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Biochemical Characterization of Compatible Plant Virus Interaction: A Case Study with Bunchy Top Virus-Banana Host-Pathosystem

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ABSTRACT
Virus infection can result in the alteration of physiological, biochemical and metabolic processes within plants leading to symptom development. *Banana bunchy top virus* (BBTV) is one of the most destructive viral diseases in Tropical Asia, Pacific Indian Oceania (PIO) regions and Africa leading to 100% yield loss in banana and plantains. Though molecular characterization and their diversity were studied in depth in recent years, information on physiological and biochemical changes during banana-BBTV interaction is still not convincingly explained. Therefore, the present investigation was conducted to find out the quantifiable changes in physiological and biochemical parameters such as proteins, pigment and carbohydrate content, phenolic compounds, polyphenol oxidase (PPO), peroxidase (POX), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), catalase (CAT) and superoxide dismutase (SOD) activities in leaves of banana cultivars Grand Nain (AAA) and Virupakshi (AAB). The amount of carbohydrate contents, phenolic compounds, PPO, POX, APX, GPX, CAT were significantly higher in BBTV infected leaves of both the cultivars over the healthy, whereas total protein content, pigments and SOD activity showed an opposite trend. Overall the results suggest that BBTV infection induces significant changes in enzyme levels leading to irreversible symptom development. Further studies would lead to identification of biochemical markers for studying plant-virus compatible and incompatible interactions.

Key words: BBTV, banana, biochemical changes, pigments, carbohydrate, antioxidant enzymes

INTRODUCTION

Environmental stresses of both biotic and abiotic nature produce characteristic changes in physiology and metabolic processes of higher plants (Miteva *et al*., 2005). Among these stresses, infection by the pathogens causes substantial alterations of biochemical changes leading to harmful effects on plant health. Banana cultivation is subjected to many natural calamities including pest and pathogen attacks which constitute the major production problem. Among plant diseases, viral diseases may cause considerable loss on production by retarding plant growth and reducing yield. Four viral diseases viz., Banana Bunchy Top Disease (BBTD) caused by *Banana bunchy top virus* (BBTV), Bract Mosaic Disease (BBrMD) caused by *Banana bract mosaic virus* (BBrMV), Banana Streak Disease (BSD) caused by different species of *Banana streak virus* (BSV) and banana mosaic or infectious chlorosis caused by *Cucumber mosaic virus* (CMV) occur in most of the banana growing regions of the world. Among banana viral diseases, Banana Bunchy Top Disease (BBTD) is one of the most destructive viral diseases in Tropical Asia, Pacific Indian Oceania (PIO) regions and

Africa. Grand Nain, Virupakshi (Hill banana), Robusta, Nendran, Rasthali, Poovan, Ney Poovan, Monthan and Red Banana are severely affected by BBTD. There is no resistance gene source available in germplasm of bananas and plantains (Shekhawat *et al*., 2012). The BBTV affected plants show intermittent dark green dots, dash, streaks of variable length on leaf sheath, midrib, leaf veins and petioles of infected plants. Leaves produced are progressively shorter, brittle in texture, narrow and gives the appearance of bunchyness at the top leading to 100% yield loss (FAO., 2009). Plants have evolved various pre-existing physical and chemical barriers, as well as inducible defense responses that interfere with pathogen colonization (Jones and Dangl, 2006; Zhao *et al*., 2008; Vanitha *et al*., 2009). However, this requires comprehensive studies and understanding of the adaptive mechanisms and responses to BBTV infection in banana. Because viruses cannot be cultured *in vitro*, our knowledge about their interactions with host and changes on physiology, biochemistry and molecular biology of the host is limited. Moreover, a comprehensive report regarding biochemical alterations in banana plants infected by BBTV is still not clear. Therefore, the present investigation was conducted to find the quantitative estimations of physiological and biochemical parameters such as protein, pigment and carbohydrate contents, phenolic compounds, polyphenol oxidase (PPO), peroxidase (POX), catalase (CAT), ascorbate peroxidise (APX), guaiacol peroxidise (GPX) and superoxide dismutase (SOD) activities, indicating their role in BBTV inoculated and non-inoculated plants of Virupakshi (hill banana), a unique flavoured elite dessert banana cultivar and Grand Nain, which belongs to a Cavendish sub group occupying approximately 50% of area in the world.

