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Differential Biochemical Response among Banana (*Musa* spp.) Genotypes against Banana Bunchy Top Virus (BBTV)

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Authors' contributions

This work was carried out in collaboration among all authors. Authors NT and AR designed the study. Author NT performed the experiment and the statistical analysis. Authors NT and KKK wrote the manuscript and authors SV and KS corrected the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Banana bunchy top virus (BBTV) is one of the major viruses causing high yield loss in bananas. The study was carried out to gain a better understanding of the host and virus interaction and to explore the adaptive mechanism and biochemical responses in 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, polyphenol oxidase, catalase, ascorbate peroxidase, guaiacol peroxidase and

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superoxide dismutase. The virus infected samples of both cultivars showed a significant increase in the defense enzymes over the healthy sample. Higher total phenols in healthy Rasthali 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 content. 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.

ABBREVIATIONS

APX : Ascorbate Peroxidase
CAT : Catalase
GPX : Guaiacol peroxidase
PPO : Poly Phenol Oxidase
POX : Peroxidase
SOD : Superoxide dismutase

1. INTRODUCTION

Plants are frequently exposed to infection by a wide array of pathogens that lead to different responses in the host plant. During compatible plant-pathogen interaction, along with the development of visible symptoms, the pathogen adversely affects the growth and development, physiological status and yield of a plant [1]. The constitutive defense mechanism in the plants such as pre-existing physical and chemical barriers, along with inducible defense responses restrict pathogen colonization [2,3,4]. Bananas and plantains are important agricultural produce which are attacked by various pests and pathogens causing major production losses. *Grand Naine (AAA subgroup Cavendish) is* 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. Banana bunchy top disease (BBTD) is one of the most damaging viral diseases affecting various banana cultivars. There is no resistant germplasm available in bananas and plantains; however, the level of susceptibility varies among the banana cultivars.

BBTD infects the fruit and foliage. It is caused by a single-strand DNA virus, the banana bunchy top virus (BBTV; Genus *Babuvirus*; Family *Nanoviridae*). The virus colonizes in the phloem tissue and damages the host cells. The etiology of the disease comes from the typical symptoms which occurs in banana plants, in which the newly emerging leaves are narrow, chlorotic and reduced leaf size, which causes a "bunchy" appearance at the top. In addition, few distinctive symptoms are 'morse code streaking,' 'green J hooks' and 'keikis'. Viruses uses the host machinery for their replication and multiplication, which initiate significant changes in their usual physiological processes such as loss of pigment contents, increasing respiration rates, soluble sugar and starch accumulation and production of higher levels of enzymatic antioxidants. Due to viral infection, changes occur in the host plants at the molecular level, thereby leading to biological and physiological changes. Hence, it is of value to estimate the physiological and biochemical changes in banana cultivars Rasthali and Grand Naine and to measure 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. MATERIALS 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. Leaf samples from 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 isolated from 100 mg leaf samples of healthy and infected

(showing characteristic symptoms of BBTV) samples of Rasthali and Grand Naine using the cetyl trimethyl ammonium bromide (CTAB) method with some modification as described by Doyle and Doyle [5] and subjected to PCR using the BBTV specific primers designed for Replicase gene F-5' ACGACAGAATGG CGCGA3' and R- 5'TCAGCAAGAAACCA ACTTTATTC3'. 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

BBTV infected samples were collected from the most recent fully expanded leaf for biochemical analysis. 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 chlorophyll 'a', chlorophyll 'b' and total chlorophyll content of healthy and infected leaves were estimated according to the non-destructive DMSO method as explained by Hiscox and Israelstam [6]. The absorbance was recorded at 663 and 645 nm, respectively in a spectrophotometer. Chlorophyll a, b and total chlorophyll were calculated by the following formulas:

Chlorophyll a (mg g^{-1} tissue) = (12.7(OD663) - 2.69(OD645)/ x V x W) / 1000

Chlorophyll b (mg g^{-1} tissue) = (22.9(OD645) - 4.68(OD663)/ x V x W) / 1000

Total Chlorophyll (mg g^{-1} tissue) = (8.02(OD663) + 20.20(OD645)/ x V x W) / 1000

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 Dubios et al., [7] and the total starch content as suggested by McCready et al., [8]. The absorbance of the samples was recorded at 625 nm in a spectrophotometer along with the blank sample. The amount of total sugars and total starch was estimated by using a standard curve prepared for D-glucose. The content of reducing sugar and total starch was expressed as mg g^{-1} fresh weight.

