

Study on Phenolics and Their Oxidative Enzyme in *Capsicum annuum* L Infected with Geminivirus



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Abstract : The contents of total phenol, o-dihydroxyphenol, peroxidase and poly phenoloxidase were recorded in healthy and diseased leaf of chilli. The total phenols were found to be higher in diseased leaves as compared to those of healthy leaves where as lower o-dihydroxy phenols were recorded. Enhanced peroxidase activity and polyphenol oxidase activities found to occur in diseased leaves as compared to healthy leaves.

Key words : *Capsicum annuum*, total phenol, o-dihydroxyphenol, polyphenol oxidase, peroxidase, chilli leaf curl disease.

Introduction

Chilli (*Capsicum annuum* L) is an important spice crop in India and ranks first among all the spice with a share of 33.7% in the total production (Anonymous 2003). The crop is infected by several fungal, bacterial and viral diseases. Among all the viral diseases leaf curl disease greatly affect the crop productivity of chilli. The disease is characterized by severe adaxial and abaxial curling of the leaves, shrinking of the leaves and stunted plant growth. This is accompanied by puckering and swelling of veins and the buds are stimulated to produce cluster of leaves. The whole plant assumes a bushy appearance with stunted growth, fewer flowers and fruits developed due to disease (Ansari *et al.*, 2006). Peroxidase catalyses the oxidation of various hydrogen donors in the presence of H_2O_2 and oxidized the phenolics substance. Their reaction products were highly reactive and toxic to pathogens. They played important role in host parasite interaction, disease development and defence reaction of infected plants. The enhanced phenol synthesis and peroxidase activity in various host parasite combination

was correlated with disease resistance (Ghosal *et al.*, 2004). Hence, present study is an attempt on investigation on the changes in phenols (total and o-dihydroxy phenols) and enzymes like peroxidase and poly phenol oxidase due to the infection of geminivirus.

Materials and Methods

Healthy and diseased leaves of chilli were collected. Estimation of Phenolics and their oxidative enzymes in diseased and healthy leaves was carried out by standard methods. Bray and Thorpe (1954) method was used for estimation of total phenol, Worthington Enzyme Manual (1972) for Peroxidase activity, Shinshi and Noguchi (1975) for Polyphenol oxidase. For the estimation 500mg of healthy and diseased leaves were taken. Total phenols were estimated and O.D. was recorded at 725 nm. O-dihydroxy phenol was estimated by Bhattacharya and Chenchaiyah (2007) method and O.D. was recorded at 515 nm. Total phenols were calculated with the help of standard curve prepared using catechol. Isozymes of peroxidase (E.C. 1.11.1.7) were analysed by polyacrylamide gel

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electrophoresis, PAGE (Davis 1964). PAGE was prepared and samples were prepared by homogenizing 1g of diseased and healthy leaf in 5 ml phosphate buffer pH=7, 0.1 M. Samples were poured in wells of running gel by micropipette. The gel tray was placed on buffer electrode filled upto 2 cm depth. Peroxidase was incubated in equal volume of acetate buffer (pH =4.8, 1 M) and 0.1% o-dianisidine for one hour and enzyme bands were visualized under 5 Mm solution of H₂O₂ and guaiacol after inoculation. The gels were photographed and Rm values of each band were calculated by making zymograms.

Results and Discussion

Total phenol was significantly high in diseased leaf as compared to healthy leaf (Fig.1) The increased quantity of total phenol might be attributed to defence mechanism. The resistance to disease caused by pathogen was attributed to the presence of high amount of phenol (Jain and Yadav, 2003; Kushawaha and Narain, 2005 and Parashar and Lodha, 2007). Hence, the increased quantity of phenolics in the infected plant parts of the chilli may be contributing to the resistance against pathogen (viral infection).

Phenolics compounds play an important role in the defence mechanism of the plant and this is supported by their accumulation in the cell followed by viral infection. Increased level of phenolic suggested an acceleration of phenols synthesizing pathway following pathogen infection. The increase in the phenols in severely infected plant parts could be attributed to the induced resistance for further invasion by the pathogen. Hence, the increase in phenolics in relation to resistance was reported in *Brassica* (Singh; 2004). Activation of PAL and subsequent increase in phenol contents were general responses associated with resistance mechanism in plants as reported (Ghosal et al, 2004).

