plant of the banana may be contributing to the resistance against the infection of viral pathogens [24].

3.5 Total Protein

Protein content was found to decrease significantly in the BBTV infected plants of both the cultivars (Fig. 4b). Results in our study showed that the total protein content changes regarding viral infection in both the cultivars thereby confirming the accumulation of protein as a response to viral infections. The involvement of host protein components in plant disease resistance has been documented in various plant pathogenic interactions [4, 36]. This result is in accordance with the results of Tobacco mosaic virus-infected tobacco plants [19], Tomato yellow leaf curl virus-infected tomato plants [9], Banana bunchy top virus-infected cultivars of banana [7], geminivirus infected *Capsicum annum* [25] and cotton with CLCuBuV [34].



(a)

(b)

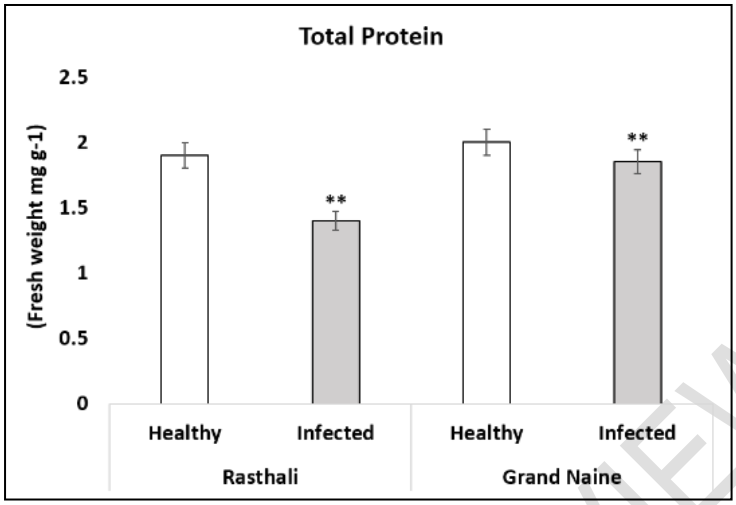


Fig. 4. (a) Total phenol content and (b) Total protein of healthy and infected Rasthali and Grand Naine plants.

*Data represents the mean ± standard error of mean of four independent replications. Significant differences in healthy and infected from each cultivar analysed by Student's t test (*P<0.05, **P<0.01) are shown.*

3.6 Enzyme activities

3.6.1 Peroxidase

The POX activity was increased significantly in BBTV infected plants of both cultivars, in comparison to healthy (Fig. 6). In an earlier report, a similar increase in the activity of POX was observed in Virupakshi and Grand Naine cultivar [1]. The peroxidases are enzymes whose primary function is to oxidize hydrogen donors at the expense of peroxides which is known to be involved in oxidative damage in response to stress to the plant. POX activity was found to be increased in chilli against chilli leaf curl virus as reported by [30].

3.6.2 Poly Phenol Oxidase

The polyphenol oxidase is involved in the formation of insoluble polyphenols in plants by the oxidation of the soluble phenols. The higher poly phenoloxidase activity was observed in Rasthali in healthy plants which marginally reduced after BBTV infection, in contrast, Grand Naine showed lower activity in healthy plants which increased during BBTV infection (Fig. 6). Higher total soluble phenols, together with higher PPO play a role in resistance to viral pathogens [1, 28].

3.6.3 Catalase

A significant elevation was observed in the CAT activity of BBTV-infected samples in both the banana cultivars tested (Fig. 5). Changes in catalase activity have been found to be a

significant monitoring index of plant responses under abiotic or biotic conditions. An increase in foliar CAT activity was observed in leaves of *Arachis hypogaea* infected with Peanut mottle virus [20] and cotton plants infected with the Cotton leaf curl burewala virus [34].

3.6.4 Ascorbate Peroxidase

The activity of ascorbate was significantly higher in BBTV infected plants of both cultivars when compared to the healthy (Fig. 5). APX acts as an antioxidant response triggered by the increasing presence of H_2O_2 within cells. One of the major peroxide detoxifying system in plant cells is the ascorbate-glutathione cycle, in which ascorbate peroxidase (APX) enzyme has a key role catalyzing the conversion of H_2O_2 into H_2O, using ascorbate as a specific electron donor. The increase in APX activity in BBTD infected banana was similar to that reported for *Hibiscus cannabinus* infected with begomovirus *Nicotiana benthamiana* infected with Pepper mild mottle virus [15] and sunflower infected with sunflower chlorotic mottle virus [31].