MATERIALS AND METHODS

Two cultivars of banana, Virupakshi (hill banana) and Grand Nain were used in the present investigation. Three month old suckers were planted in pots containing a mixture of sand, loam soil and compost (1:1:2). The pots were kept in insect-proof glass house. Virus vector aphids (fifteen adult or late instar aphids) were collected and transferred to healthy banana plants having four to five well developed leaves for inoculation access for 48 h. Inoculation access feeding was conducted at 25±0.5°C and 12 h light/dark photoperiod. At the completion of inoculation-access period all plants were sprayed with insecticide imidacloprid (0.1%) and kept in the greenhouse to monitor the symptom expression. All the plants showed typical BBTD symptoms after 20-30 days of inoculation. The un-inoculated plants (healthy plants) of each cultivar were maintained as control. Leaf samples were harvested from both controls and inoculated plants after symptom development to determine the enzyme activities.

Estimation of chlorophylls: Total chlorophyll, chlorophyll 'a' and chlorophyll 'b' contents of healthy and infected leaves were estimated as per the non-destructive DMSO method (Hiscox and Israelstam, 1979). The leaf discs (500 mg) were collected in test tubes, to this 10 mL of DMSO were added. The tubes were kept overnight in dark for 1-2 h. The absorbance was recorded at 663 and 645 nm in a spectrophotometer. Chlorophyll a, b and total chlorophyll were calculated by using following formulas:

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

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

$$\text{Total Chlorophyll (mg g}^{-1}\text{ tissue)} = \frac{20.2\ (OD)+8.02\ (OD645)\times V}{1000}\times W$$

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

Estimation of total sugars and starch: The estimation of total sugars was done according to the method of DuBois *et al.* (1956) and total starch content by method of McCready *et al.* (1950). Briefly, 500 mg of healthy and infected leaves were taken, washed thoroughly with tap water followed by distilled water and blotted to dry in between filter paper folds. The midribs of leaf samples were removed, cut into bits and macerated with 5 mL of 80% ethanol. The macerates were transferred to centrifuge tubes and centrifuged at 5000 rpm for 15 min. The pellet was washed thrice with 80% ethanol. The supernatants were pooled and made upto known volume with 80% ethanol. The samples were heated in water bath at 85°C until the alcohol was completely lost from the samples. The supernatants were pooled and used for estimation of sugars. The pellet was subsequently used for extraction and estimation of starch.

For sugar determination, 20 mL of healthy and virus infected pooled supernatants were taken separately into the test tubes. One milliliter of distilled water and 4 mL of cold anthrone reagent were rapidly added to each tube, shaken well and incubated for 10 min on ice bath and cooled at room temperature. The blank was prepared by taking 1 mL of distilled water and 4 mL of cold anthrone reagent. The absorbance of the samples was read at 625 nm in a spectrophotometer. Amount of total sugars was estimated by using a standard curve prepared for D-glucose.

For starch determination, the pellet which was collected while preparing the extract for total sugar was solubilized in 5 mL of 52% perchloric acid (PCA) and boiled at 80°C for 10 min. The solution was filtered through glass wool. The filtrate was measured and made up to 10 mL with PCA. Twenty microliter of healthy and infected sample extracts were

taken separately, added 3 mL of distilled water and 5 mL of anthrone reagent and incubated for 10 min in ice bath. The absorbance of the samples was read at 625 nm in a spectrophotometer. The amount of starch was calculated by using glucose standard curve.

Estimation of phenol: The phenol content was estimated using Folin-Ciocalteau reagent. 80% ethanol was used for extraction of phenols. One gram plant material was ground, divided into two 5 mL portions of 80% ethanol and centrifuged. The extracts were pooled and made up to 10 mL. Then, 0.1 mL of ethanol extract was evaporated on a water bath, to which 6 mL water was added and shaken well before addition of 0.5 mL Folin-Ciocalteau reagent. After 5 min, 2 mL of 20% sodium carbonate solution were added. After incubation for 30 min, absorbance at 660 nm was measured. Using pyrocatechol as standard, the phenol content in the leaf extract was calculated (Folin and Ciocalteu, 1927).

Preparation of enzyme extract: One gram of 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] with mortar and pestle. The homogenate was then centrifuged at 12000 rpm for 20 min at 4°C and the supernatant was used as the crude extract for the estimation of POX, PPO, catalase, APX, GPX and SOD activities.