2.6 Phenolic Content

Phenol content was measured using Folin-Ciocalteau reagent and estimated using the method described by Folin and Ciocalteau [9]. The absorbance of the samples 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.

2.7 Measurement of Total Protein Content

Total protein was estimated by using the Bradford method [10] 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 according to the method given by Malick and Singh [11]. The absorbance of the reaction mixture of the sample was recorded at 430 nm at 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 Ngadze et al., [12]. 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 Maechlay and Chance [13]. 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 Chen and Asada [14]. 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. [15]. 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 Dhindsa et al*.,* [16]. The absorbance of the reaction mixture was recorded spectro-photometrically 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.)

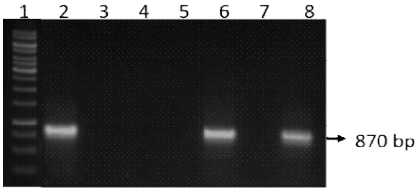


Fig. 1. PCR amplification of BBTV Rep gene in symptomatic Rasthali and Grand Naine plants

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 plants exhibited a two- fold reduction in photosynthetic pigment contents (chlorophyll a, chlorophyll b and total chlorophyll) compared to healthy plants (Fig. 2). The decrease in the chlorophyll content in the infected plant reduces the photosynthetic capacity and plant growth resulting in the symptoms such as stunting and chlorosis. This change in the chlorophyll content can be due to the stimulation of enzymes like chlorophyllase that degrades chlorophyll [17], or it may be due to the effect of the virus on pigment synthesis [18,19]. A recent study suggests the possibility of BBTV utilizing the chloroplast for the synthesis of viral proteins [20]. They found during BBTV infection, outer membranes of chloroplasts are disrupted and crystalline aggregation of virus-like particles accumulate in it.

3.3 Carbohydrates

The total sugars and starch were significantly higher in infected plants compared to healthy in both the cultivars of banana (Fig. 3 a-b). Our study suggests that sugars increase during BBTV infection may control photo inhibitory processes and produce symptoms. The carbohydrate content reported by Anuradha et al. [21] 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. Viruses appear to alter both their rate of synthesis and rate of translocation and have little effect on carbohydrates which affects the overall growth of the plant [22].

