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Biochemical alterations in banana cultivars infected systemically by banana bract mosaic virus (BBrMV)

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Abstract

Virus infection in plants lead to the alteration of physiological, biochemical and metabolic processes such as total protein synthesis, activation or synthesis of defence peptides and proteins, rapid production of reactive oxygen species (ROS) as well as synthesis of phenols. Banana Bract Mosaic Disease (BBrMD) is one of the most destructive viral diseases of banana, however, information on physiological and biochemical changes during banana-BBrMV interaction is still unexplained. Therefore, the present investigation was conducted to find out the quantifiable changes in biochemical parameters such as total proteins, phenolic compounds, peroxidase (PO), polyphenol oxidase (PPO), catalase (CAT) ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and superoxide dismutase (SOD) activities in leaves of commercial banana cultivar Poovan (AAB) and a plantain cv. Nendran (AAB). The amount of phenols, total protein, GPX and SOD activities were significantly higher in leaves of BBrMV infected plants of both the cultivars over the healthy plants, whereas PO, PPO, CAT and APX activities reduced in infected than healthy plants. Overall the results suggest that BBrMV infection induces significant changes in biochemical and enzyme levels leading to irreversible symptom development and significant yield loss.

Keywords: anti-oxidant enzymes, BBrMV, banana, biochemical changes

Introduction

Plants undergo many characteristic physiological and metabolic changes upon virus infection [1]. Plant virus interaction triggers defence response which lead to the accumulation of reactive oxygen species (ROS) such as superoxide ions, hydrogen peroxide, hydroxyl radicals and the singlet oxygen. The burst of ROS is a common feature of incompatible and compatible plant-virus interactions and this could be a key step in the activation of the defence against virus infection. ROS is responsible for eliciting pathogen restriction and death of plant cells at the infection site and it also induces defence response in adjoining cells. Early ROS accumulation at the virus infection site determines the outcome of the defence in host plants. A slow response in the host may lead to virus infection resulting in oxidative stress and programmed cell death (PCD). The concentration of ROS varies in different patho-systems and is influenced by antioxidant capacity of the plant. Both enzymatic and non-enzymatic systems play an important role in maintaining the levels of ROS in plants upon pathogen entry. Among these, enzymatic ROS scavenger ameliorates the effects caused by the presence of cellular oxidants [2].

Banana and plantain are the most important food crops of the world. Banana cultivation is subjected to many natural calamities, but pests and diseases constitutes major problem. Among pest and diseases, viral diseases cause serious yield losses [3, 4]. Banana bract mosaic disease (BBrMD) caused by Banana bract mosaic virus (BBrMV), a member of the genus *Potyvirus* and family *Potyviridae* is one of the most important viral diseases which was first observed in Philippines in the year 1988. The virus occurrence was discovered in few countries in Asia and South Pacific, including India, the Philippines, Samoa, Sri Lanka, Thailand, Vietnam, Colombia and Ecuador [5, 6]. In Hawaii (USA), BBrMV was detected in ornamental ginger plants (*Alpinia purpurata*) but not in *Musa* and it has also been reported in small cardamom in India [7]. BBrMV is found in plants of all age group and primarily transmitted through infected suckers and secondary spread is through aphid vectors with non-persistent transmission. The incidence of disease ranges from 5 to 36 per cent and more in cv. Nendran in Kerala. The infected plant yields bunch, but the fingers are smaller in size and malformed. Very severely affected plants may fail to flower and may die by stunted growth and necrosis of pseudostem. The male buds are dark purple in color with mosaic patches.

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There are varietal differences in the symptomatology of the disease. The disease has a great impact on the yield of the crop with maximum yield reduction in cv. Robusta (AAA) (70%), followed by cv. Nendran (AAB) (52%). Besides yield reduction, the fingers become malformed and curved, which reduce the market acceptability of fruits [6].

With the emergence of BBrMV in India, identifying the resistance source and breeding for resistance is the need of the hour. Disease resistance against BBrMV in banana is associated with genetic background and activation of defense enzymes that interferes with pathogen establishment. However, the biochemical alteration in the host induced by the infection of BBrMV remains unexplored. Hence, the present investigation was undertaken to determine the biochemical and physiological changes upon virus infection indicating their role in two widely grown susceptible commercial bananas.

Materials and Methods

Virus inoculation and sample collection

Banana cultivars, Nendran (AAB) and Poovan (AAB) were used in the present investigation. Three-month-old suckers were planted in pots containing a mixture of sand, loam and compost (1:1:2). These pots were kept in insect-proof glass house. The transmission was carried out as described by Selvarajan *et al.* (2006) [8]. The banana black aphids, *Pentalonia nigronervosa* (Fifteen adult or late instar aphids) were collected, allowed to starve for 30 min and transferred to bract mosaic infected banana plants having four to five well developed leaves for acquisition access period of 5-10 min. Then the viruliferous aphids were allowed for inoculation feeding for 30 min at 25±1.0 °C with 12h light/dark photoperiod. At the completion of inoculation-feeding period plants were sprayed with insecticide imidacloprid (0.1%) and kept in the insect-proof glasshouse for monitoring the symptoms. All the plants showed typical BBrMV symptoms after 30-40 days of inoculation. The mock inoculated healthy plants of each cultivar were maintained as control. Leaf samples were collected from BBrMV inoculated plants after symptom development and healthy control plants to determine the enzyme activity.