Enzyme assays: Peroxidase activity was assessed following the oxidation of O-dianisidine using the method of Malic and Singh (1980). For the assay 3.5 mL of phosphate buffer (pH-6.5) were taken in a clean dry cuvette and to it 0.2 mL of enzyme extract and 0.1 mL of freshly prepared O-dianisidine solution was added. Then 0.2 mL of 0.2M H_2O_2 were added and immediately absorbance of the reaction mixture was read at 430 nm at every 30 sec interval upto 3 min.

Polyphenol oxidase activity was measured according to the method described by Ngadze *et al.* (2012).

Catalase activity was assayed by measuring the rate of disappearance of H_2O_2 using the method of Maehly and Chance (1959). The reaction mixture contained 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 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.

Ascorbate peroxidase activity was determined according to the method of Chen and Asada (1989) with minor modification. The 1 mL reaction mixture was composed 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 the decrease in the absorbance at 240 nm.

Guaiacol peroxidase activity was determined according to Upadhyaya *et al.* (1985). The reaction mixture contained 2.5 mL of 50 mM phosphate buffer (pH 6.1), 1 mL of 1% H_2O_2, 1 mL of 1% guaiacol and 20 µL of enzyme extract. The increase in absorbance at 420 nm was observed for 1 min.

Superoxide dismutase activity was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) using the method of Dhindsa *et al.* (1981). The 3 mL reaction mixture contained 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 shaken and placed 30 cm below a light bank consisting of two 15 W fluorescent lamps for 10 min. The absorbance of the reaction mixture was read at 560 nm.

Estimation of total protein content: Total protein was estimated colorimetrically by using Bradford method (Bradford, 1976) recording absorbance at 595 nm. Bovine serum albumin was used as standard. Protein content in leaf samples was recorded as µg of protein per gram of leaf tissue.

Statistical analysis: All experiments were repeated twice with similar results. Data was subjected to an analysis of variance and means and standard errors calculated.

RESULTS AND DISCUSSION

The physiology of virus infected plant tissues is of prime interest to understand the processes involved in the development of symptoms. There are several reports in the literature indicating many changes in the physiology and biochemistry of host plants as a consequence of viral disease (Radwan *et al*., 2007). Many investigators (Matthews, 1970) in different host virus combinations have estimated and correlated the virus concentration to altered metabolic processes and symptom production and severity.

Chlorophyll: Leaf chlorophyll content can be directly related to stress physiology affecting the growth and yield. Virus infection induces changes in leaf pigmentation, hence the symptoms. The relative chlorophyll content of banana leaves was compared between BBTV infected and healthy plants. Drastic reduction of chlorophyll a, chlorophyll b and total chlorophyll was observed in infected Virupakshi and Grand Nain leaves (Fig. 1a-c). From the values of a/b ratio it can be deduced that chlorophyll b is more sensitive to viral infection than chlorophyll a. Reduced chlorophyll content has been reported in virus infected plants (Sinha and Srivatsava, 2010) and is attributed to stimulation of cell enzymes like chlorophyllase that degrades chlorophyll (Goodman *et al*., 1967), or it may be the effect of virus on pigment synthesis (Porter, 1959; Sadasivan, 1963; Granick and Beale, 1983; Balachandran *et al*., 1997) and disturbed physiological processes like photosynthesis and utilization of plastid proteins or their precursors for the synthesis of virus protein

