



Changes in antioxidative enzymes and isozyme pattern due to *Exobasidium vexans* (blister blight) infection in tea cultivars

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ABSTRACT

Assessment of antioxidative enzymes and their isoenzyme pattern were studied with field grown tea plants having highly resistant (SA-6 & SMP-1) and highly susceptible (UPASI-14 & TES 31) traits to *Exobasidium vexans*. Antioxidative enzymes were assayed in healthy and infected leaves collected from selected tea clones. Peroxidase, ascorbate peroxidase and glutathione reductase have higher activity in resistant clones but catalase activity was very low while there was no trend in SOD activity. The zymograms of peroxidase and catalase in the resistant genotype showed amplified expression than susceptible tea clones. Induction and /or amplified expression of particular isoforms of peroxidase and catalase against blister blight infection in resistant tea clones led to the apparent conclusion that amplified isoenzyme expression linked with blight resistance. This might be considered as an important criterion in breeding programme to identify the *Exobasidium* resistant genotype in tea.

Keywords: Isoenzyme, antioxidative enzymes, blister blight

1. INTRODUCTION

Tea is the most popular and inexpensive beverage produced from tender shoots of the commercially cultivated tea plants (*Camellia* spp.). Being perennial crop, tea is affected by various pest and diseases (1). The crop loss in tea due to pests and diseases is estimated around 10-20% according to severity in Southern India (2). Chen and Chen (3) described nearly 400 tea pathogens where the foliar diseases are important because they affect the crop yield as well as the quality of final produce (made tea) (4,5).

Among the biotic stress, blister blight caused by a biotrophic fungus, *Exobasidium vexans* Masee is the most destructive foliar disease (6). When a plant recognizes an invading pathogen, active defence mechanism are induced which can include a hypersensitive response, accumulation of antimicrobial phytoalexins, synthesis and hydrolyses of pathogenesis related proteins, reinforcement of cell walls through callose deposition and lignification besides activation of defence related genes (7, 8). The production of reactive oxygen species (ROS) is an important defence mechanism in plants against

pathogens. In plant cells, ROS, mainly H_2O_2 , superoxide anion (O_2^-) and hydroxyl radical (OH) are generated in the cytosol, chloroplasts, mitochondria and the apoplasticspace (9, 10). A higher concentration of intracellular ROS has been shown to create cytotoxic conditions, including oxidative damage to lipids, proteins, and nucleic acids. Thus, excessive production of ROS may cause the disruption of cellular functions, finally leading to cell death (11). To overcome the negative consequence of ROS, plants have evolved various protective mechanisms either to reduce or to completely eliminate it (12) through antioxidative system of producing antioxidative enzymes and low molecular mass antioxidants (13). The enzymatic antioxidative system operates with a sequential and simultaneous action of many enzymes such as superoxide dismutase, peroxidase and catalase, which react with ROS and keep them at low levels while the low molecular weight antioxidants such as ascorbate and glutathione scavenge H_2O_2 at the expense of NADPH or NADH (14). There is no report on changes in antioxidative enzyme activity and their isozymes pattern among the resistant and susceptible tea cultivars in relation to leaf blister blight disease. Hence an attempt has been made to find out the changes in antioxidative enzyme activities, mainly those participating in the metabolism of ROS during pathogenesis.

2. MATERIALS AND METHODS

2.1. Plant material

Tea leaf samples were collected randomly from susceptible (UPASI-14 & TES-34) and resistant tea clones (SA-6 & SMP-1) grown at experimental field of UPASI Tea Research Institute, Valparai, located alongside the western Ghats of Southern India ($10^\circ 30'$ N, $77^\circ 0'$ E, altitude 1050 m above MSL). During the course of study period, five times sampling were done between June and October 2008.

