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Host-Pathogen Interaction of Cassava (*Manihot esculenta* Crantz) and Cassava Mosaic Viruses (ICMV and SLCMV)

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ABSTRACT

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As a response of host-pathogen interaction, the induction of systemic resistance (ISR) was studied in cassava upon challenge inoculation with cassava mosaic viruses (ICMV and SLCMV). Due to the viral infection, the reduced amount of chlorophyll a, chlorophyll b and total chlorophyll was recorded in virus infected cassava seedlings compared to control (meristem derived healthy plants). The accumulation of total phenol, total sugars and total soluble proteins were found to be reduced in virus infected seedlings than control. Moreover, the activity of defense enzymes viz., peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL) and catalase was found to be increased in infected cassava seedlings compared to control. In addition an increased expression of specific isoforms of PO (PO2 and PO3) and PPO (PPO2 and PPO3) were observed in native PAGE analysis with response to challenge inoculation of pathogen due to induced systemic resistance (ISR) induction.

Introduction

Cassava, *Manihot esculenta* Crantz belonging to the family Euphorbiaceae, is one of the most important staple food crops (El-Sharkawy, 2007; Lopez, 2005; Mann, 1997) for more than 700 million subsistence farmers in tropical and sub-tropical Africa, Asia and Latin America (IFAD and FAO, 2000) and grown widely under diverse environmental conditions. About 70 per cent of world

cassava root production (which is estimated to be over 45 m metric tonnes of dry root annually) is used for human consumption either directly after cooking or in processed forms; the remaining 30 per cent is used for animal feed and other industrial products such as starch, glucose, and alcohol (El-Sharkawy, 2004). The major constraint in cassava production is cassava mosaic disease (CMD),

caused by Cassava infecting geminiviruses (CIGs) belonging to the family of *Geminiviridae* and Genus *Begomovirus*, which is transmitted by whitefly, *Bemisia tabaci* Genn. CMD was first described in 1894. In India, this disease is caused by *Indian cassava mosaic virus* (ICMV) and *Sri Lankan cassava mosaic virus* (SLCMV) and was first noted in 1956 (Abraham, 1956), although it is likely to have occurred much earlier and the disease was described later by Alagianagalingam and Ramakrishnan (1966). It has since received considerable attention in the southern states of Kerala and Tamil Nadu, which are the main cassava growing areas of India. The infected plant stunted gradually in growth by means of reduces the plant height, petiole length, mid leaflet length and width (Thankappan and Chacko, 1976). Initially, the leaves showed faint greenish yellow specks surrounded by normal green area, which were mostly confined to the lower portion of the lamina. In fully developed diseased leaves, the chlorotic areas were yellow intermingled with normal green colour and showed mosaic mottling. In case of severe infection, the leaf gets reduced in size, misshapen, and twisted with bright yellow areas separated normally by green areas (Jos *et al.*, 1984; Mathew, 1989).

Plants can acquire local and systemic resistance to diseases through various biological agents including necrotizing pathogens, non-pathogens (Van Loon *et al.*, 1997). This type of resistance, known as induced systemic resistance, is mediated by triggering various defense mechanisms including production phytoalexins, synthesis of phenolics (M'Piga *et al.*, 1997; Chen *et al.*, 2000), accumulation of pathogenesis-related proteins (Meena *et al.*, 2000) and deposition of structural barriers (Benhamou *et al.*, 1996). The defense gene products peroxidases (PO) and polyphenol oxidases (PPO) catalyze the formation of lignin an phenylalanine

ammonia-lyase (PAL) is involved in the synthesis of phytoalexins and phenolics (Karthikeyan *et al.*, 2005). The present study was carried out to compare those compounds in healthy and cassava mosaic virus infected cassava (cv. H226) plants.