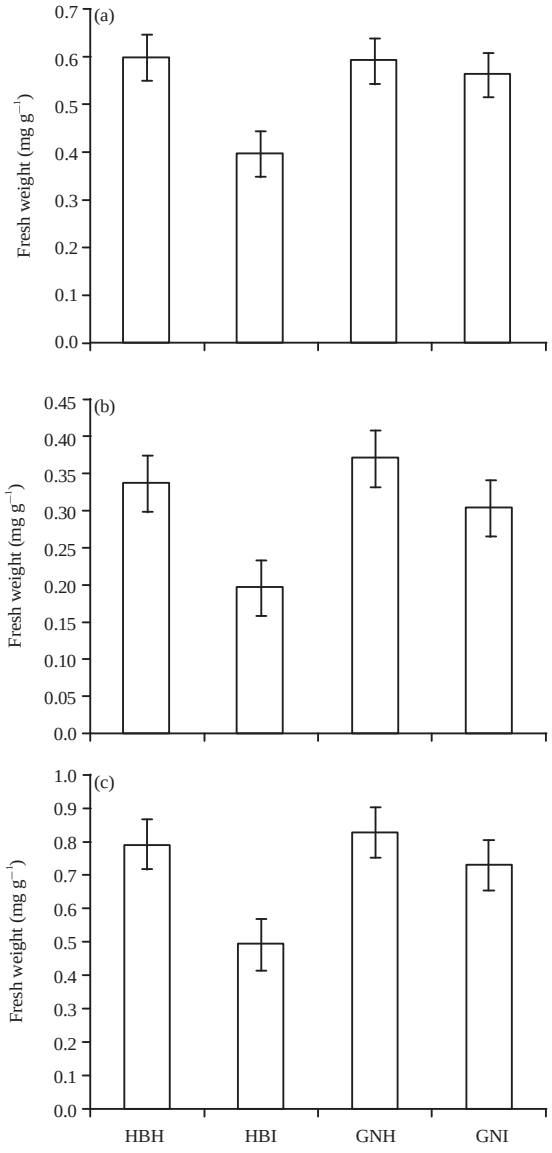

Fig. 1(a-c): Chlorophyll content (a) Chlorophyll a (b) Chlorophyll b and (c) Total chlorophyll of healthy and infected Virupakshi (HB) and Grand Nain (GN) plants

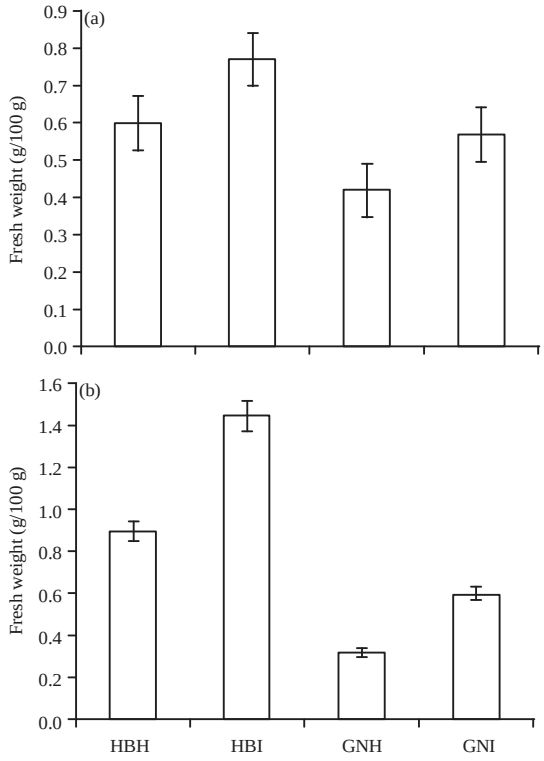

Fig. 2(a-b): Carbohydrate content (a) Total sugars and (b) Starch of healthy and infected Virupakshi (HB) and Grand Nain (GN) plants

(Fraser, 1987). Other reasons for the decrease in chlorophyll content following virus infection may be accumulation of carbohydrates in the leaves (Watson, 1955). As in case of many different virus-host interactions, altered ratio of chlorophylls due to BBTV was observed probably affecting the photosynthetic efficiency (Endo *et al*., 2001). According to Sheffield (1933), the virus of tomato aucuba mosaic did not affect the chlorophyll in older leaves but prevented the formation of plastids in young growing leaves, on the other hand cucumber mosaic in tomato produced stripe chlorosis showing destruction of already formed chlorophyll. Subsequent studies with improved techniques have shown that the chlorophyll 'a' associated with the reaction centre of photosystem II (i.e., CPa complex) was reduced to a greater extent than the antenna chlorophyll of light harvesting system (i.e., LHCPY, 1, 2, 3 and II d complexes). Changes in pigments are often considered to be the secondary effects on the host plant since many viruses appear to multiplying and accumulate in the cytoplasm of the cell. Although, these changes appear to be secondary as far as synthesis is considered, they are important for disease induction or resistance process, considering the plant as a whole (Crosbie and Matthews, 1974).