2.2. Biochemical analysis

All the biochemical parameters were done with freshly collected tea shoots (two leaves and a bud) extracted with alcohol. Catechins were estimated adopting the method described by Swain and Hillis (15), while the polyphenols were quantified following the method described by Dev Choudhury and Goswami (16). Amino acids and reducing sugars were quantified following the methods described by Moore and Stein and Hedge and Hofreiter (17), respectively. All the results were expressed as per cent dry weight

2.3. Assay of enzyme activities

Peroxidase (POX) activity was determined using pyrogallol as the substrate with the amount of purpurogaline produced (18) and expressed as unit mg^{-1} protein. Catalase (CAT) activity was determined by involving the decomposition of hydrogen peroxide (19) and expressed as $\mu\text{mol H}_2\text{O}_2$ oxidized $\text{min}^{-1} \text{mg}^{-1}$ protein. Super oxide dismutase (SOD) activity was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium chloride (NBT) (20). One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the NBT photoreduction rate and the results were expressed as units per mg protein. Ascorbate peroxidase was determined according to Nakano and Asada (21). The decrease in ascorbate concentration was followed as a decline in optical density at 290 nm [$\epsilon = 2.8 (\text{mmol L}^{-1})^{-1} \text{cm}^{-1}$] and expressed as $\mu\text{mol ascorbic acid oxidized min}^{-1} \text{mg}^{-1}$ protein. Glutathione reductase activity was assayed by monitoring the glutathione dependant oxidation of NADPH (22). GR activity was calculated using the extinction coefficient for NADPH of $6.2 (\text{mmol L}^{-1})^{-1} \text{cm}^{-1}$ and expressed as $\mu\text{mol NADPH oxidized min}^{-1} \text{mg protein}^{-1}$. Total protein content measured spectrophotometrically at 595 nm where bovine serum albumin were used as a standard (23).

2.4. Native PAGE and visualization

The isoform profiles of peroxidase (POX) and catalase (CAT) were examined by discontinuous native polyacrylamide gel electrophoresis (native-PAGE) (24). Native PAGE was carried out by using 10% separating gel and 4.0% stacking gel. Equal amount of protein from each extract was loaded along with sample loading dye (Tris, pH 6.8; 0.1% bromophenol blue and 15% glycerol) in the wells. The electrophoresis was carried out at 20 mA constant current until the blue dye reached the bottom of the gel. Immediately after electrophoresis, staining activity was carried out by incubating the gel in substrate solution. All the steps were carried out at 4°C . Staining of POD isoenzymes was achieved by incubating the gels in sodium acetate buffer (pH 4.5) containing 2 mM benzidine (dissolved in DMSO). The reaction was initiated by adding 3 mM H_2O_2 and the reaction was allowed to continue for 20 min. (28).

2.5. Statistical analysis

Generated data were subjected to statistical analysis wherever possible and presented with standard error mean, critical difference and co-efficient of variation.

3. RESULTS AND DISCUSSION

Due to pathogen infection polyphenol and catechin contents were significantly higher in infected shoots than healthy shoots but reverse trend was observed in amino acids and reducing sugars. Tea clones exhibited significantly wide variation in polyphenol content and the ranged from 30.39 to 21.01% (Table 1). Identical trend was noticed with catechins, as the total catechins are a part and parcel of polyphenols. Amino acids and reducing sugars were significantly higher in healthy leaves than infected leaves. Accumulation of certain biochemical constituents due to pathogen infection in tea leaves were reported earlier (29, 30). Reports were also available on prominent reduction in all the constituents in certain tea clones due to their susceptibility to the disease (31). The reduction in sugar content of diseased leaves might be due to the increase in the rate of utilization by the pathogen as respiratory substrate during pathogenesis (32).

Table 1. Analysis of biochemical constituents

Clone	PP (%)	Cat (%)	AA (%)	RS (%)
UP-14(I)	25.33	14.72	2.55	7.51
UP-14(UI)	21.01	11.43	3.40	8.74
TES-34(I)	25.27	16.19	2.37	6.62
TES(UI)	23.85	14.37	3.24	7.49
SMP 1(I)	30.39	23.19	4.04	5.89
SMP 1(UI)	27.43	20.33	4.21	6.95
SA-6 (I)	27.47	18.53	4.36	8.31
SA-6 (UI)	24.83	16.55	4.83	9.73
SE	0.42	0.42	2.96	0.26
CD	0.89	0.89	0.09	0.54
P=0.05				
CV%	2.02	2.02	0.18	4.14