Preparation of enzyme extract

One gram of leaf sample was homogenized at 4 °C in 1 ml of extraction buffer [0.1M sodium phosphate buffer (pH 7.0), 1% Triton X-100 and 7mM 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 different biochemical parameters.

Estimation of total protein content

Total protein was estimated calorimetrically by using Bradford method [9] 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.

Estimation of phenol

The phenolic content was estimated using Folin-Ciocalteu reagent. 80% ethanol was used for extraction of phenols. One gram of plant material was ground in two 5 ml portions of 80% ethanol and centrifuged. The extracts were pooled and made up to 10ml. 0.1ml 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-Ciocalteu reagent. After 5 min, 2 ml of 20% sodium carbonate solution was 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 [10].

Enzyme assays

Peroxidase (PO) activity was measured by following the protocol of Malik and Singh (1980) [11]. In this method, 3.5 ml of phosphate buffer (PH-6.5) and 0.2ml of enzyme extract were added to a mixture of 0.1ml of freshly prepared O-dianisidine solution and 0.2ml of 0.2M H₂O₂ in a test tube. The development of an orange color indicates the presence of the peroxidase enzyme and immediately absorbance of the reaction mixture was read at 430 nm at every 30 sec interval upto 3 min.

The specific activity of all the enzymes assayed in this study were expressed as units /min/mg of protein on fresh weight basis.

Polyphenol oxidase (PPO) activity was measured as described by Ngadze *et al.* (2012) [12]. One ml of enzyme extract supernatant was transferred to a test tube and mixed with 2.9 ml of 0.05 M sodium phosphate buffer and 1 ml of 0.1 M catechol (Sigma). The mixture was aliquoted into three portions for measurement of PPO activity. The absorbance at 546 nm was measured for 4 min at 20 sec intervals.

For the estimation of CAT activity, assay solution (3 mL) was prepared by mixing 50 mM phosphate buffer (pH 7.0), 5.9 mM H₂O, and 0.1 mL enzyme extract. Reduction in absorbance of the reaction solution at 240 nm was recorded after every 20 sec [13].

Ascorbate peroxidase (APX) activity was determined according to the method of Chen and Asada (1989) [14] with minor modification. Reaction mixture (1 ml) contained 50 mM phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.5 mM ascorbate, 1.54 mM H₂O₂ and 50 µl of enzyme extract. Decrease in the absorbance at 240 nm indicates the oxidation of ascorbate. One unit of APX was defined as the amount of enzyme that oxidized 1 µmol of ascorbate per min at 25°C.

Guaiacol peroxidase (GPX) activity was measured as per the protocol described by Upadhyaya *et al.* (1985) [15]. The reaction mixture contained 2.5 ml of 50 mM phosphate buffer (pH-6.1), 1 ml of 1% H₂O₂, 1 ml of 1% guaiacol and 20 µl of enzyme extract. The increase in absorbance was monitored spectrophotometrically for 1 min at 470 nm.

Superoxide dismutase (SOD) activity was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) using the method of Dhindsa *et al.* (1981) [16]. Three ml reaction mixture contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75M 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. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reaction of NBT.

Statistical analysis

The experiment was carried out following two factorial completely randomized design (FCRD) with three replicates. The data set of biochemical analysis were submitted to two-way analysis of variance (two-way ANOVA), followed by the LSD at 5% for mean comparison among the factors and their interactions. The two-way ANOVA, of the data set were performed using OPSTAT (<http://14.139.232.166/opstat/>) [17].

Results and Discussion

The total protein content in banana cultivar Poovan was significantly higher than that of Nendran (Fig. 1A). In infected plants, the total protein content increased significantly in comparison to the un-inoculated control irrespective of the cultivars. An increase of 1.5-fold and 1.01-fold total protein was recorded in BBrMV infected Nendran and Poovan cultivars in comparison to un-inoculated control, respectively. Infected plants showed higher protein content, which could be due to both the activation of host defence mechanisms^[2] and synthesis of virus specific proteins during pathogenesis and accumulation of virus particles. Similar findings have been reported by several workers in many host – virus systems *viz.*, *Cucurbita pepo* infected with Zucchini yellow mosaic virus (ZYMV)^[18], black gram infected with Urdbean leaf crinkle virus (ULCV)^[19], mungbean infected with Mungbean yellow mosaic virus (MYMV)^[20], cucurbits infected with Cucumber mosaic virus (CMV)^[21] and Sunflower necrosis tospovirus (SNV) infected sunflower^[22].

