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Alterations in zymogram of peroxidase and esterase isoenzymes in leaves of root (wilt) diseased coconut (*Cocos nucifera* L.) cv. West Coast Tall

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Root (wilt) disease (RWD) of coconut, which occurs contiguously in eight southern districts of Kerala, is caused by phytoplasma - a plant infecting, non-helical, non-culturable pleomorphic mollicutes (Solomon *et al.,* 1983). Ribbing of leaflets, yellowing and marginal necrosis of leaves, necrotic blackening of inflorescence in certain cases and gradual decline in yield are symptomatic characteristics of the disease. Unlike other phytoplasma diseases of crops and palms, RWD of coconut is a debilitating malady and not a lethal disease resulting in quick death of palms.

Many biochemical changes take place in diseased plants during infection of pathogens. Peroxidase (PO) has been extensively studied and implicated in plant resistance to various diseases. PO catalyses dehydrogenation of a large array of compounds like hydroxycinnamyl alcohols, phenolics and aromatic amines etc. (Montalbini *et al.,* 1995). Physiological roles of this haemoprotein are lignin synthesis, cross-linking cell wall polysaccharides and wound healing (Gross, 1980 and Lagrimini *et al.,* 1993). From a phytopathological point of view, role of peroxidase has been implicated in the defence response of diseased plants during infection of fungus (Cadena-Gomez and Nicholson, 1987; Krstic *et al.,* 1997) and virus (Montalbini *et al.,* 1995 and Ye *et al.,* 1990).

Esterases (EST) are fatty acid hydrolases involved in lipid synthesis and are responsible for changes in membrane lipids. Involvement of esterase from fungal pathogen during various stages of infection has been reported (Podila *et al.,* 1995; Muller and Ishii, 1997). It modifies the lipid composition of membrane in response to infection, rendering the membrane a stiff barrier to invading pathogens.

In this investigation, the profiles of PO and EST isoenzymes from tender leaves of healthy, apparently healthy (showing field tolerance to RWD and various stages of diseased coconut palms.

Tender, non-chlorophyllous slightly whitish to yellowish leaves from unopened fronds of coconut cv. West Coast Tall were used for isoenzyme studies. Healthy leaf samples were collected from palms from disease-free areas in Tirunelveli and Madurai districts of Tamil Nadu. These samples were serologically tested and confirmed to be disease-free. Apparently healthy leaf materials collected from elite mother palms from heavily diseased tract and found to be serologically negative were selected for breeding programme. Palms of varying intensity of disease *viz.* early, middle and advanced stages, were selected from the farm of CPCRI, Kayangulam.

The extraction buffer used was 0.05 M Tris-HCl (pH 7.5) with 1 µl 2-mercaptoethanol/ml of buffer (Fernando and Gajanayake, 1997). One gram of thoroughly washed leaf tissues were finely cut into small pieces separately and ground in 2 ml of extraction buffer using liquid nitrogen. While grinding, 40 mg insoluble PVP were mixed with the material. The clear supernatant obtained after centrifugation at 12,000 g for 10 min, was used as enzyme source for electrophoresis.

The electrophoretic run for isozymes was according to the method of Laemmli (1970). A

discontinuous native-polyacrylamide gel system, which consisted of 4% stacking gel and 7% separating gel was used throughout. The enzyme source was mixed (1:1v/v) with sample buffer (0.5 M Tris-HC1, pH 6.8, 20% glycerol, 0.1% 2- mercaptoethanol and 1% bromophenol blue as tracking dye). Twenty five µl of sample was loaded from each sample for the study. The run was performed at constant current of 40 mA until the bromophenol blue had crossed the stacking gel and thereafter the run was completed at 50mA. All the operations were carried out at 4 °C.

The staining solution for PO was 1% Tetramethyl Benzidine in 3% H_2O_2 (Winterhatter and James, 1983) whereas painting of esterase was revealed in a mixture of α- and β- naphthyl acetates and Fast Blue RR salt in 0.05 M sodium phosphate buffer (Smith *et al.,* 1970) at room temperature. The band profile in stained gels were immediately recorded and gels were fixed in 7% acetic acid solution.

The investigation revealed clear alterations in zymogram of the PO enzyme system in leaves of diseased coconut palms. In total, two isoenzymes of PO were observed and designated as 1 and 2 in increasing order of migration in the healthy and apparently healthy palms (Fig. 1). Although both the PO1 and PO2 could be detected in disease early palms, the relative intensity of PO2 was greatly reduced and only partially detectable. In palms of middle and advanced stages of disease. PO2 was not found. However, PO1 could be detected in diseased palms irrespective of severity of the RWD. The partial detection of PO2 in early stage of disease and its total absence with the progress of the disease severity indicates its association with host resistance/susceptibility.