3.6.5 Guaiacol Peroxidase

Guaiacol peroxidase (GPX) activity was found to be significantly higher in BBTV infected cultivars when compared with healthy (Fig. 5 and 6). GPX is an essential group from peroxidase enzymes, which oxidize guaiacol and is found in cellular cytoplasm and apoplasm fractions, involved in a range of processes related to plant growth and development. GPX activity was found be higher in mesta plants infected with yellow vein mosaic virus as reported by [5]

3.6.6 Superoxide dismutase

The superoxide dismutase (SOD) is an enzyme that breaks down superoxide radical generated during stress into molecular oxygen or hydrogen peroxide thereby preventing cell damage. The SOD activity was significantly higher in the leaves of healthy plants of Rasthali in contrast to healthy plants of Grand Naine (Fig. 6). Upon BBTV infection, Rasthali showed increased SOD activity, whereas, Grand Naine showed decrease in SOD activity. In contrast, early reports show increase in SOD activity upon BBTV infection in Grand Naine [1]. SOD constitutes the front-line of defense against ROS and oxidative stress in plant cells and it is one of the most important scavenging enzymes. It is also reported that the induction of antioxidant enzymes, including SOD, is vital for the development of plant stress tolerance. Based on the present result, it can be concluded that higher SOD activity in Rasthali compared to Grand Naine might contribute to increased level of tolerance to BBTV infection in Rasthali and Grand Naine.

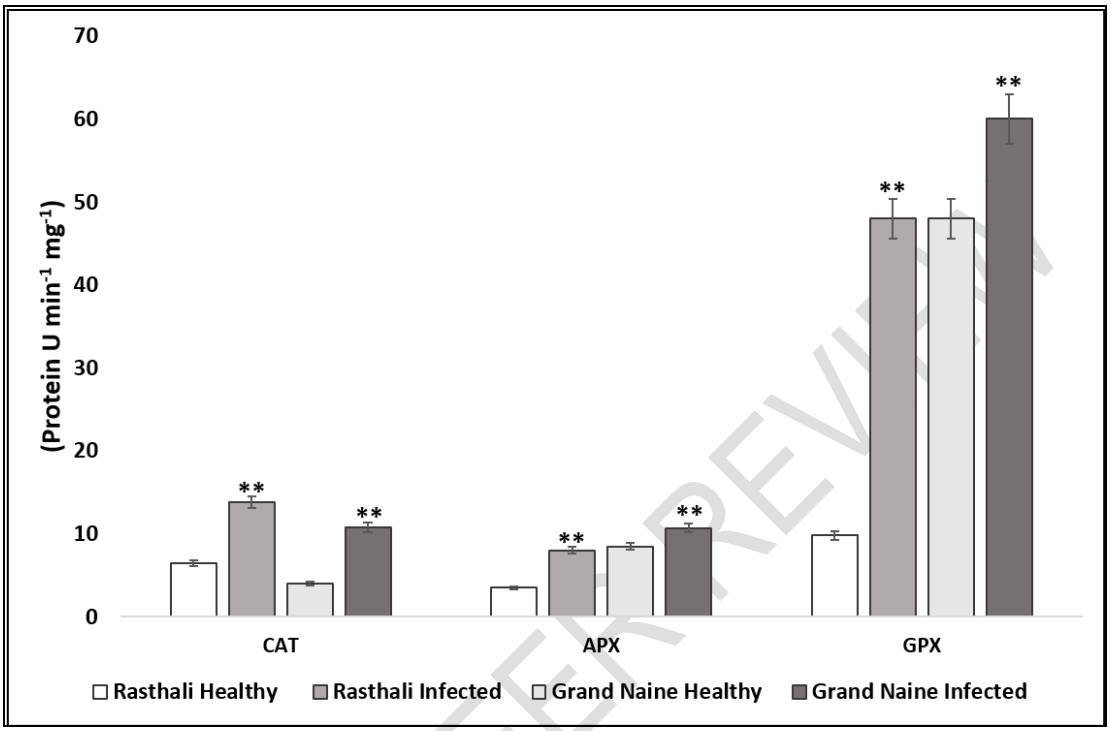


Fig. 5. Changes in enzyme activities of CAT, APX and GPX in healthy and BBTV infected Rasthali and Grand naine banana plants.