The total phenolic content was significantly elevated in response to BBrMV infection as compared to control in both the cultivars. We also recorded that there was a natural variation in the total phenolic content among the cultivars. In cultivar Nendran the phenolic content was significantly higher than Poovan and similar observations have been reported in banana^[23]. In BBrMV infected Nendran and Poovan, 2.3-fold and 1.36-fold increase in total phenol was observed respectively as compared to the healthy (Fig. 1B). Increased level of phenolics could be due to the acceleration of phenol synthesizing pathway upon virus infection and it also attributes to induced resistance for restricting further invasion by BBrMV. The phenolic accumulation may be regarded as a sign of avoidance of virus infection and establishment^[24, 25, 26]. Both the cultivars studied were relatively susceptible to BBrMV, but the symptom development was faster in Poovan than cultivar Nendran (Selvarajan, unpublished). In the present study, 2.3-fold increase in phenol was observed in Nendran after BBrMV infection which might be the reason for delayed symptom development and this corroborates with the results of Tanuja *et al.* (2019)^[27] in banana infected with Banana bunchy top virus (BBTV). The increase in phenolics in response to virus has also been reported in mothbean infected with Yellow mosaic virus (YMV)^[28], *Capsicum annum* infected with Gemini virus^[29], black gram infected with ULCV^[19], papaya infected with Papaya meleira virus (PMeV)^[30] and CMV infected cucurbits^[21].

The changes in the enzyme activities (PPO, PO, CAT, APX, GPX and SOD) due to BBrMV infection in Nendran and Poovan are furnished in Fig. 2.

PPO activity was significantly higher in control plants as compared to BBrMV infected leaf samples of both cultivars. PPO activity was significantly lowered by 0.4- and 0.5-fold in Nendran and Poovan plants inoculated with BBrMV in comparison to control (Fig. 2A). It has been demonstrated that higher soluble phenols, together with higher PPO may play a role in resistance to viral pathogens^[31], but reverse trend have been observed in this study. The decrease in the activity of PPO can be correlated with accumulation of phenols in BBrMV infected leaves. Tanuja *et al.* (2019) have reported similar results in banana upon BBTV infection.

The PO activity was significantly decreased by 0.4-fold and 0.72-fold in Nendran and Poovan plants inoculated with BBrMV as compared to control (Fig. 2B). POs are involved in plant defense by the production of pathogenesis-related proteins and in the formation of lignin around the cell which

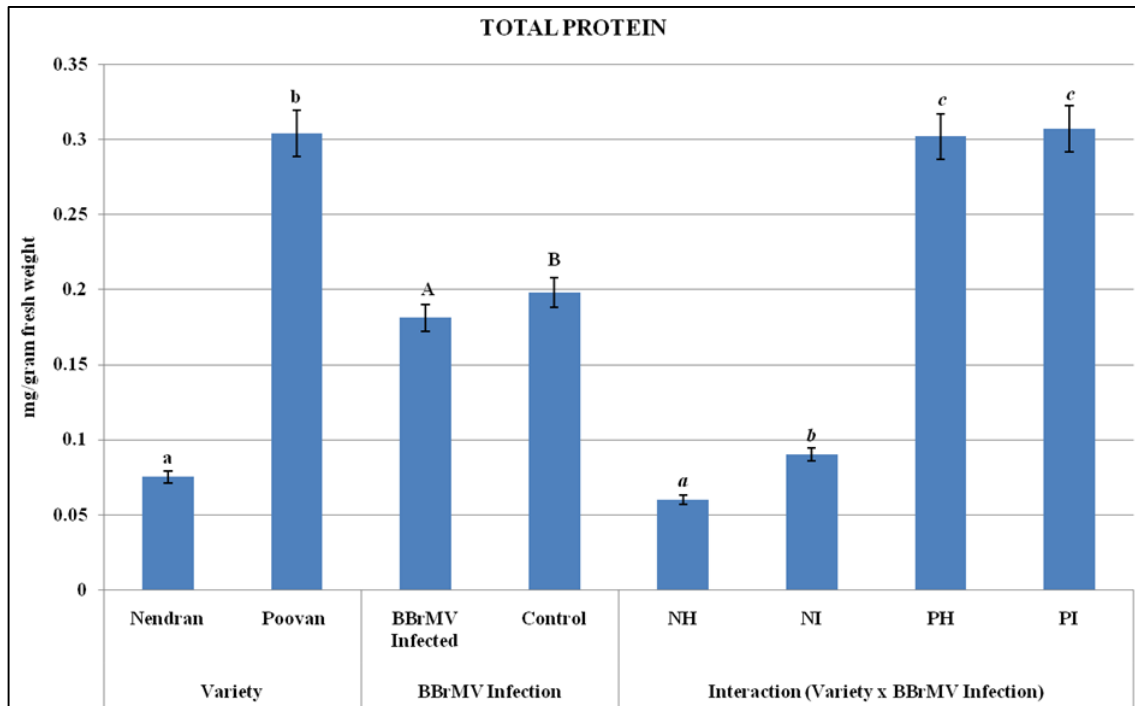
limits pathogens to cross from the place of penetration^[32]. Similar reduction in the PPO activity results were observed in kenaf and roselle plants infected with Mesta yellow vein mosaic virus (MeYVMV)^[33] and cotton infected with Cotton leaf curl viruses (CLCuBV)^[34]. PO and PPO enzyme probably affects synthesis of compounds effective in conferring resistance.