In our previous report, virus free cassava plants cv. H226 were produced *in vitro* through apical meristems at Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University (Raghu *et al.*, 2012). The virus free healthy cassava plants were confirmed by polymerase chain reaction (PCR) using geminivirus specific degenerate primers (Deng *et al.*, 1994) and these plants were used for combined transmission of *Indian cassava mosaic virus* (ICMV) and *Sri Lankan cassava mosaic virus* (SLCMV) in cassava plants cv. H226 by an insect vector *Bemisia tabaci* (whitefly) (Raghu *et al.*, 2012). In this study, those virus free healthy (control) and infected cassava plants cv. H226 were used to study the interaction of cassava mosaic virus with cassava through the estimation of chlorophyll, total phenol, total soluble proteins and total sugars, biochemical assay of defense enzymes including PO, PPO, PAL and catalase and native PAGE analysis of PO and PPO.

Materials and Methods

Host-pathogen interaction alters the host physiology. Hence, studies were undertaken to assess changes in physiology of healthy cassava plants (meristem derived) as well as cassava mosaic virus infected cassava plant of cv. H226. General method for virus acquisition and transmission employed as described our previous report (Raghu *et al.*, 2012). Fresh leaf samples were collected from infected and healthy cassava plants for the assay of chlorophyll, total phenol, total soluble proteins and total sugars and to assay

the changes in activities of defense related enzymes *viz.*, PO, PPO, PAL and catalase. Different isoforms of PO and PPO were analyzed through native PAGE analysis. A separate negative control was maintained without inoculation of virus.

Estimation of chlorophyll

Chlorophyll a, b, and total chlorophyll were estimated using the procedure of Witham *et al.*, (1971). One g of leaf samples were macerated with 20 ml of 80% acetone and the

homogenate was centrifuged at 5000 rpm for 5 min. The supernatant was transferred to a 50 ml volumetric flask and the residue was again extracted with acetone till the residue became colourless. The volume of the chlorophyll extract was made up to 100 ml with 80% acetone. The optical density was measured in a spectrophotometer at 663, 652 and 645 nm using 80 per cent acetone as blank. The contents of a, b and total chlorophyll were calculated using the following formulae,

$$\text{Chlorophyll a} = 12.7 (A_{663}) - 2.69 (A_{645}) \frac{V}{1000 \times W}$$

$$\text{Chlorophyll b} = 22.9 (A_{645}) - 4.68(A_{663}) \frac{V}{1000 \times W}$$

$$\text{Totalchlorophyll} = 20.2 (A_{645}) - 8.02 (A_{663}) \frac{V}{1000 \times W}$$

Where,

A= absorbance at specific wave lengths,
V = final volume of chlorophyll extract in 80% acetone and

W = fresh weight of tissue extracted.

The results were expressed as mg/g on fresh weight basis.

Estimation of total phenol

Total phenol was estimated by adopting the protocol of Malik and Singh (1980). Leaf samples (1 g) were homogenized with 10 ml of 80% and were centrifuged at 10,000 rpm for 20 min. The supernatant was evaporated to dryness and the residue was dissolved in known volume of water. To 1 ml of the extract, 5 ml of distilled water and 0.5 of ml Folin- Ciocalteau reagent were added and the solution was kept at 25°C. After 3 min, 1 ml of 20% sodium carbonate and 1 ml of distilled water were added and the reaction mixture was incubated for 1 h at 25°C. The intensity of blue colour was read at 650 nm in a

spectrophotometer against a reagent blank. Catechol at different concentrations served as the standard. The amount of phenol was expressed as catechol equivalent in mg/g of fresh tissue.

Estimation of total sugars

Total sugar content was determined by anthrone method (Hedge and Hofreiter, 1962). Leaf samples (100 mg) were homogenized with 5 ml of 80% ethanol. The homogenate was centrifuged at 10000 rpm for 10 min and the supernatant was collected. Two hundred micro litres of the supernatant was taken in a boiling tube and the alcohol was evaporated using a water bath. The residue was dissolved in 1 ml of water. To this 4 ml of anthrone reagent was added and heated for 8 min in a boiling water bath. Later, the tubes were cooled rapidly and the absorbance was read at 630 nm in a spectrophotometer. D-glucose was used as a standard and the total sugar content was expressed in terms of mg of

glucose equivalent per g of leaf tissue on fresh weight.