*Data represents the mean ± standard error of mean of four independent replications. Significant differences in healthy and infected from each cultivar analysed by Student's t test (*P<0.05, **P<0.01) are shown.*

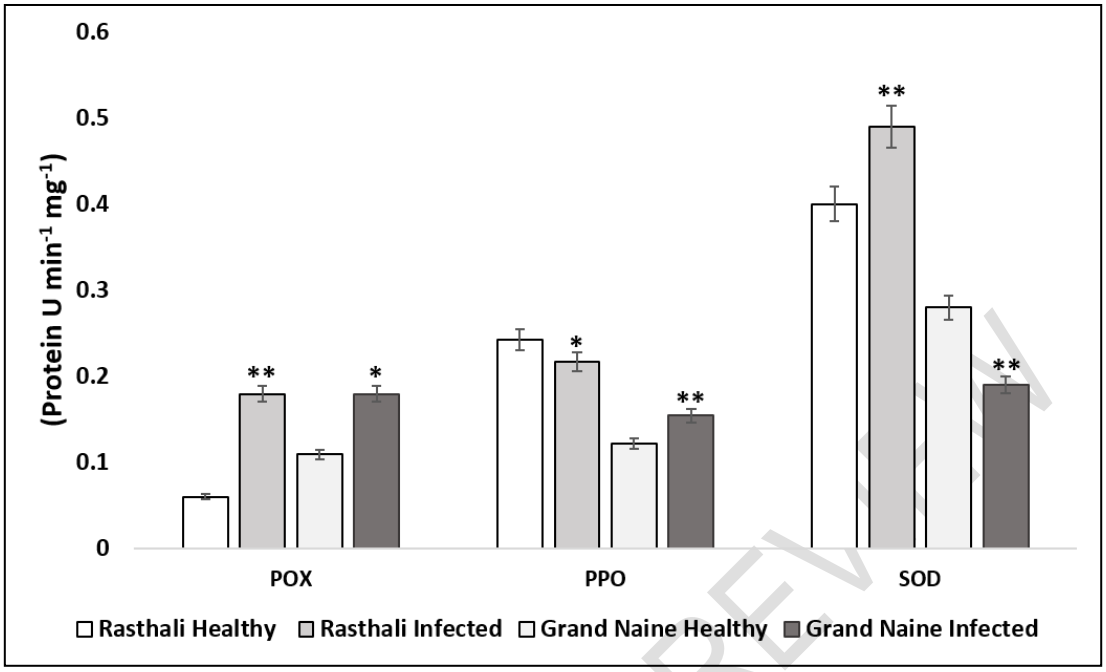


Fig.6. Changes in enzyme activities of POX, PPO and SOD in healthy and BBTV infected Rasthali and Grand Naine banana plants.

*Data represents the mean ± standard error of mean of four independent replications. Significant differences in healthy and infected from each cultivar analysed by Student's t test (*P<0.05, **P<0.01) are shown.*

4. CONCLUSION

It is well known that plant defense mechanism is complex, and the evolution of new strains of pathogens makes it a very difficult task to study. Various physiological and biochemical parameters were analyzed in BBTV infected and healthy banana cultivars Grand Naine and Rasthali. Our results indicated significant increase in defense enzyme activities in the BBTV infected cultivars compared to the healthy. There was a significant increase in amount of phenol and polyphenols in Rasthali in comparison to Grand Naine. The level of difference of biochemical constituents between the genotypes reverberates the variation of genotypes in defense against the BBTV. The findings of this study will help in better understanding of various physiological changes that occur in banana species against the BBTV and will contribute to plant resistance mechanisms which in turn will provide new tools for crop improvement.

REFERENCES

1. Anuradha C, Selvarajan R, Vasantha S, Suresha GS. Biochemical Characterization of Compatible Plant Virus Interaction: A Case Study with Bunchy Top Virus-Banana Host-Pathosystem. 2015:14 (4): 212-222.