In this study, catalase activity was significantly reduced by 0.37-fold and 0.69- fold in BBrMV infected samples than healthy samples of Nendran and Poovan, respectively (Fig. 2C) suggests that, a sharp decrease in activity of CAT may be due to inhibition of the enzyme substrate - H₂O₂^[35]. CAT enzyme acts as a monitoring index for plant response to viruses. Catalase scavenges hydrogen peroxide (H₂O₂) and it directly dismutates it to H₂O and O₂^[27, 31]. CAT activity has also, been reported to decrease in cells undergoing hypersensitive reaction (HR)^[36]. Reduced catalase activity was observed in *Phaseolus vulgaris* infected with White clover mosaic virus (WCLMV)^[37], ULCV infected urdbean^[38], Plum pox virus (PPV) infected apricot^[39], Kenaf and Roselle plants infected with MeYVMV^[33] and *Nicotiana benthamiana* infected with Pepper mild mottle virus (PMMoV)^[40]. Decrease in catalase and peroxidase activity in both the cultivars upon BBrMV infection can be correlated well with disease severity and indicates breakdown of resistance against pathogen^[41].

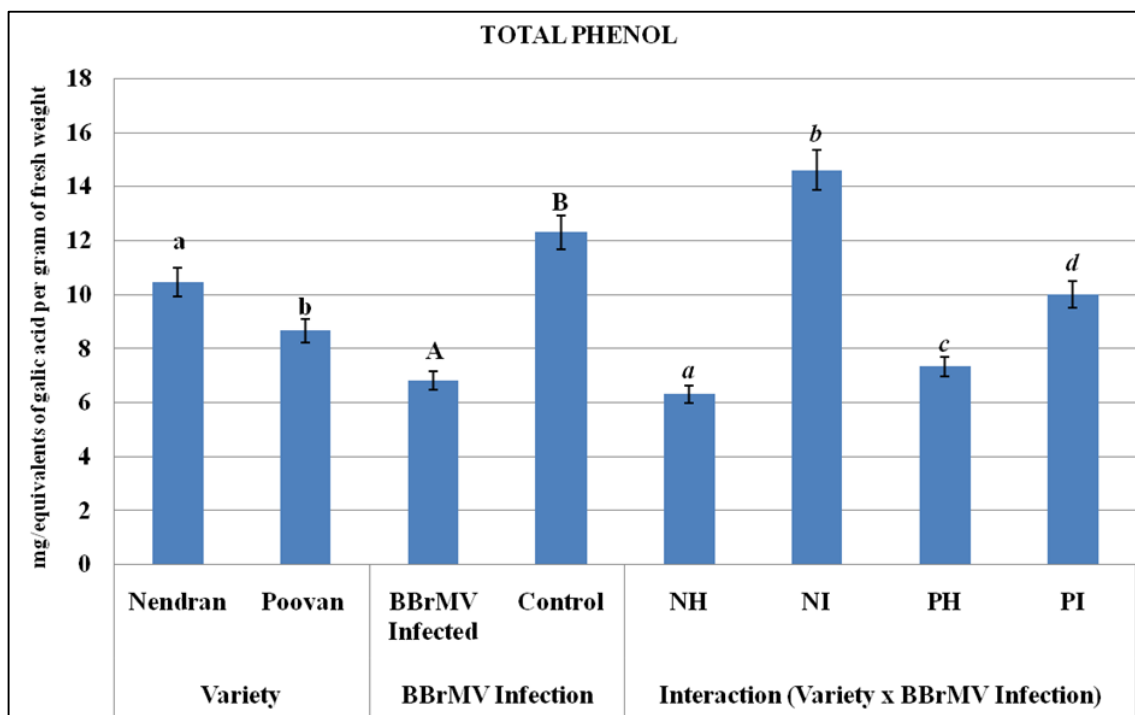
APX activity was significantly lowered by 0.97-fold and 0.38-fold in BBrMV infected Nendran and Poovan cultivars than the healthy, respectively (Fig. 2D) thereby reducing the capability of cells to scavenge H₂O₂ resulting in HR cell death. Similar result was observed by Vanacker *et al.* (1998)^[42] in barley. Activity of GPX was increased significantly in the BBrMV infected leaf by 1.2-fold and 1.55-fold in comparison with control plants of Nendran and Poovan, respectively (Fig. 2E). Induced GPX activity can be attributed primarily to resistance against the viral pathogen. Similar results were observed in peanut mottle virus infected peanut plants^[43], mungbean infected with MYMV^[44] and banana infected with BBTV^[31].