Estimation of total soluble proteins

Total soluble protein content was estimated as described by Bradford (1976). *Preparation of stock solution:* Coomassie brilliant blue G250 (100 mg) was dissolved in 50 ml of 95% ethanol and to that 100 ml of concentrated ortho-phosphoric acid was added. The volume was made up to 200 ml with water and kept at 4°C. Stock solution was diluted four times with water to get the working solution. *Sample preparation:* Leaf samples (1 g) were homogenized in 5 ml of 0.1 M phosphate buffer (pH 7.0). The homogenate was centrifuged at 10000 rpm for 10 min at 4°C and the supernatant was used for the assay of soluble protein. *Assay:* To 0.5 ml of the extract, 0.5 ml of water and 2.5 ml of the working dye solution were added and incubated for five min at room temperature. One ml of water added with 2.5 ml of dye served as the blank. The intensity of blue colour was measured at 595 nm in a spectrophotometer. Bovine serum albumin at various concentrations served as the protein standard. Soluble protein content was expressed as mg albumin equivalent of soluble protein per g on fresh weight basis.

Peroxidase (PO)

Leaf sample (1 g) was homogenized with 1 ml of 0.1 M phosphate buffer pH 7.0 and was centrifuged at 10 000 rpm for 20 min at 4°C. The supernatant was used to assay activities of PO and PPO and catalase. The activity of peroxidase was determined as detailed by Hammerschmidt *et al.*, (1982). The reaction mixture consisting of 1.5 ml of 0.05 M pyrogallol and 0.1 ml of enzyme extract was taken in a cuvette. To initiate the reaction 0.5 ml of 1% H₂O₂ was added. The change in absorbance was recorded at 420 nm at 30 s interval for 3 min from zero s of incubation at

room temperature. The boiled enzyme served as blank. The enzyme activity was expressed as change in absorbance/min/g of fresh tissue.

Polyphenol oxidase (PPO)

The reaction mixture consisting of 1.5 ml of 0.1 M sodium phosphate (pH 7.0) and 0.1 ml of enzyme extract was taken in a cuvette. To this, 0.2 ml of catechol (0.01M) was added to initiate the reaction. The change in absorbance was recorded at 495 nm at 30 s interval for three min and the results were expressed as change in absorbance min⁻¹ g⁻¹ of fresh tissue (Mayer *et al.*, 1965).

Catalase

Catalase activity was assayed in the leaf samples collected from healthy and infected cassava plants as per the method described by Beers and Sizer (1952). Reaction mixture consisting of 0.5ml of 0.1 M phosphate buffer (pH 7.0), 0.2 ml of enzyme extract and 0.1ml of 1% H₂O₂ was incubated at 28±1°C. At the start of the enzyme reaction the absorbance of the mixture was set zero at 230nm in a spectrophotometer and changes in the absorbance was recorded at 15 sec interval for 3 min. Catalase activity was expressed as change in the absorbance of the reaction mixture/ min/ g of leaf tissue on fresh weight basis.

Phenylalanine ammonia lyase (PAL)

Leaf samples (500 mg) were homogenized in 5 ml of cold 25 mM borate HCl buffer (pH 8.8). The homogenate was centrifuged at 10000 rpm for 15 min and the supernatant was used as enzyme source. The assay mixture consisted of 0.2 ml of enzyme extract, 1.3 ml water and 0.5 ml borate buffer. The reaction was initiated by the addition of 1ml of 12 mM L-phenylalanine. The reaction mixture was incubated for 1 h at 32°C. The reaction was stopped by the addition of 0.5 ml of 2 N HCl.