Higher peroxidase activity was observed in diseased leaf as compared to healthy leaf

(Fig.3). Devanathan et al (2005) observed high peroxidase activity in bunchy top Banana virus infected cultivars of banana. Increased peroxidase activity was associated with resistance reaction which could be due to increased phenol concentration, where phenols were cofactor of peroxidase and hence influenced resistance in the host. Polyphenol oxidase was responsible for oxidation of o-dihydroxy phenols. In the present study high polyphenol activity was observed (Fig. 4) and lower O-dihydroxy phenol in diseased leaf as compared to healthy (Fig. 2). which was responsible for phenol accumulation as oxidation of o-dihydroxy phenol was due to this enzyme

Enzymes involved in phenol metabolism were most easily considered as one of the important biochemical parameters for disease resistance. Polyphenol oxidases catalyses the oxidation of monophenol and o-dihydroxy phenol. Polyphenol oxidase was known as tyrosinase, catechol oxidase and potato oxidase (Maheshwari et al, 2006). The enhanced polyphenol activity might result in the augmented rate of oxidation of phenolics substance that participates in the defence reaction of host. These reactive quinones were known to inhibit polyphenol oxidase. Table-1 shows Rm values of isoperoxidase bands as is evident from diagrammatic sketch of peroxidase banding patterns (Fig. 5A,B). The electrophoretic studies revealed three isoperoxidase bands in diseased leaves whereas two in healthy leaves. The Rm values in diseased leaves were 0.22, 0.46 and 0.87 whereas in healthy ones 0.25 and 0.46. Rm values. 0.46 was similar in healthy and diseased leaf. Intensity of peroxidase activity was found to be very high in diseased leaf as compared to that of healthy leaf.

Acknowledgement

The first author (Rishikesh Meena) is highly thankful to CSIR, New Delhi for giving Senior Research Fellowship.

Table 1 : Rm value of isozymes of peroxidase activity of healthy and diseased leaf of chilli analysed by PAGE