2. Balachandran S, Hurry VM, Kelley SE, Osmond CB, Robinson SA, Rohozinski J, Seaton GG, Sims DA. Concepts of plant biotic stress. Some insights into the stress physiology of virus-infected plants, from the perspective of photosynthesis. Physiol Plant. 1997:100(2):203-13.
3. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Ana biochem. 1976:72(1-2):248-54.
4. Carvalho DD, Ferreira RA, Oliveira LM, Oliveira AF, Gemaque RC. Proteins and isozymes electrophoresis in seeds of Copaifera Langsdorffii Desf. (*Leguminosae caesalpinioideae*) artificially aged. Revista Árvore. 2006:30(1):19-24.
5. Chatterjee A, Ghosh SK. Alterations in biochemical components in mesta plants infected with yellow vein mosaic disease. Brazilian journal of plant physiology. 2008:20(4):267-75.
6. Chen GX, Asada K. Ascorbate peroxidase in tea leaves: occurrence of two isozymes and the differences in their enzymatic and molecular properties. Plant Cell Physiol. 1989:30(7):987-98.
7. Devanathan M, Ramaiah M, Sundar AR, Murugan M. Changes of peroxidase and polyphenol oxidase in bunchy top nana virus infected and healthy cultivars of banana. AoB Plants. 2005:19(1):114.
8. Dhindsa RS, Plumb-Dhindsa P, Thorpe TA. Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. J Exp Bot. 1981:32(1):93-101.
9. Dieng H, Satho T, Hassan AA, Aziz AT, Morales RE, Hamid SA, Miake F, Abubakar S. Peroxidase activity after viral infection and whitefly infestation in juvenile and mature leaves of *Solanum lycopersicum*. J Phytopathol. 2011:159(11-12):707-12.
10. Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure from small quantity of fresh leaf material. Phytochemical Bulletin. 1987:119: 11–15.
11. Dubois M, Gilles KA, Hamilton JK, Rebers PT, Smith F. Colorimetric method for determination of sugars and related substances. Anal Chem. 1956:28(3):350-6.
12. Folin O, Ciocalteu V. On Tyrosine and Tryptophan determinations in proteins. J Bio chem. 1927:73(2):627-50.

13. Gaddam SA, Kotakadi VS, Reddy MN, Saigopal DV. Antigenic relationships of citrus yellow mosaic virus by immunological methods. Asian J. Plant Sci. Res. 2012:566-9.
14. Goodman RN, Király Z, Zaitlin M. The biochemistry and physiology of infectious plant disease. The biochemistry and physiology of infectious plant disease.1967.
15. Hakmaoui A, Pérez-Bueno ML, García-Fontana B, Camejo D, Jiménez A, Sevilla F, Barón M. Analysis of the antioxidant response of *Nicotiana benthamiana* to infection with two strains of Pepper mild mottle virus. J Exp Bot. 2012:63(15):5487-96.
16. Hiscox JD, Israelstam GF. A method for the extraction of chlorophyll from leaf tissue without maceration. Can J Bot. 1979 Jun 15:57(12):1332-1334.
17. Jain AK, Yadava HS. Biochemical Constituents of Finger Millet Genotypes Associated with Resistance to Blast Caused by *Pyricularia grisea* Sacc. Ann Plant Prot Sci. 2003:11(1):70-4.
18. Jones JD, Dangl JL. The plant immune system. Nature. 2006:444(7117):323.
19. Király Z, Barna B, Kecskés A, Fodor J. Down-regulation of antioxidative capacity in a transgenic tobacco which fails to develop acquired resistance to necrotization caused by TMV. Free Radic Res. 2002:36(9):981-91.
20. Kobeasy MI, El-Beltagi HS, El-Shazly MA, Khattab EA. Induction of resistance in *Arachis hypogaea* L. against Peanut mottle virus by nitric oxide and salicylic acid. Physiological and Mol Plant Pathol. 2011:76(2):112-8.
21. Kushwaha KP, Narain U. Biochemical changes in pigeon-pea leaves infested with *Alternaria tenuissima*. Ann Plant Prot Sci. 2005:13(2):415-7.
22. Maechlay AC, Chance B. The assay of catalase and peroxidase. Methods of Biochemical Analysis. Interscience Inc., New York. 1954:357-424.
23. Malick CP, Singh MB. Plants enzymology. New Delhi: Kalyani Publishers. Anubis, with *Schostosoma mansoni* scadinarion. Journal of laboratory animal. 1980:34:119-26.
24. Manohar Jebakumar R, Selvarajan R. Biopriming of micropropagated banana plants at pre-or post-BBTV inoculation stage with rhizosphere and endophytic bacteria determines their ability to induce systemic resistance against BBTV in cultivar Grand Naine. Biocontrol Sci Technol. 2018:28(11):1074-90.