The absorbance was measured at 290 nm. PAL activity was determined as the rate of conversion of L-phenylalanine to trans-cinnamic acid. The enzyme activity was expressed as n mol of trans-cinnamic acid/min/g of leaf tissue (Dickerson *et al.*, 1984).

Native-polyacrylamide gel electrophoresis (PAGE) analysis

The isoforms profile of PO and PPO were examined by discontinuous native polyacrylamide gel electrophoresis (native-PAGE) (Laemmli, 1970). Samples collected from healthy meristem derived and cassava mosaic virus infected plants were homogenized with 1 ml of 0.1 M sodium phosphate buffer pH 7.0 and centrifuged at 10000 rpm for 20 min at 4°C. The protein content of the sample was determined by the Bradford (1976) method. Samples (50 µg protein) were loaded onto separating gel of 8% and stacking gel of 4% polyacrylamide concentration (Sigma, USA) for the analysis to find out the expression of PO and PPO isoforms. After electrophoresis, the gel was stained in 0.2 M acetate buffer at pH 4.2 containing 0.05% benzidine for 30 min in the dark. Then drops of H₂O₂ (0.03%) were added slowly with constant shaking to visualize the PO isoforms. After staining the gel was washed with distilled water (Nadlony and Sequerira, 1980). For assessing PPO isoforms profile, the gels were equilibrated for 30 min in 0.1% p-phenylene diamine in 0.1 M potassium phosphate buffer (pH 7.0) followed by addition of 10 mM catechol in the same buffer and kept in a shaker with gentle shaking. The appearance of dark brown protein bands was noticed after some time (Jayaraman *et al.*, 1987).

Results and Discussion

Estimation of chlorophyll: Meristem derived healthy cassava plants of cv. H226 were having more chlorophyll content. Chlorophyll

a, b and total chlorophyll were significantly more in healthy cassava plants than in ICMV and SLCMV infected cassava plants. Such increase in healthy plants over infected was 18.38 per cent in chlorophyll a, 27.99 per cent in chlorophyll b and 24.13 per cent in total chlorophylls (Table 1 and Figure 1a). *Estimation of total phenols:* Meristem derived healthy cassava plants of cv. H226 exhibited 32.24 per cent increase in accumulation of total phenol as compared to that in Cassava mosaic virus infected plants (Table 2 and Figure 1b). *Estimation of total sugars:* Meristem derived healthy cassava plants of cv. H226 exhibited a significant increase in total sugar content (26.43 per cent) as compared to that in Cassava mosaic virus infected plants (Table 2 and Figure 1b). *Estimation of total soluble proteins:* Total soluble proteins in meristem derived healthy cassava plants of cv. H226 increased to the tune of 33.91 per cent as compared to that in Cassava mosaic virus infected plants (Table 2 and Figure 1b).

Peroxidase (PO): Infection of the cassava plants (cv. H226) by cassava mosaic virus led to increased peroxidase activity (Table 3). When compared to Cassava mosaic virus infected cassava plants peroxidase activity significantly decreased in meristem derived healthy cassava plants of cv. H226 (Figure 1c) to the tune of 23.55 per cent. *Polyphenol oxidase (PPO):* Data presented in table 3 revealed that the effect of virus infection on Polyphenol oxidase activity, which is a defence related enzyme. Infection of the cassava plants (cv. H226) by cassava mosaic virus led to increased Poly phenol oxidase activity. The activity of the enzyme was found to be significantly less in meristem derived healthy cassava plants of cv. H226 (Figure 1c) to the tune of 33.28 per cent as compared to that in infected plant. *Catalase:* When compared to meristem derived healthy cassava plants, activity of catalase was increased in infected plants and less in healthy plants. The percentage decrease of catalase

activity in healthy cassava plants of cv. H226 was 23.52 per cent (Figure 1c and Table 3). *Phenylalanine ammonia lyase (PAL)*: Data presented in table 3 indicated an increased activity of PAL in Cassava mosaic virus infected plants. The activity of the enzyme significantly decreased in meristem derived healthy cassava plants of cv. H226 (Figure 1d) to the tune of 11.67 per cent as compared to that in infected plant.