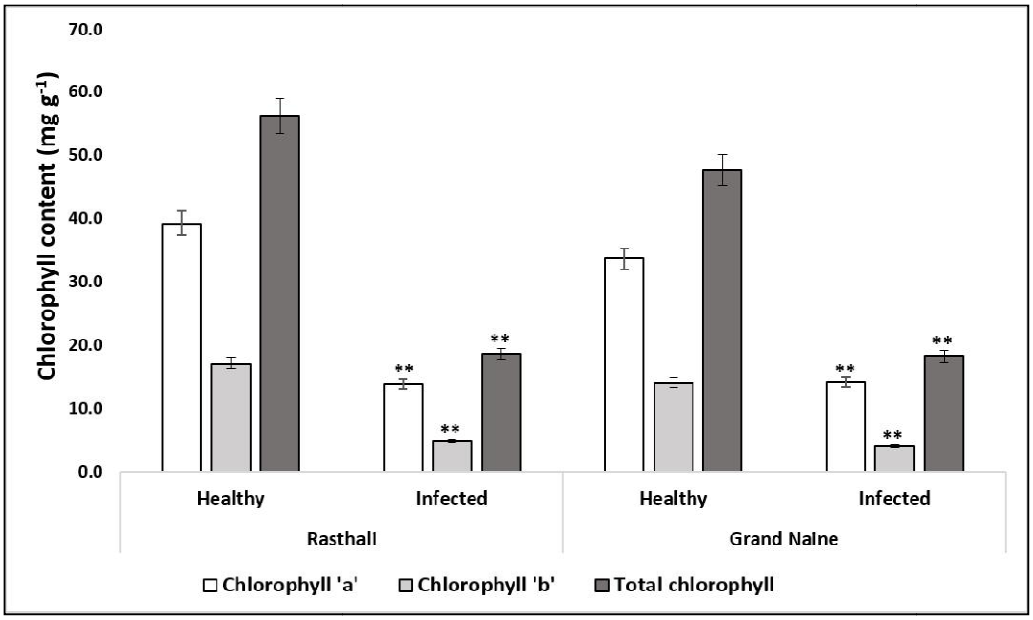


Fig. 2. Chlorophyll content of healthy and infected Rasthali and Grand Naine plants

*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*

3.4 Total Phenol

In the present investigation a significant variation in the total phenolic compounds of banana cultivars in response to infection with BBTV was apparent (Fig. 4a). The total phenol content was significantly higher in virus-infected leaves in both the cultivars tested. Increased quantities of phenols might be attributed to a defense mechanism where plant polyphenols act as secondary metabolites. It has been reported that resistance to disease caused by pathogens can be attributed to the presence of a high amount of phenol [11,19,23,24,25]. It has been reported that Rasthali viral infection occurs later than in the Grand Naine cultivar because of the difference in the genomes [26]. 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, but to a lower level after BBTV infection. Hence, the increased phenolics in the infected plant may be contributing to the resistance against the infection of viral pathogens [27].

3.5 Total Protein

Protein content was found to decrease significantly in the BBTV infected plants of both cultivars (Fig. 4b). The involvement of host proteins in disease resistance has been demonstrated in various plant pathogenic interactions [15,28]. The results obtained are in accordance with the results of Tobacco mosaic virus-infected tobacco plants [29], Tomato yellow leaf curl virus-infected tomato plants [30], Banana bunchy top virus-infected cultivars of banana [31], geminivirus infected *Capsicum annum* [32] and cotton with CLCuBuV [33].

3.6 Enzyme Activities

3.6.1 Peroxidase

Peroxidase 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 [21]. 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 Rai et al. [34].