SOD activity was found to be significantly higher by 1.8-fold in Nendran and 1.12-fold in Poovan infected with BBrMV than healthy (Fig. 2F). As such the SOD activity was higher in Nendran than Poovan under natural conditions which might be the reason for delayed expression of symptoms due to BBrMV (data not shown). The higher SOD activity in infected leaves indicates a probable mechanism of overcoming the stress situation^[33]. Increased SOD activity has been proved upon virus infection in *prunus*^[45], mesta^[33], sunflower^[46], peanut^[43] and papaya^[30]. Overall, the enzyme activity in case of Nendran, is significantly higher as compared to Poovan which may be attributed to their genetic makeup.

The activities of anti-oxidative enzymes were studied in banana cultivars Nendran and Poovan which showed significant changes in response to BBrMV infection. There has been a significant increase of GPX, SOD and substantial decrease in CAT, PPO, PO and APX activity upon challenge inoculation with BBrMV in banana. Similar trend was observed in many plant species infected with viruses. Therefore, we conclude that the BBrMV infection in banana triggers ROS accumulation and induce changes in the antioxidative enzymes. These anti-oxidative enzymes could be shortlisted for developing biomarker for the use in diagnosis and to study the virus response in banana. The putative biomarker could be validated in 320 core banana germplasm collections being maintained in the field gene bank of ICAR-NRCB, Trichy for resistance to BBrMV.



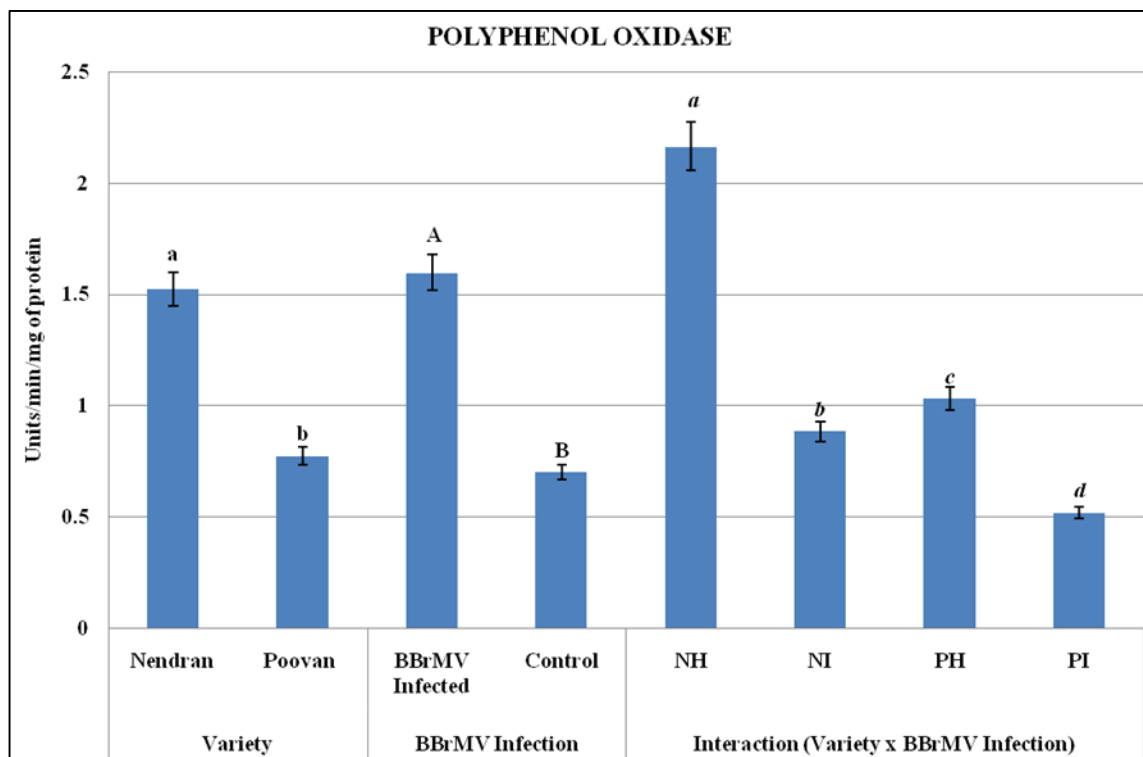
(A)