Native PAGE analysis of PO and PPO isoforms in meristem derived and Cassava mosaic virus infected cassava plants of cv. H226

Native PAGE analysis revealed the presence of four PO (PO1, PO2, PO3 and PO4) and four PPO (PPO1, PPO2, PPO3 and PPO4) isoforms in cassava mosaic virus infected and meristem derived cassava plants of cv. H226 whereas, two PO (PO1 and PO4) and two PPO (PPO1 and PPO4) isoforms were present in meristem derived cv. H226 cassava plants. Two PO (PO2 and PO3) and two PPO (PPO1 and PPO3) isoforms were found in CMD infected cv. H226 cassava plants (Figure 2).

Plant virus infection leads to measurable changes in the physiological process of the host. Any biochemical or physiological study on infected plants may contribute to understand the virus disease (Bawden and Pirie, 1952). Porter (1959) reported that changes in metabolism of host cell induced by the virus leads to various macroscopic and microscopic symptoms. To measure these changes the CMD infected plants will compared with a completely virus free plantlets derived from meristem culture. The present study gives direct evidence on the induction of plant defense mechanism in the control of cassava mosaic disease caused by cassava mosaic virus. Induction of systemic resistance has been established as a new mechanism by which plants defend

themselves against pathogen attack (Van Loon *et al.*, 1998). There are various reports confirm the induction of systemic resistance (Yedidia *et al.*, 1999; Meena *et al.*, 2000).

As a response of induction of systemic resistance (ISR), accumulation of reactive oxygen species (ROS) is central to plant response to several pathogens. One of the sources of ROS is the chloroplast because of the photoactive nature of the chlorophylls. Chlorophyllase I, induced after tissue damage caused by cassava mosaic virus, is degrade the chlorophyll molecules. In the present study, Chlorophyll a, b and total chlorophyll were significantly more in meristem derived healthy cassava plants than in cassava mosaic virus infected cassava plant. This is in agreement with the results of Alagianagalingam and Ramakrishnan (1978). They reported that mosaic infected cassava leaves had less chlorophyll and carotene and lower photosynthetic rate. Similar results were obtained by Vasanthi (1998). The loss in chlorophyll content might be due to the diversion of plastid protein into virus protein (Bawden and Pirie, 1952) or a result of normal cell degrading enzymes that utilize chlorophyll as a substrate (Goodman *et al.*, 1977). This has also been attributed to either the inhibition of chloroplast development or the distribution of pigments in matured chloroplasts (Matthews, 1981). In meristem derived plants because of the absence of the virus there was no degradation of chlorophyll.

The results revealed a significant increase in total sugars in the meristem culture derived healthy cassava plants of cv. H226 (26.43 per cent), whereas significant reduction in total sugar content was observed in cassava mosaic virus infected plants. The reduced levels of total sugar content of the diseased plants may be due to higher utilization of sugars for phosphate ester formation required for virus nucleic acid synthesis (Singh and Srivastava,

1974). Conversion of sugars into amino acids and organic acids may be yet another possible reason for the reduced level of sugars in virus infected plant (Maggyaarosy *et al.*, 1973). In meristem derived plants because of the absence of the virus there was no chance of conversion of sugars into amino acids and organic acids.

The total soluble protein content was significantly increased in meristem derived healthy cassava plants of cv. H226, whereas in diseased plant total soluble protein content was decreased. This is in contrary with the findings of Nambisan (1994), Vasanthi (1998) and Madhubala (2003). Where they reported that total soluble protein content was significantly increased in cassava mosaic virus infected cassava plants.