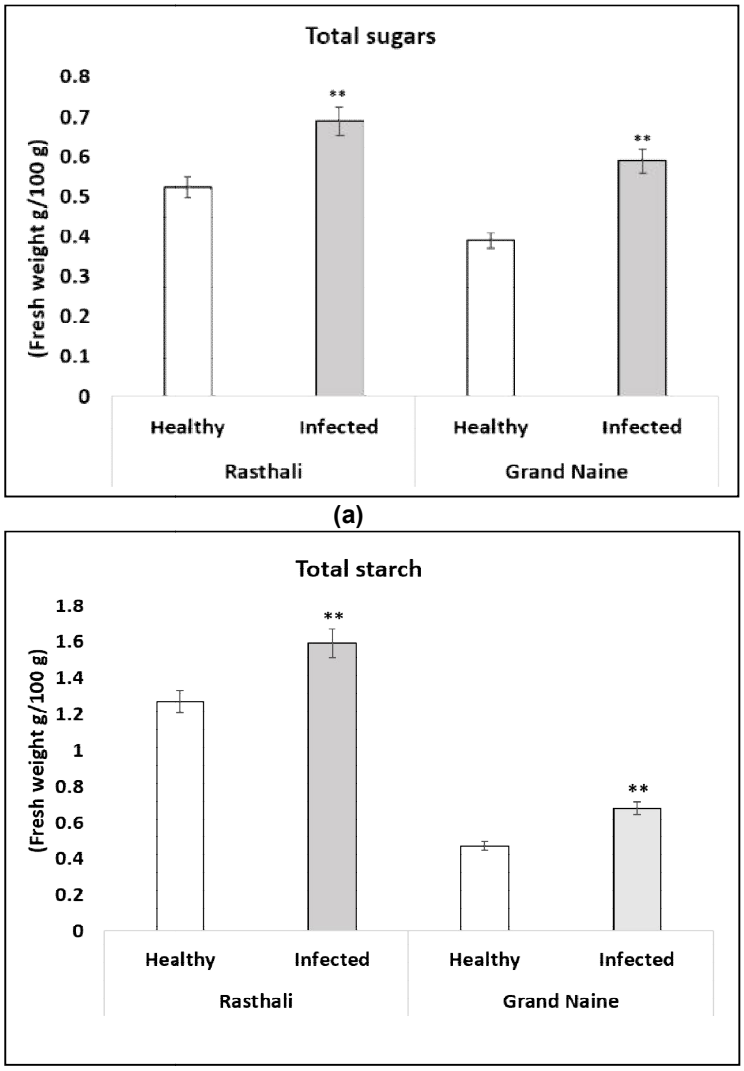


Fig. 3. Carbohydrate content (a) Total sugars and (b) Starch of healthy and infected Rasthali and Grand Naine plants

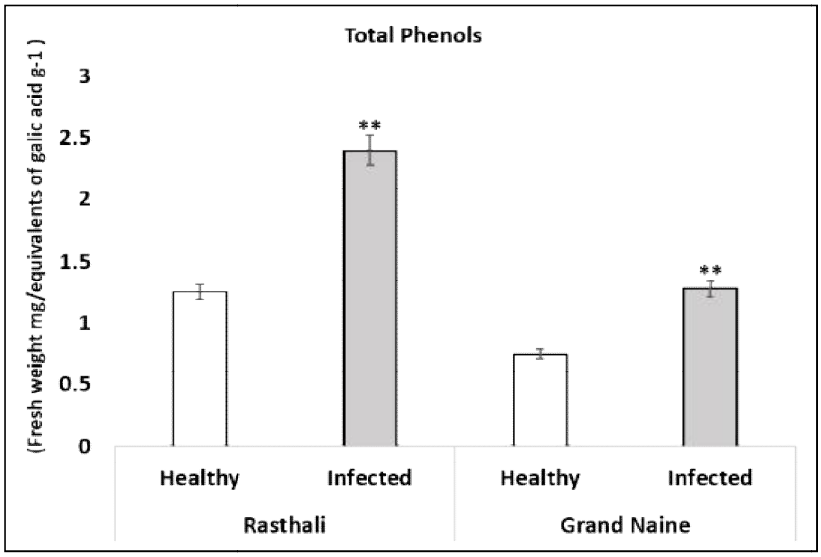
*Data represents the mean ± standard error of mean of four independent replications. Significant differences in healthy and infected from each cultivar analysed by Student's t test (*P<0.05, **P<0.01) are shown*