Carbohydrates: Influence of plant pathogenic viruses on the carbohydrate metabolism of the infected host is very important with regard to economic damage caused to host. 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 (Gaddam *et al*., 2012). In the present investigation, the total sugars and starch were high in infected plants compared to healthy (Fig. 2a-b). Regarding viruses, carbohydrates appear to have a role in plant defences and it has been known since the 1930s that accumulation of starch precedes the presence of virus symptoms (Holmes,1931) and for example, marrow plants infected with *Zucchini yellow mosaic virus* (ZYMV) (Blua *et al*., 1994), mosaic infected acid

lime (Gaddam *et al*., 2012), ZYMV of *Cucurbita pepo* (Radwan *et al*., 2007), *Sunflower mosaic virus* (Bhavani *et al*., 1998), *Papaya meleira virus* (Buss *et al*., 2011) and *Yellow virus* infected sugar beet (Watson, 1955) show increased sucrose content. A link of carbohydrates with defences against oxidative stress has been observed in other studies (Sulmon *et al*., 2004; Loreti *et al*., 2005) and carbohydrates are essential in the production of many anti-oxidant defences (Couee *et al*., 2006).

Our results suggest that sugar increases during BBTV infection may modulate photoinhibitory processes which probably induce the symptoms. Virus infection also induces singlet oxygen generation resulting from the photoinhibitory process. Singlet oxygen (O_2^1) is a potent agent of oxidative damage that could also contribute to chlorotic symptom development (Fryer *et al*., 2002). The BBTV infection could in turn increases ROS generation in other cellular compartments (Diaz-Vivancos *et al*., 2008; Song *et al*., 2009) in association with respiratory and photorespiratory processes. These alternative ROS sources might contribute to the development of chlorotic symptoms.

Total protein: Involvement of protein components in plant diseases resistance has been documented in plant pathogenic interactions (Tornero *et al*., 2002; De Carvalho *et al*., 2006). Usually, infected plants show a high protein content, which could be due to both the activation of the host defense mechanism and the pathogen attack mechanism (Agrios, 1997). However, protein content was found to decrease significantly in the BBTV infected plants of both the cultivars (Fig. 3a). A possible explanation may be that the disease might have caused denaturation or break down of proteins as well as polypeptide chains and bound amino acids. Similar results have also been reported in Mesta infected with yellow vein mosaic disease (Chatterjee and Ghose, 2008) and in cotton with CLCuBuV (Siddique *et al*., 2014).

Total phenol: Upon infection by pathogens, the host phenolic compounds may increase and contribute to enhance the mechanical strength of host cell walls by the synthesis of lignin and suberin that are involved in the formation of physical barriers that can block the spread of pathogens (Ngadze *et al*., 2012; Singh *et al*., 2014). In the present study, the amount of total phenol was significantly higher in virus infected leaves (Fig. 3b) and the increased quantity of phenols might be attributed to a defence mechanism (Rai *et al*., 2010). The resistance to disease caused by pathogen was attributed to the presence of high amount of phenol (Jain and Yadav, 2003; Kushwaha and Narain, 2005; Parashar and Lodha, 2007; Meena *et al*., 2008; Rai *et al*., 2010; Siddique *et al*., 2014). Hence, the increased quantity of phenolics in the infected plant of the banana may be contributing to the resistance against the infection of viral pathogen. Increased levels of phenolics also suggest an acceleration of phenol synthesizing pathway following pathogen infection.

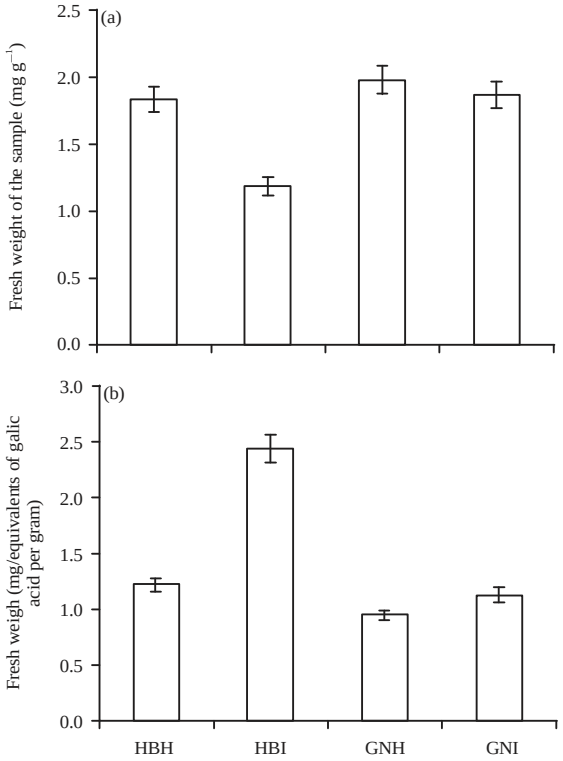

Fig. 3(a-b): (a) Total protein and (b) Total phenol content of healthy and infected Virupakshi (HB) and Grand Nain (GN) plants