The phenolic compounds as constituents of lignin may contribute to enhance the mechanical strength of the host cell wall and may also inhibit pathogen growth as they are fungitoxic in nature. The induction of phenols might be due to the activation of the shikimic acid pathway, through which the aromatic amino acids phenylalanine and tyrosine are formed and channelled for the synthesis of phenolics (M'Pign *et al.*, 1997). The present investigation revealed that total phenol content was more in meristem derived healthy cassava plants, whereas total phenol content was less in cassava mosaic virus infected cassava plants of cv. H226. This finding is in contrary with the observations of Vasanthi (1998) and Madhubala (2003), where they reported that total phenol content was more in cassava mosaic virus infected cassava plants. In general phenols are mainly responsible for resistance in plants against stresses. Similar kind of findings was reported for blackgram against dry root rot (Sundaravadana, 2002) and groundnut against *C. Personatum* (Meena *et al.*, 2000). The result of the present study revealed that the virus infection in cassava

plants (cv. H226) led to increase in PO, PPO, PAL and catalase activity. This is in agreement with the findings of Alagianagalingam and Ramakrishnan (1974), Vasanthi (1998) and Madhubala (2003). They noticed significant differences in PO, PPO, PAL and catalase activity in the infected plants. PO is an early response in plants to pathogen attack and plays a key enzyme in the biosynthesis of lignin which limits the extent of pathogen spread (Bruce and west, 1989), It also catalyses the oxidation of phenols to quinines which are highly fungitoxic. The product of this enzymes in the presence of a hydrogen donor and hydrogen peroxide have antimicrobial activity and even antiviral activity (Van Loon and Callow, 1983) increase activity of cell wall bound PO have been elicited in deferent plants such as cucumber (Chen *et al.*, 2000) and tobacco (Ahl Goy *et al.*, 1992) due to pathogen infection. In bean, rhizosphere colonization by various bacteria induced PO activity (Zolor and Anderson, 1992). In the present study, a rapid increase in PO activity was recorded in cassava infected with cassava mosaic virus. The present study also indicates that expression four PO isoforms, PO1, PO2, PO3 and PO4 was more prominent in cassava plants infected with cassava mosaic virus. Moreover, the isoforms PO2 and PO3 were expressed only in the infected plants rather than healthy.

PAL is the first enzyme in the phenyl propanoid metabolism and has a definite role in defense mechanism of plants (Friend and Threshfall, 1976). It plays an important role in the biosynthesis of various defense chemicals in phenylpropanoid metabolism. Activity of PAL could be induced in plant-pathogen interaction (De Mayer *et al.*, 1999). Several studies have shown that PAL activity is induced in plants upon infection (Chen *et al.*, 2000; Sundaravadana, 2002; Saravanakumar, 2003).

Table.1 Estimation of chlorophyll content in meristem derived and cassava mosaic virus infected cassava plants (cv. H226)

Treatments	Chlorophyll a* (mg/g of fresh weight)	Decrease over infected (%)	Chlorophyll b* (mg/g of fresh weight)	Decrease over infected (%)	Total Chlorophyll * (mg/g of fresh weight)	Decrease over infected (%)
	(Mean ± SE)	(Mean ± SE)	(Mean ± SE)	(Mean ± SE)	(Mean ± SE)	(Mean ± SE)
Control	0.979 ± 0.015	-	0.567 ± 0.000	-	1.785 ± 0.020	-
Infected	0.827 ± 0.009	18.38 ± 0.002	0.443 ± 0.005	27.99 ± 0.192	1.438 ± 0.031	24.13 ± 0.739

*Mean of five replications

Table.2 Estimation of total phenol, total sugars and total soluble proteins contents of meristem derived and cassava mosaic virus infected cassava plants (cv. H226)

Treatments	Total phenols* (mg/g of fresh weight)	Decrease over infected (%)	Total sugars* (mg D-glucose equivalent/g of fresh weight)	Decrease over infected (%)	Total soluble proteins* (mg/g fresh weight)	Decrease over infected (%)
	(Mean ± SE)	(Mean ± SE)	(Mean ± SE)	(Mean ± SE)	(Mean ± SE)	(Mean ± SE)
Control	1.415 ± 0.032	-	11.736 ± 0.345	-	1.011 ± 0.008	-
Infected	1.07 ± 0.009	32.24 ± 0.314	9.283 ± 0.244	26.43 ± 0.809	0.755 ± 0.005	33.91 ± 0.509