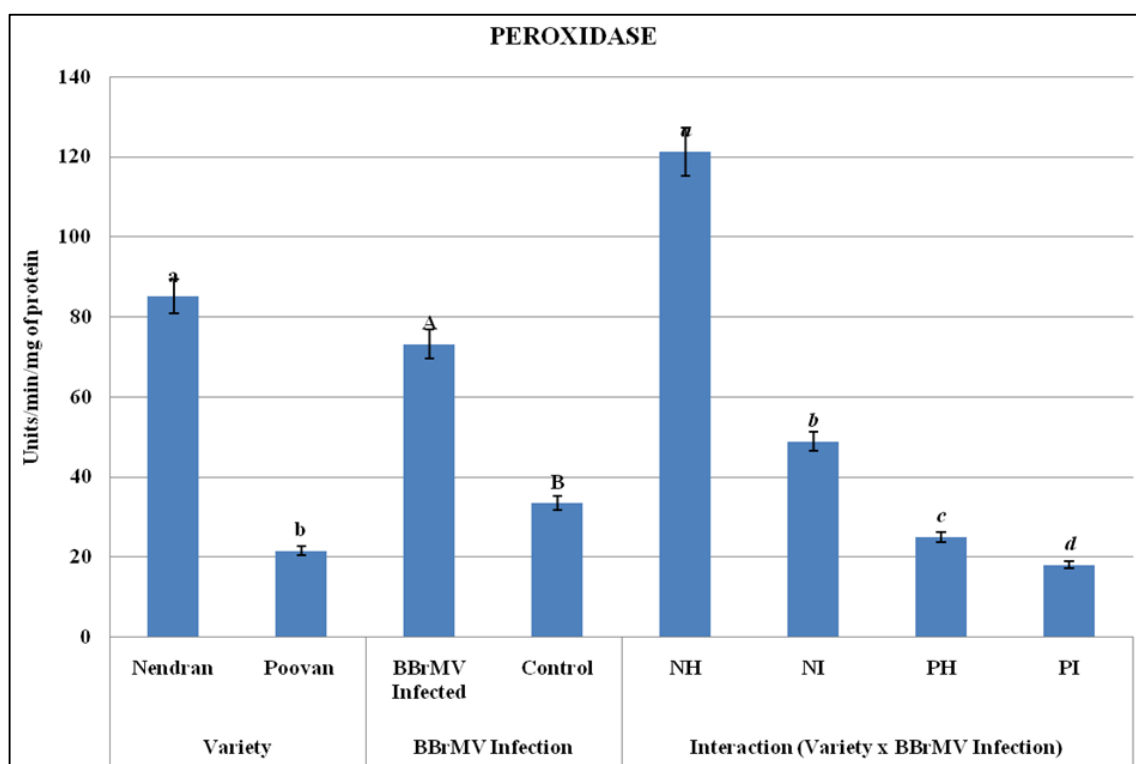
(B)

Results are expressed as mean \pm SE ($n = 3$) using two-way ANOVA. [Means followed by the same small alphabet (Variety), by the same capital alphabet (upon BBrMV infection) and by the same lower case alphabet in italics (Interaction = Variety x BBrMV) are not significantly different ($p \leq 0.05$) by LSD test; NH = Nendran Healthy; NI = Nendran Infected; PH = Poovan Healthy; PI = Poovan Infected].

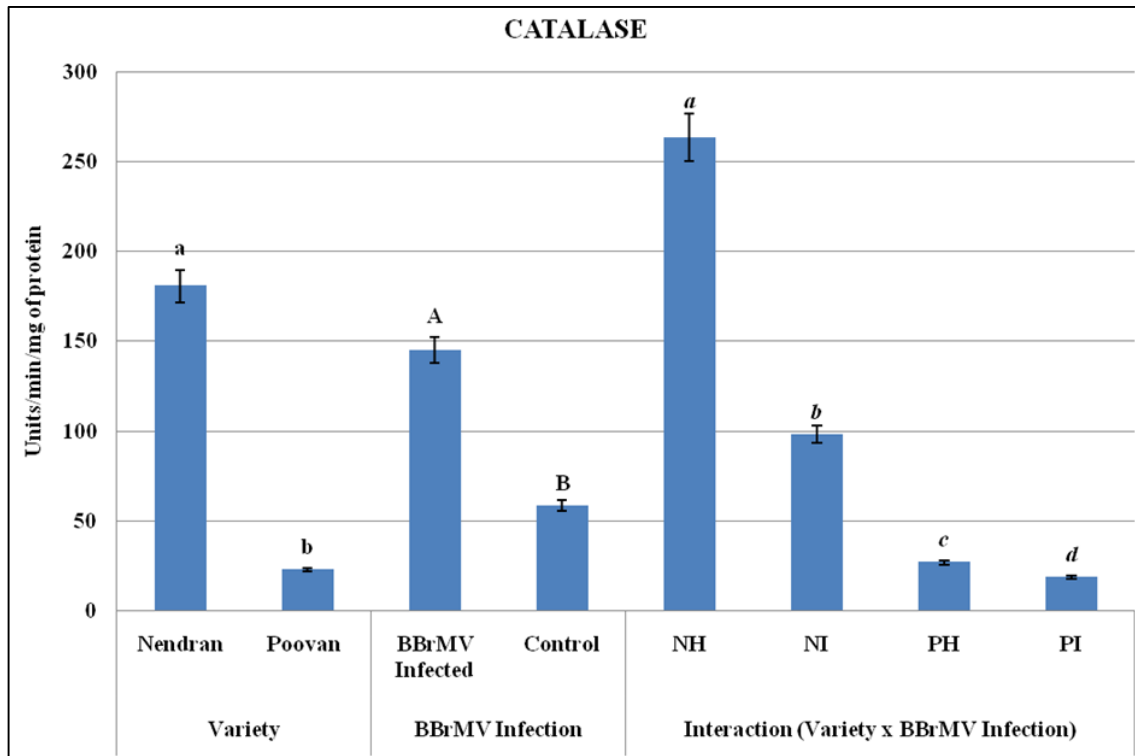
Fig 1: Total protein (A) and total phenol (B) content of healthy and BBrMV infected Nendran and Poovan leaf tissue.