Peroxidase: Peroxidase (POX) is one of the first enzymes responding and providing fast defense against plant pathogens (Sulman *et al*., 2001). The POXs are involved in the lignification, suberification, polymerization of hydroxy-proline-rich glycoproteins, regulation of cell wall elongation, wound healing and resistance against pathogens in plants (Hammond-Kosack and Jones, 1996; Yoshida *et al*., 2003; Maksimov *et al*., 2014). The POX activity was significantly higher in BBTV infected plants of both cultivars, as compared to healthy (Fig. 4a). This result is in accordance with the results of *Tobacco mosaic virus* infected tobacco (Lagrimini and Rothstein,1987), plum pox virus infected peaches, apricots (Diaz-Vivancos *et al*., 2006), *Beans yellow mosaic virus* infected beans (Radwan *et al*., 2010), *Potato virus* Y infected potato (Milavec *et al*., 2001), tomato and bell pepper infected with *Tobacco mosaic virus* and *Tomato mosaic tobamo virus* (Madhusudhan *et al*., 2009), *Cucumber mosaic virus* and *Zucchini yellow mosaic virus* infected *Cucumis sativus* and *Cucurbita pepo* plants (Riedle-Bauer, 2000), *Tobacco mosaic virus* infected tobacco plants (Kiraly *et al*., 2002), *Tomato yellow leaf curl virus* infected tomato plants (Dieng *et al*., 2011), *Banana bunchy top virus* infected cultivars of banana (Devanathan *et al*., 2005), geminivirus infected *Capsicum annum* (Meena *et al*., 2008) and cotton with CLCuBuV (Siddique *et al*., 2014). Virus infection appears to stimulate POX activity in all hosts in