*Mean of five replications

Table.3 Defense enzyme status in meristem derived and cassava mosaic virus infected cassava plants (cv. H226)

Treatments	PO activity* (Change in abs/min /g)	Increase over control (%)	PPO activity* (Change in abs/min/g)	Increase over control (%)	PAL activity* (n moles of transcinnamic acid/g)	Increase over control (%)	Catalase*	Increase over control (%)
	(Mean ± SE)	(Mean ± SE)	(Mean ± SE)	(Mean ± SE)	(Mean ± SE)	(Mean ± SE)	(Mean ± SE)	(Mean ± SE)
Control	0.74 ± 0.006	-	0.397 ± 0.002	-	106 ± 3.114	-	0.608 ± 0.001	-
Infected	0.968 ± 0.005	23.55 ± 0.250	0.595 ± 0.014	33.28 ± 0.624	120 ± 1.110	11.67 ± 0.129	0.795 ± 0.003	23.52 ± 0.328

*Mean of five replications

Fig.1 Biochemical assays between control (black colour bars) and infected (ash colour bars) cassava plants challenged with the pathogen cassava mosaic virus: (a) induction of chlorophyll a,b and total chlorophyll; (b) induction of total phenol, total sugars and total soluble proteins; (c) changes in the activities of peroxidase (PO) and polyphenol oxidase (PPO); (d) n moles of transcinamic acid/g of phenylalanine ammonia lyase; (e) Catalase