3.6.2 Poly phenol oxidase

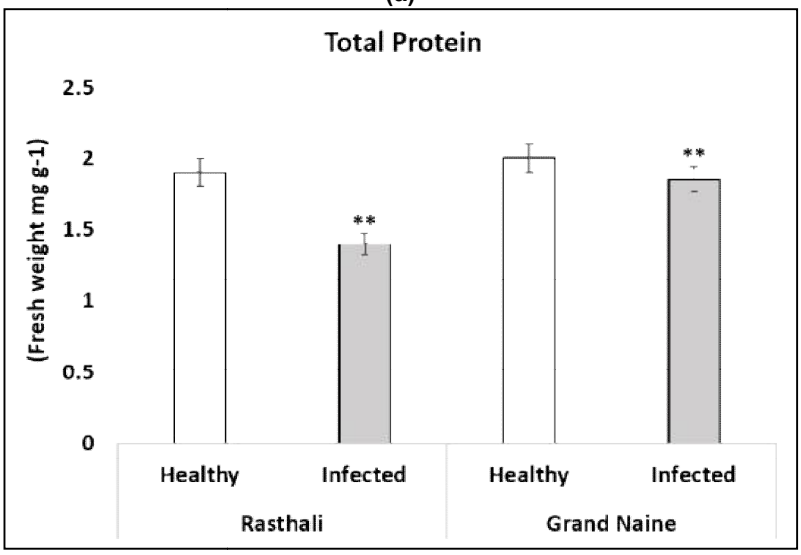
Polyphenol oxidase is involved in the formation of insoluble polyphenols in plants by the oxidation of soluble phenols. Higher poly phenol oxidase activity was observed in Rasthali in healthy plants which marginally reduced after BBTV infection, in contrast, Grand Naine showed lower activity in healthy plants which increased during BBTV infection (Fig. 6). Higher total soluble phenols, together with higher PPO have been demonstrated to play a role in resistance to viral pathogens [12,21].

3.6.3 Catalase

A significant elevation was observed in the CAT activity of BBTV-infected samples in both banana cultivars tested (Fig. 5). Changes in catalase activity have been found to be a significant monitoring index for plant responses under abiotic or biotic conditions. An elevation in the CAT activity was reported in leaves of *Arachis hypogaea* infected with Peanut mottle virus [35] and cotton plants infected with the Cotton leaf curl burewala virus [33].



(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*

3.6.4 Ascorbate peroxidase

The activity of ascorbate peroxidase was significantly higher in BBTV infected plants of both cultivars when compared to the healthy (Fig. 5). Peroxidases acts as an antioxidant response activated by the increasing presence of H_2O_2 within cells. Among the major peroxide detoxifying systems in plant cells ascorbate-glutathione cycle, is the one in which ascorbate peroxidase enzyme plays a key role catalyzing the conversion of H_2O_2 into H_2O. The increase in APX activity in BBTD infected banana was similar to reports of *Hibiscus cannabinus* infected with begomovirus *Nicotiana benthamiana* infected with Pepper mild mottle virus [36] and sunflower infected with sunflower chlorotic mottle virus [37].

3.6.5 Guaiacol peroxidase

Guaiacol peroxidase (GPX) activity was recorded to be significantly higher in BBTV infected cultivars when compared with healthy