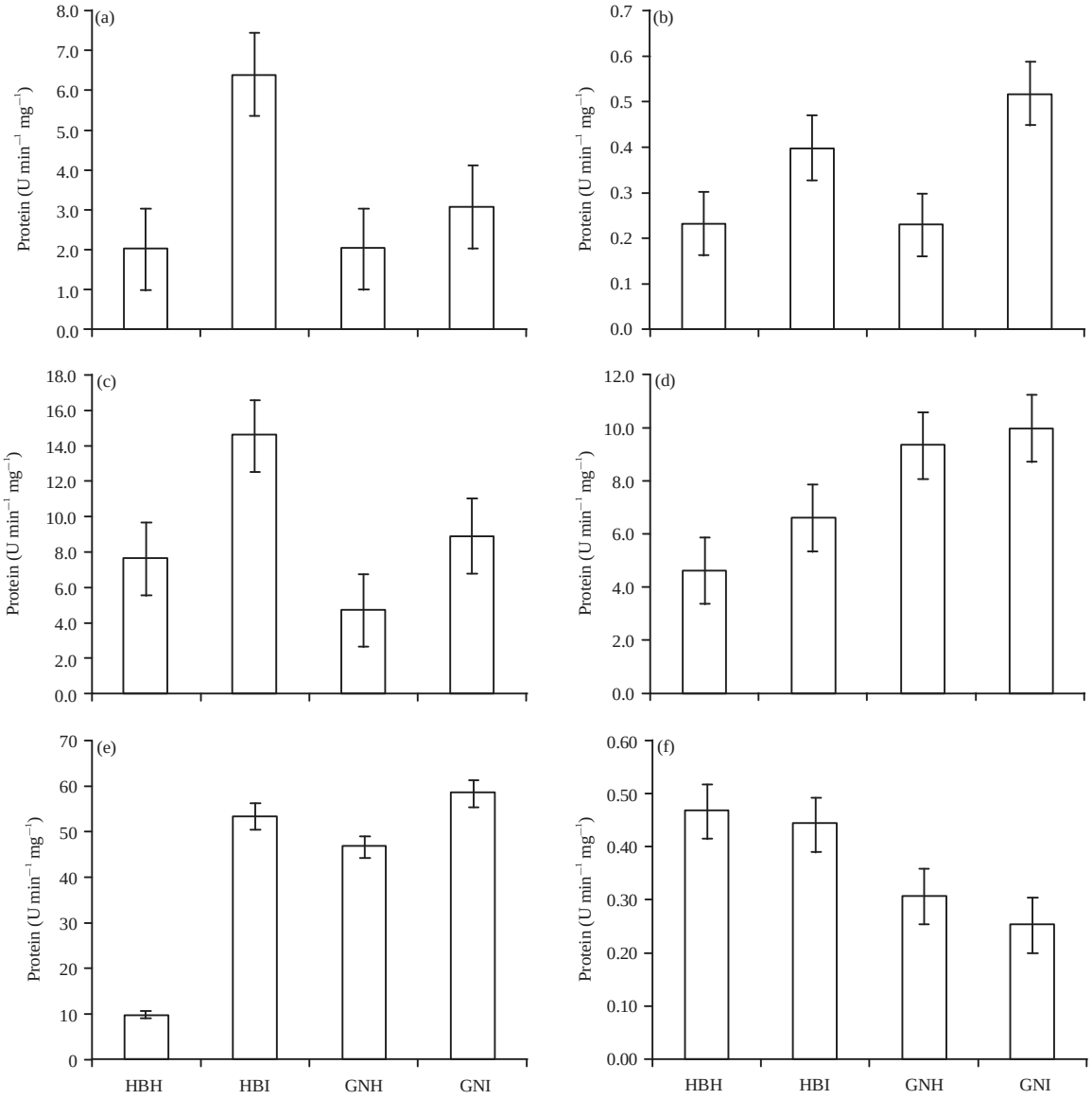

Fig. 4(a-f): Changes in enzyme activities of (a) POX, (b) PPO, (c) CAT, (d) APX, (e), GPX (E) and (f) SOD in healthy and BBTV infected Virupakshi (HB) and Grand Nain (GN) plants

which necrotic or chlorotic symptoms are induced, the degree of stimulation correlating with severity of symptoms (Wood, 1990). The POX is known to be involved in the Active Oxygen Species (AOS) mechanisms. The AOX accumulation causes oxidative damage through actions such as lipid peroxidation and membrane destruction. The AOX is proposed to be responsible for chlorophyll degradation and POX levels increase during senescence (Kuroda *et al*., 1990). So, there is a correlation between the decrease in chlorophyll content and increase in POX activity in the green leaves infected with virus (Milavec *et al*., 2001). Also, POX is known to catalyze the final polymerization step of lignin synthesis and is directly associated with the increased ability of systemically protected tissues to lignify (Chittoor *et al*., 1999). Therefore, higher activity of POX leads to lignification process which is considered as a resistance mechanism against pathogen attack. By way of oxidation of indole-3-acetic acid, upregulated peroxidases might also be responsible for growth reductions and malformations in virus-infected plants (Riedle-Bauer, 2000).

Polyphenol oxidase (PPO): Polyphenol oxidase (PPO) is important in the initial stage of plant defense where membrane damage causes release of phenols such as chlorogenic acid. The PPO catalyzes the oxidation of phenolics to free radicals that can react with biological molecules, thus creating an unfavorable environment for pathogen development (Jockusch, 1966; Mohamed *et al*., 2012). The PPO activity

was found to increase in leaves of BBTV-infected plants of tested cultivars (Fig. 4b). Total soluble phenols together with PPO play a role in resistance to viral pathogens (Thipathi and Verma, 1975; Kumar *et al*., 1991; Li and Steffens, 2002; Ngadze *et al*., 2012).

Catalase: Catalase is an oxygen-scavenging enzyme that plays the role of specific peroxidative, protecting cells from the toxic effects of substrates (H_2O_2) during development, which are otherwise lethal (Choodamani *et al*., 2009; Patel *et al*., 2011; Hameed and Iqbal, 2014). The CAT activity was found to increase in leaves of BBTV-inoculated plants of both the tested cultivars (Fig. 4c). Similar increase in foliar CAT activity was observed in leaves of *Arachis hypogaea* infected with peanut mottle virus (Kobeasy *et al*., 2011), cotton leaves inoculated with *Cotton leaf curl burewala virus* (Siddique *et al*., 2014) and in *Hibiscus cannabinus* infected with *Yellow vein mosaic virus* (Sarkar *et al*., 2010).