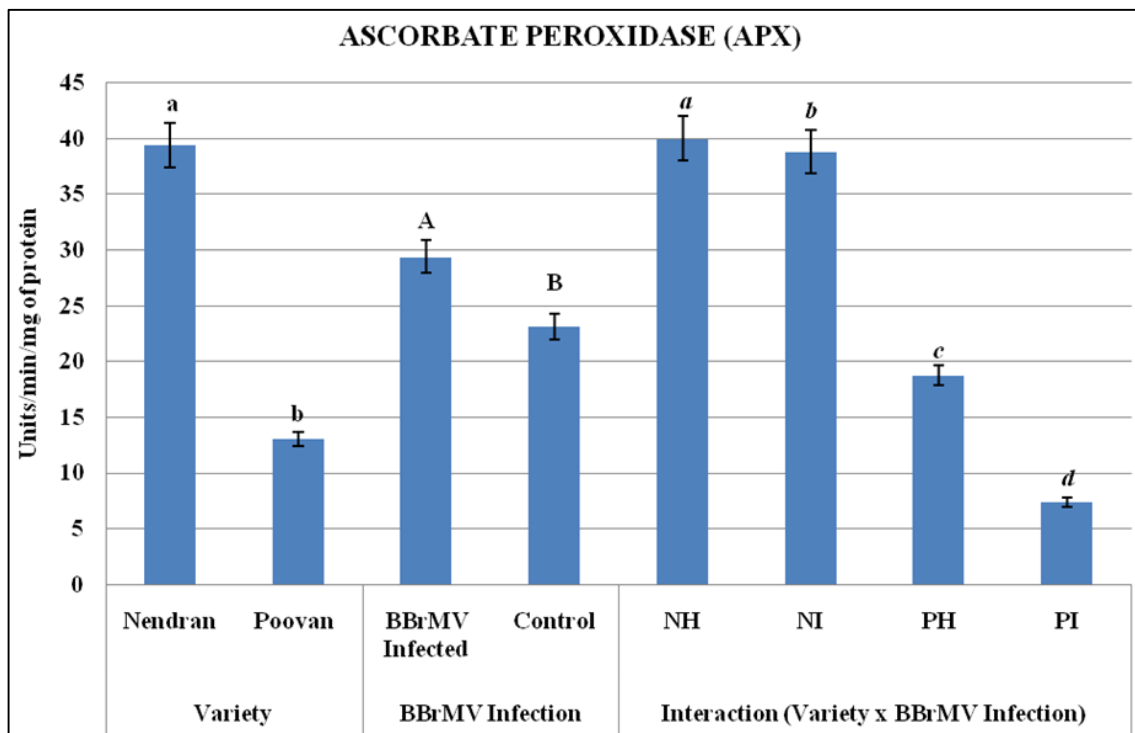
(A)