Healthy leaf	Diseased leaf
0.25	0.22
0.46	0.46
-	0.87

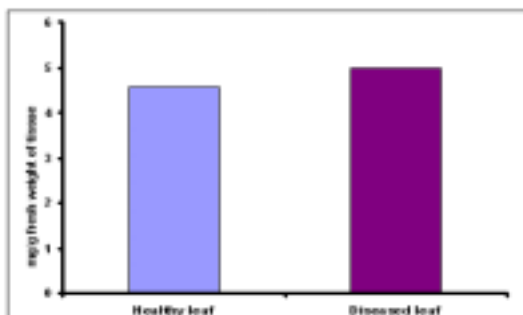


Fig. 1 : Estimation of total phenols in healthy and diseased leaf

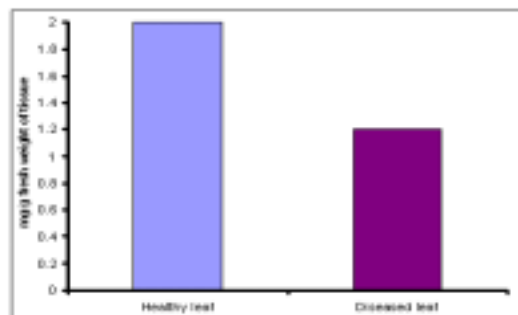


Fig. 2 : Estimation of O-dihydroxyphenol in healthy and diseased leaf

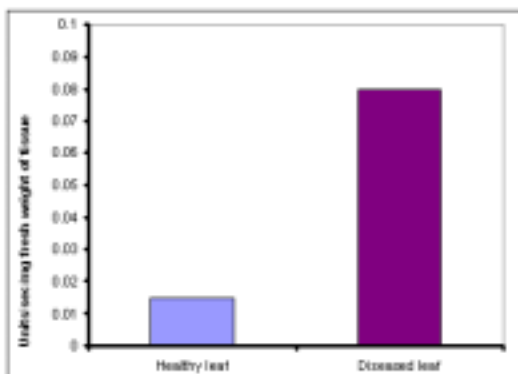


Fig. 3 : Estimation of peroxidase activity in healthy and diseased leaf

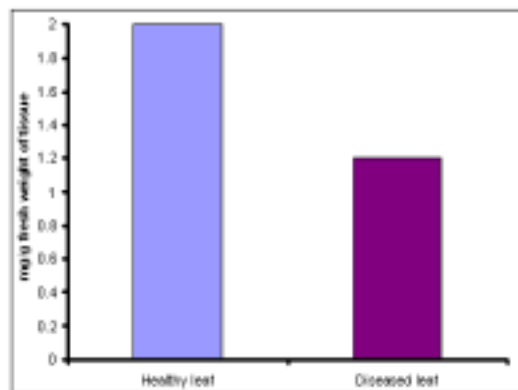


Fig. 4 : Estimation of polyphenol-oxidase activity in healthy and diseased leaf

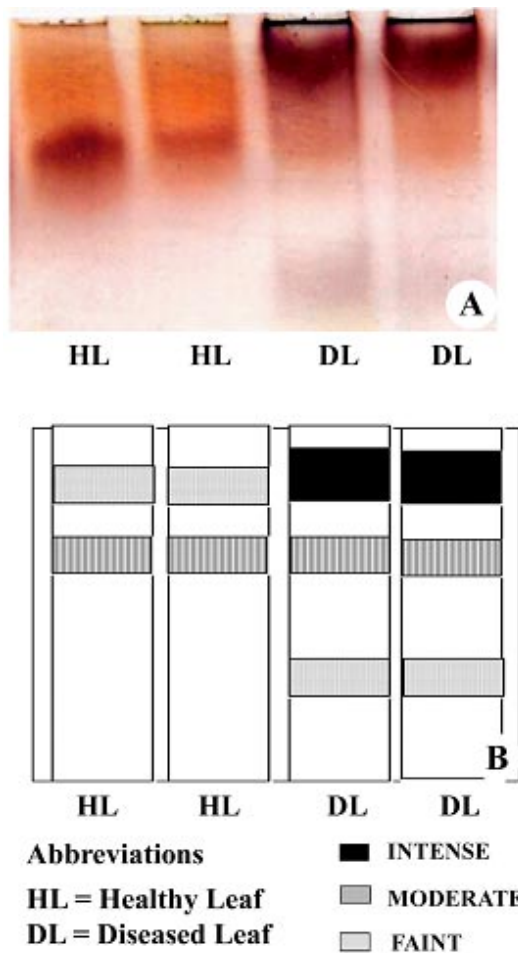


Fig. 5 A : Banding pattern of Peroxidase isozyme by PAGE B Zymogram of isozyme profile

References

- Anonymous (2003): Economics Survey of India (2002-03) Ministry of Finance and Company Affairs Economics Division, Indian. pp.245.
- Ansari N.A., Mishra V.N. and Tiwari J.P. (2006): Host range of tomato leaf curl virus. *Ann. Pl. Protec. Sci.*, **14**, 509-510.
- Bhattacharya A.K. and Chenchaiiah K.C. (2007): Seed coat phenolic compound of red gram *Cajanus cajan* as chemical barrier in formulation of artificial diet of *Spodoptera litura*. *Ann. Pl. Protec. Sci.*, **15**, 92-96.

Bray H.C. and Thorpe W.V. (1954): Analysis of phenolic compounds of interest in metabolism. *Meth. Biochem. Analysis*. **1**, 27-52.

Devanathan M., Ramaiah M., Sundar A.R. and Murugan M.(2005): Changes of peroxidase and polyphenol oxidase in bunchy top nana virus infected and healthy cultivars of banana. *Annals of Plant Physiology*. **19**, 114-116

Davis B.J. (1964): Disc electrophoresis II. Method and application to human proteins. *Ann. N.Y. Acad. Sci.* **121**, 404

Ghosal T.K., Dutta S., Senapati S.K. and Deb D.C. (2004): Role of phenol contents in legume seeds and its effect on the biology of *Collosbrchus chinensis*. *Ann. Pl. Protec. Sci.* **12**, 442-444.

Jain A.K. and Yadav H.S. (2003): Biochemical constituents of finger millet genotype associated with resistant to blast caused by *Pyricularis grisea*. *Ann. Pl. Protec. Sci.*, **11**, 70-74.

Kushwaha K.P.S. and Udit Narain (2005): Biochemical changes to pigeon pea leaves infected with *Alternaria tenuissinia*. *Ann. Pl. Protec. Sci.*, **13**, 415-417.

Maheshwari M.U., Suresh S. and Emmanuel N. (2006): Biochemical basis of resistance in rice hybrids and conventional varieties against brown plant hopper. *Ann. Pl. Protec. Sci.*, **14**, 69-72.

Parashar A. and Lodha P. (2007) : Phenolics estimation in foeniculum vulgare infected with Ramularia blight. *Ann. Pl. Protec. Sci.* **15**, 396-398.

Shinshi H. and Noguchi M. (1975): Relationship between peroxidase, IAA-oxidase and polyphenol oxidase. *Phytochemistry*. **14**, 1255-1258.

Singh H.V. (2004): Biochemical transformation in Brassica spp. due to *Peronospora parasitica* infection. *Ann. Pl. Protec. Sci.*, **12**, 301-304.

Worthington-Enzyme Manual (1972): Enzymes, Enzyme Reagents, related Biochemicals, Worthington Corporation Freehold, New Jersey, U.S.A. pp. 216.