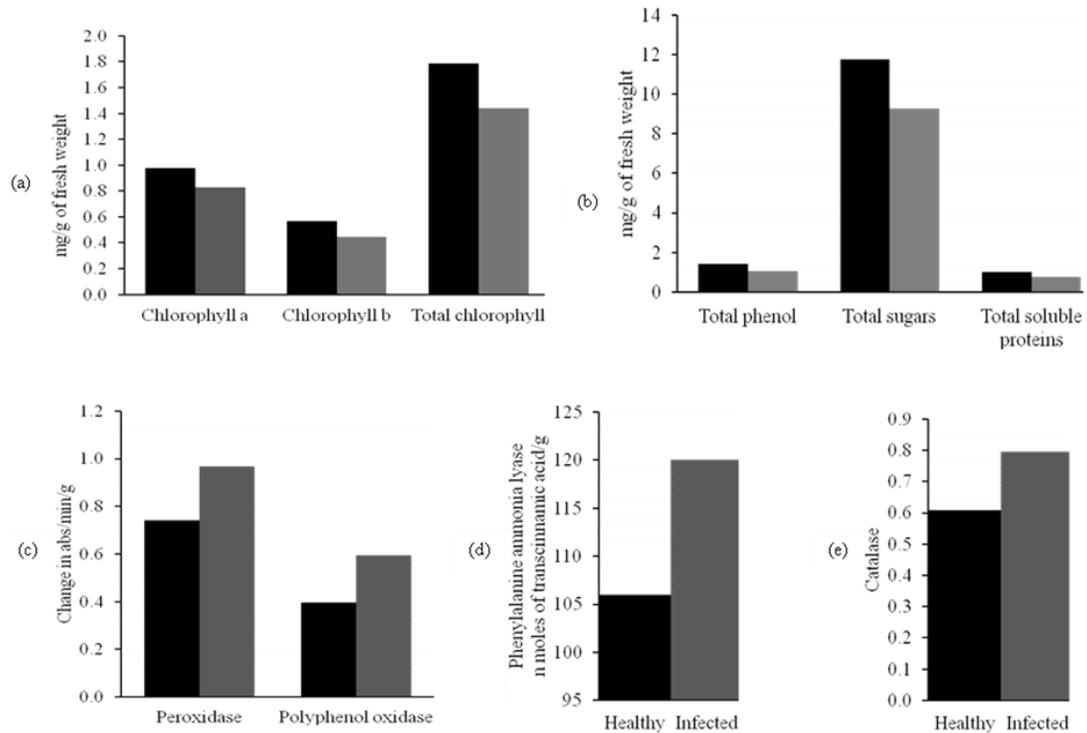
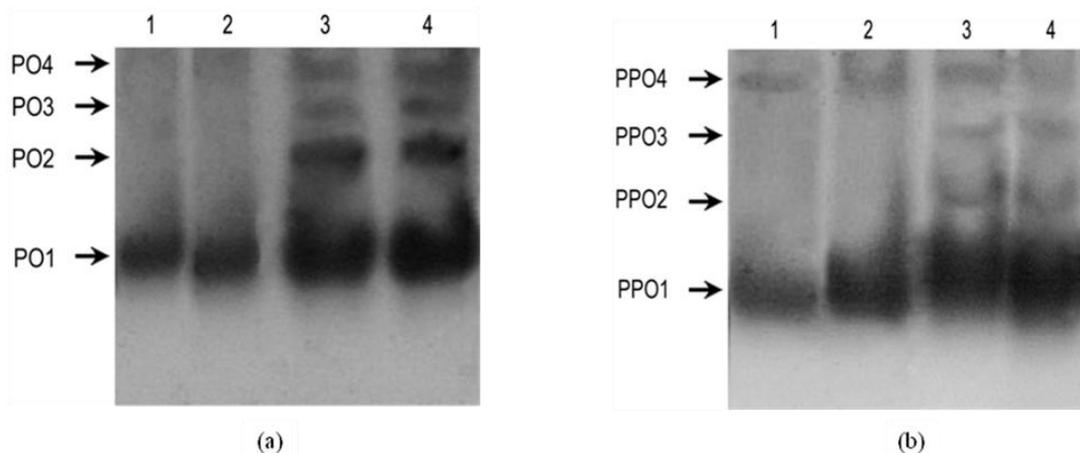


Fig.2 Native PAGE analysis of defense related enzyme induced in cassava cv. H226 challenged with or without the pathogen cassava mosaic virus: (a) peroxides (PO) isoforms profile; (b) polyphenol oxidase (PPO) isoforms profile. Lane 1 and 2 is control plants; Lane 3 and 4 is infected plants



PPO catalyses the oxidative phenolic compounds and plays major role in defense. Similar to other enzymes, PPO activity also increased by infection with cassava mosaic virus, expression of four PPO isoforms, PPO1, PPO2, PPO3 and PPO4 was very clear in cassava infected with cassava mosaic virus. The induced PPO2 and PPO3 isoforms and a higher level of expression of the other isoforms were implicated in induced defense responses against cassava mosaic virus in cassava. The virus infected plant accelerates the synthesis of catalase to remove excess hydrogen peroxide produced due to increased respiration (Lodh *et al.*, 1973). The abrupt rise in the enzyme activity may be due to alteration in redox potential of the host as a result of virus infection (Vidhayasekaran, 1995). Since the meristem derived plants are free from the virus infection there was no induction of the above mentioned enzymes.

Induced systemic resistance is an important mechanism of bio-control that received considerable attention in recent times. Pathogenesis-related (PR) proteins, isoenzymes (different molecular form of same enzyme specificity) of phenylpropanoid metabolism including Peroxidase (PO) and Polyphenol oxidase (PPO) that catalyze the formation of lignin and phenyl ammonia-lyase (PAL) that involves in phytoalexin and phenolics biosynthesis are found to be associated with induced systemic resistance to some plant pathogens (Shannon, 1968). In the present study four different isoforms of both PO and PPO were noticed during host-pathogen interaction between cassava and cassava mosaic virus.

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