Ascorbate peroxidase (APX): Ascorbate peroxidase (APX) is thought to play the most essential role in scavenging ROS and protecting cells in higher plants, algae and other organisms. The APX activity was significantly higher in BBTV infected plants of both cultivars (Fig. 4d) probably as an antioxidant response triggered by the increasing presence of H_2O_2 within cells. The increase in APX activity in banana cultivars infected with BBTV was similar to that reported for *Hibiscus cannabinus* infected with begomovirus (Sarkar *et al*., 2010), *Nicotiana benthamiana* infected with *Pepper mild mottle virus* (Hakmaoui *et al*., 2012) and sunflower infected with sunflower chlorotic mottle virus (Rodriguez *et al*., 2010). It was suggested that the over-production of APX increased the Peroxidase activity which strengthens the ROS scavenging system and leads to oxidative stress tolerance. It has been reported that in a compatible response between barley and powdery mildew the cytosolic isoenzyme of APX is up-regulated in both epidermal and mesophyll cells. In these cells, that are not able to trigger a response to stop pathogens, the APX increase limits the propagation of oxidative processes allowing cells to maintain their viability, a condition required for the penetration of biotrophic powdery mildew in plant tissues (Burhenne and Gregersen, 2000). This up-regulation of APX confirms previous results reporting an increase in APX activity during successful infection of barley leaves by biotrophic compatible pathogens (El-Zahaby *et al*., 1995; Vanacker *et al*., 1998; Kuzniak and Sklodowska, 1999) and has also been reported to occur in leaves of susceptible apricot infected by *Plum pox virus* (Hernandez *et al*., 2001).

Guaiacol peroxidase: Guaiacol peroxidase (GPX) is an important group from peroxidases, which oxidize guaiacol (omethoxyphenol) as a commonly used reducing substrate. Guaiacol peroxidase (GPX) activity was higher in BBTV infected cultivars (Fig. 4e). The GPX decomposes Indole-3-Acetic Acid (IAA) and has a role in the biosynthesis of lignin and defence against biotic stresses by consuming H_2O_2.

Superoxide dismutase: Superoxide dismutase is one of the most important scavenging enzymes and catalyzes the dismutation of superoxide radicals to active oxygen species hydrogen peroxide (Ehsani-Moghaddam *et al*., 2006; Hameed and Iqbal, 2014). The SOD activity was significantly higher in leaves of healthy plants of both the cultivars (Fig. 4f). This is in agreement with results of Hernandez *et al.* (2004), who has observed a significant decrease in SOD from *Plum pox virus* inoculated peaches, similarly Clarke *et al.* (2002) in WClMV-infected *Phaseolus vulgaris* L., plants, Buonaurio and Montalbini (1993) in case of PVY infected tobacco plants, Ashfaq *et al.* (2010) in *Urdbean leaf crikle virus* (ULCV) infected black gram and Zhuang *et al.* (1993) in a resistant soybean infected with S*oybean mosaic virus*. Higher activity of SOD could be a strategy of the plant to restrict virus colonization, because the excess ROS can be removed (Govrin and Levine, 2000; Ehsani-Moghaddam *et al*., 2006) and vice versa when SOD was lower resulting in oxidative stress.

CONCLUSION

All the physiological and biochemical parameters analyzed in banana leaves showed significant changes in response to BBTV infection. Reduced chlorophyll content with increased carbohydrate content may lead to chlorotic/morse code symptom development in BBTV infected banana plants. Early and elevated expression of defence enzymes is an important feature of plant resistance to pathogens. It has been suggested that the higher activity of antioxidant enzymes can interrupt defence signals generated by ROS in several pathosystems and thus leads to compatible virus-plant interactions. Our findings indicate that there is a significant increase in total protein, phenolic compounds, POX, PPO, CAT, APX, GPX and a decrease in SOD activity which may play an active role in disease resistance against BBTV infection. Induced levels of phenolics with these enzymes may have correlation with resistance and these enzymes could be considered as biochemical markers for studying plant virus interaction in this case, BBTV in particular.

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