25. Meena RK, Patni V, Arora DK. Study on phenolics and their oxidative enzyme in *Capsicum annuum* L. infected with Geminivirus. Asian J. Exp. Sci. 2008:22(3):307-10.
26. McCready RM, Guggolz J, Silviera V, Owens HS. Determination of starch and amylose in vegetables. Anal Chem. 1950:22(9):1156-8.
27. Niyongere C, Ateka E, Losenge T, Blomme G, Lepoint P. Screening Musa genotypes for Banana Bunchy top disease resistance in Burundi. Acta horta.2011: 897.
28. Ngadze E, Icishahayo D, Coutinho TA, Van der Waals JE. Role of polyphenol oxidase, peroxidase, phenylalanine ammonia lyase, chlorogenic acid, and total soluble phenols in resistance of potatoes to soft rot. Plant Dis. 2012:96(2):186-92.
29. Parashar A, Lodha P. Phenolic estimation in *Foeniculum vulgare* infected with ramularia blight. Annals of Plant Protection Sciences. 2007:15(2):396-8.
30. Rai VP, Jaiswal N, Kumar S, Singh SP, Kumar R, Rai AB. Response of total phenols and peroxidase activity in Chilli exposed to pepper leaf curl virus disease. Vegetable Science. 2010:37(1):78-80.
31. Rodríguez M, Taleisnik E, Lenardon S, Lascano R. Are Sunflower chlorotic mottle virus infection symptoms modulated by early increases in leaf sugar concentration?. J Plant Physiol. 2010:167(14):1137-44.
32. Selvarajan R, Sheeba MM, Balasubramanian V, Rajmohan R, Dhevi NL, Sasireka T. Molecular characterization of geographically different banana bunchy top virus isolates in India. Indian Journal of Virology. 2010:21(2):110-6.
33. Sinha A, Srivastava M. Biochemical changes in mungbean plants infected by Mungbean yellow mosaic virus. Int J Virol. 2010:6(3):150-7.
34. Siddique Z, Akhtar KP, Hameed A, Sarwar N, Imran-Ul-Haq, Khan SA. Biochemical alterations in leaves of resistant and susceptible cotton genotypes infected systemically by cotton leaf curl Burewala virus. J Plant Interact. 2014:9(1):702-11.
35. Tornero P, Chao RA, Luthin WN, Goff SA, Dangl JL. Large-scale structure–function analysis of the Arabidopsis RPM1 disease resistance protein. The Plant Cell. 2002:14(2):435-50.

430 36. Upadhyaya A, Sankhla D, Davis TD, Sankhla N, Smith BN. Effect of paclobutrazol
431 on the activities of some enzymes of activated oxygen metabolism and lipid
432 peroxidation in senescing soybean leaves. J. pl. physiol. 1985:121(5):453-61.
433 37. Vanitha SC, Niranjana SR, Umesha S. Role of phenylalanine ammonia lyase and
434 polyphenol oxidase in host resistance to bacterial wilt of tomato. J. Phytopathology.
435 2009:157(9):552-7.
436 38. Zhao CJ, Wang AR, Shi YJ, Wang LQ, Liu WD, Wang ZH, Lu GD. Identification of
437 defense-related genes in rice responding to challenge by *Rhizoctonia solani*. Theor.
438 Appl. Genet. 2008:116(4):501-16.
439 39. Zhuang, Jun, Wenwu L, Christopher JC, Pengxiang S, Taiyun W, Zujjian W, Lainhui
440 X. Cleavage of the Babuvirus movement protein B4 into functional peptides capable
441 of host factor conjugation is required for virulence. Virologica sinica. 2019. 1-11.

442 **ABBREVIATIONS**

443 APX Ascorbate Peroxidase
444 CAT Catalase
445 GPX Guaiacol peroxidase
446 PPO Poly Phenol Oxidase
447 POX Peroxidase
448 SOD Superoxide dismutase

449