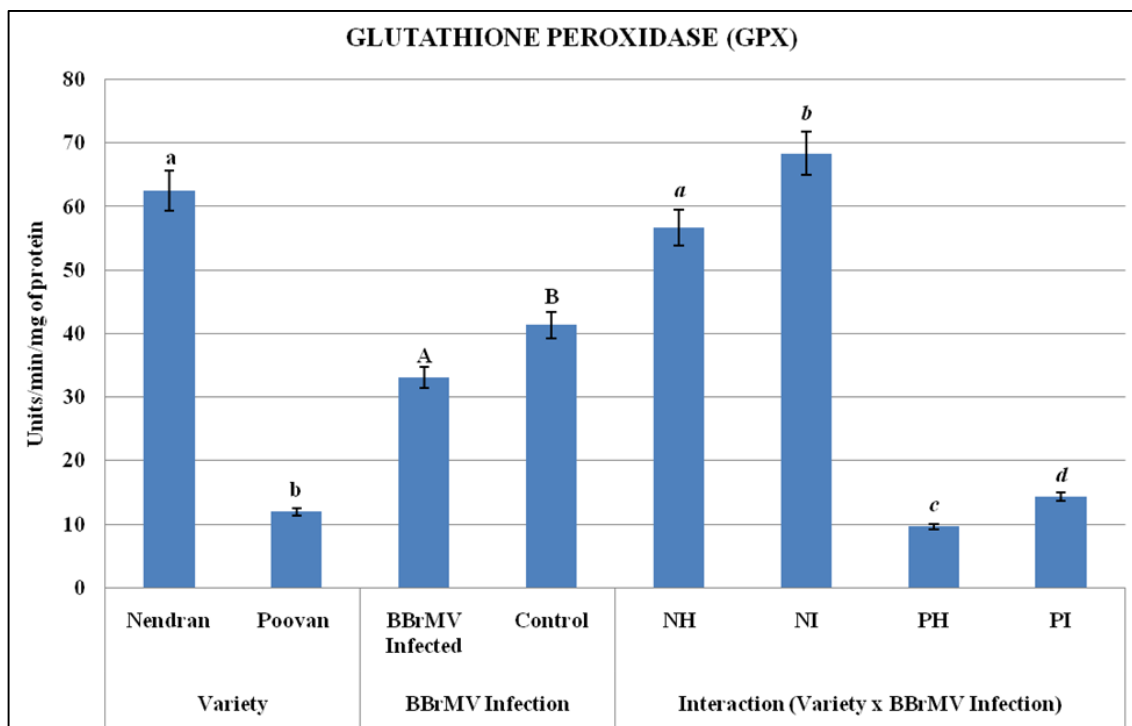
(B)



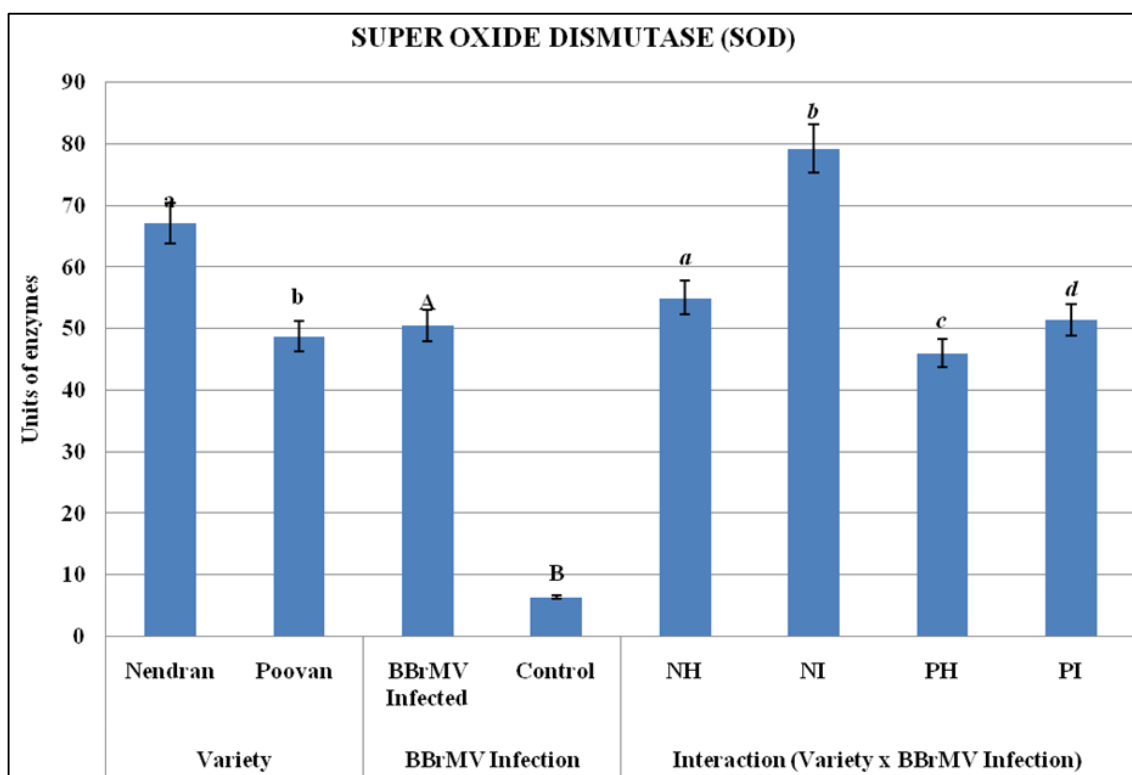
(C)



(D)



(E)



(F)

Results are expressed as mean \pm SE (n = 3) using two-way ANOVA. [Means followed by the same small alphabet (Variety), by the same capital alphabet (upon BBrMV infection) and by the same lower case alphabet in italics (Interaction = Variety x BBrMV) are not significantly different ($p \leq 0.05$) by LSD test; NH = Nendran Healthy; NI= Nendran Infected; PH=Poovan Healthy; PI= Poovan Infected].

Fig 2: Changes in enzyme activities of A) PPO, B) POX, C) CAT, D) APX, E) GPX, F) SOD in healthy and BBrMV infected Nendran and Poovan leaf tissue

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