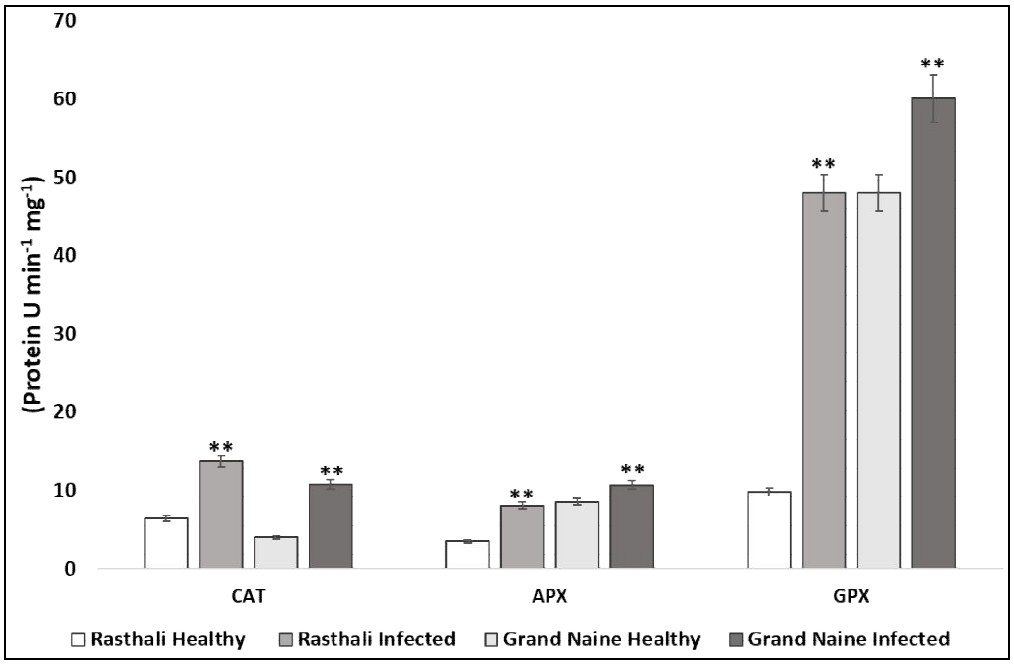


Fig. 5. Changes in enzyme activities of CAT, APX and GPX in healthy and BBTV infected Rasthali and Grand Naine Banana plants

*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*

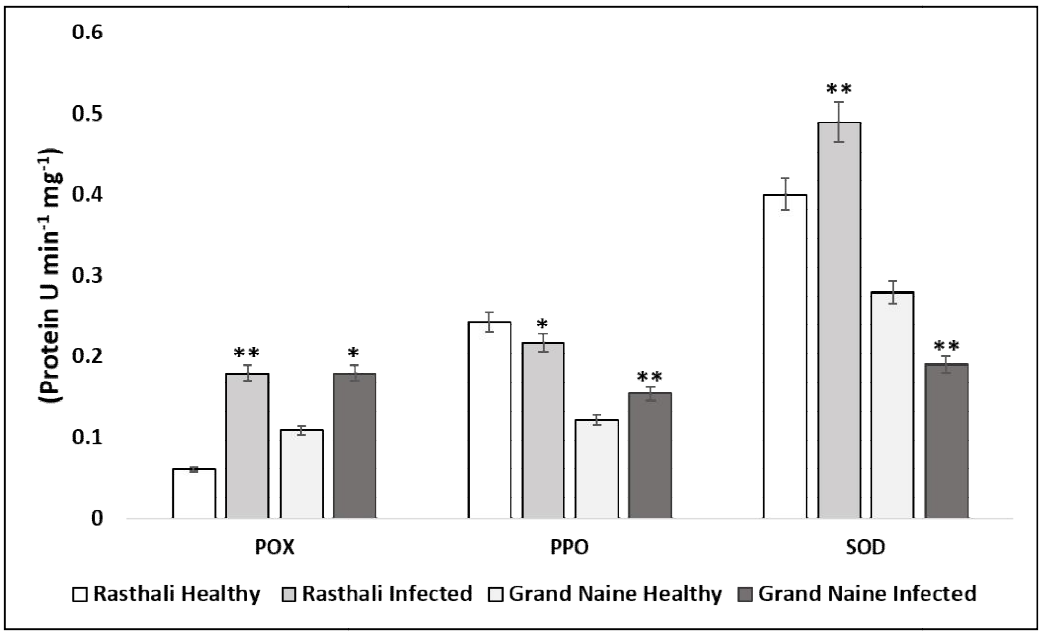


Fig. 6. Changes in enzyme activities of POX, PPO and SOD in healthy and BBTV infected Rasthali and Grand Naine banana plants

*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*

(Figs. 5 and 6). GPX is an essential group from peroxidase enzymes, which oxidize guaiacol and are found in cellular cytoplasm and apoplasm fractions. It is 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 Chatterjee and Ghosh [38].

3.6.6 Superoxide dismutase

Superoxide dismutase (SOD) is an enzyme that breaks down superoxide radical generated during stress into molecular oxygen or hydrogen peroxide thereby preventing cell damage. 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 showed an increase in SOD activity upon BBTV infection in Grand Naine [21]. SOD constitutes the front-line of defense against ROS and oxidative stress in plant cells and also is one of the 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.

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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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