UP- UPASI, PP-Polyphenol, Cat- Catechin, AA- Amino acid, RS- Reducing sugar

Quantitative assay of POD, APX and GR were higher in healthy shoots than infected leaves (Table 2) and two times enhanced activity was observed in resistant clones (SA-6 and SMP-1) when compared to that of susceptible tea clones (UPASI-14 and TES-34). The results indicated existence of diversity among the tested tea clones during blight stress conditions and marked variations between susceptible and resistant

clones. Higher activity of POD enzyme in the resistant cultivar may support the development of defence mechanism in the host to overcome infection. Similarly, peroxidases were associated with disease resistance/susceptibility in many crops (35, 36, 37 and 38). The key enzyme, APX enhanced to overcome the stress conditions. Peroxidase could serve as useful biochemical marker for the selection of tea clones which resist the *Exobasidium vexans* pathogen efficiently. When accumulation of intracellular concentrations of H₂O₂ due to the depression of the CAT scavenger are induced to detoxify H₂O₂. Because of these conditions, sensible clone have higher activity of CAT. However there was no trend in SOD activity.

Table 2. Antioxidative enzyme activity

Clone	Pox	APX	GR	SOD	CAT
UP-14(I)	22.85	4.70	1.31	0.83	1.60
UP-14(UI)	32.31	6.19	2.42	0.46	0.87
TES-34(I)	23.44	2.73	0.89	0.50	1.35
TES(UI)	28.93	6.43	2.47	0.33	0.75
SMP 1(I)	38.29	9.22	1.96	0.82	0.83
SMP 1(UI)	45.19	11.13	2.04	0.52	0.49
SA-6 (I)	53.15	9.34	1.87	0.89	0.80
SA-6 (UI)	69.54	8.75	2.98	0.74	0.38
CD	2.96	0.11	0.28	0.02	0.06
CV	9.91	3.02	22.09	6.36	36.07
SE	1.41	0.05	0.13	0.01	0.03

*POD-Peroxidase activity (μ moles product formed/min/mg protein), APX –Ascorbate peroxidase activity (μ moles product formed /mg protein), GR- Glutathione activity (μ moles of NADPH oxidised /min/ mg protein),- SOD – Superoxide dismutase (U/ mg protein),Cat-Catalase (μ mole H₂O₂ Oxi/min/mg protein)

Differential response exhibited by the tea clones against leaf blister blight disease with particular reference to the expression of POX and CAT. Peroxidases are homoproteins, present as multiple isozymes in plant tissues and are distributed throughout the cell and catalyze the reduction of H₂O₂ to H₂O. The results of the isozyme profile under leaf blight for two resistant clones (SA-6 and SMP-1) revealed two active zones. Two CAT isoforms were observed in resistant clones (Fig. 1). Since equal amounts of protein extracts were

electrophoresed in a non-denaturing gel, the band intensity equates to enzyme activity.

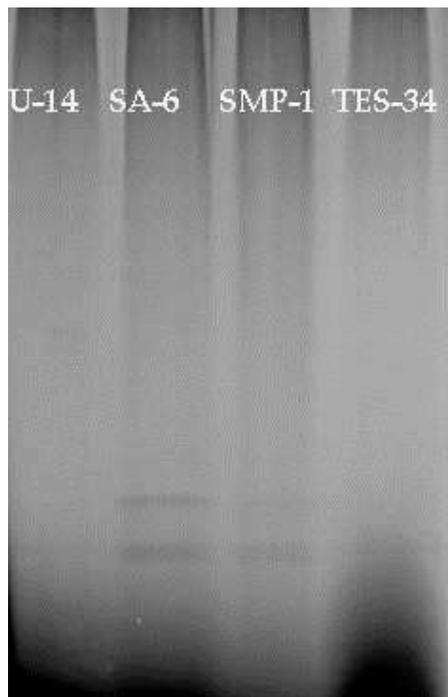


Figure 1. Isoforms of catalase native gel



Figure 2. Isoforms of peroxidase native gel

The resistant clones expressed high intensity bands while this isoforms disappeared and seemed to be absent in susceptible clones. However, blister blight susceptible clones have faint bands at the end of regions and it was absent in resistant clones. Thus,

there is an apparent correlation of higher POD activity (intense bands) with blister blight resistance, while lower activity with susceptibility. (Fig 2).The activity of CAT and POD isoforms indicated that induction and/or increased expression of either of these in the studied clones might be associated with leaf blister blight disease resistance.

Conflicts of Interest

There are no conflicts of interest.

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