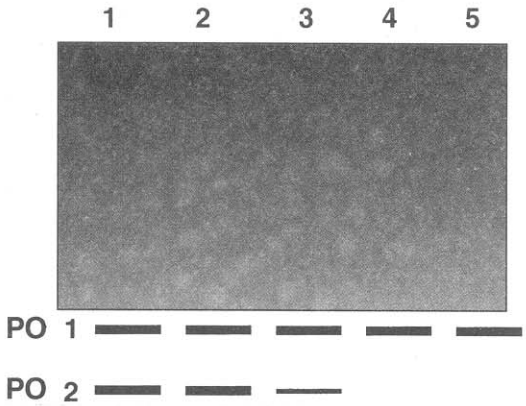

Fig. 1. Peroxidase isoenzyme profiles in leaves of root (wilt) diseased coconut, cv. West Coast Tall. Zymogram (above) and line drawing (below) showing size and intensity of bands for peroxidase; lane 1: healthy; 2: apparently healthy; lane 3: early stage of disease; lane 4: middle stage of disease and lane 5: advanced stage of disease.

There is no report on PO studies between diseased and healthy palms in case of phytoplasma disease/infection. However, while studying peroxidase polymorphism, Fernando and Gajanayake (1997) reported the presence of either single peroxidase locus or three loci among tall genotypes of coconut in Sri Lanka.

Many investigators have demonstrated increased activity and appearance of more number of PO isoforms during infection of virus (Simons and Ross, 1970; Montalbini *et al.,* 1997). These results of increased number and enhanced activity of PO were obtained during initial stages of pathogenicity and associated with necrotic reactions in the host limiting/arresting systemic spread of the infection. The qualitative and quantitative increases observed in PO following localized virus infection after inoculation with virus, is a consequence of tissue necrotization (Waigh, 1993 and Montalbini *et al.* 1995). Fig 1 indicates presence, absence and intensity of peroxidase profiles in leaves of healthy and diseased coconut palms. The presence of PO2 in the apparently healthy palms is a clear indication of characteristics of tolerance/resistance to the disease.

Isozyme profile of esterase in leaves of healthy and diseased coconut is shown in Fig. 2. In this case, seven isoesterases could be detected which are designated as 1-7 in increasing order of mobility. The esterases 1-7 could be clearly deciphered in healthy and apparently healthy palms, though the enzymes 1 and 7 intensities were found to be less. The results between these two were similar and comparable. In disease early stage, all the seven isoforms were detected, however, surprisingly bands of isoforms 3 and 5 were thick and expressed strongly. The thickness reflects strong activity of these ESTs during initial period of the infection. In middle and advanced stages of the disease, the intensity of all isoforms were reduced significantly, with ESTs 1 and 7 showing the status of disappearance.

As can be seen from the results of this study the notable result was the maintenance of activity of all seven ESTs in apparently healthy palms as in healthy palms, enhanced activity of isoESTs 3 and 5 during early stage of the disease and weakening of all the seven isoforms in the other stages of the disease.

Fernando and Gajanayake (1997) reported four esterases in two different zones of activity among tall papulation of coconut in Sri Lanka as against five esterases in Indonesian tall genotypes. However, in our study, five isoforms of esterases were round in main locus, which are comparable with results of the other

studies. In addition, two more ESTs were observed in the cv. of this study.

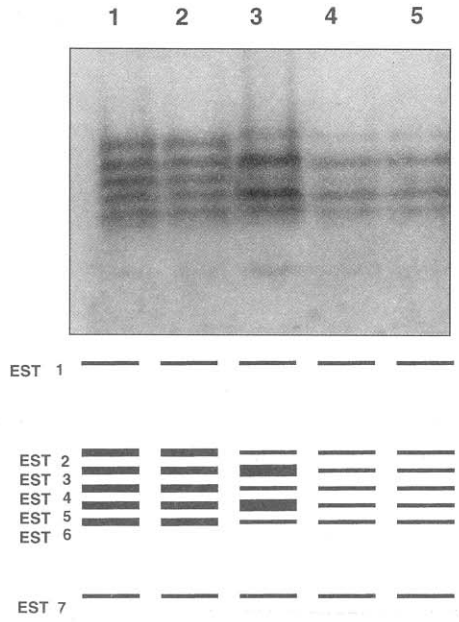

Fig. 2. Esterase isoenzyme profiles in leaves of root (wilt) diseased coconut , cv. West Coast Tall. Zymogram (above) and line drawing (below) showing size and intensity of bands for esterase; lane 1: healthy; lane 2: apparently healthy; lane 3 : early stage of disease; lane 4; middle stage of disease and lane 5 : advanced stage of disease

In a similar study of changes in EST isoenzymes following virus infection, no qualitative or quantitative changes could be detected (Waigh, 1993). In another study on esterases between healthy and lethal yellowing of palms, up to five esterases were detected, however, no consistent differences were found between healthy and diseased palms (McCoy, 1983). Furthermore, there are reports of involvements of EST from pathogen in disease development (Muller and Ishii, 1997).

Plant resistance to pathogens is a very complex phenomenon. PO is known to play a major role in the mechanism of resistance by involving in phenylpropanoid metabolism and lignification. The alterations in profiles of PO_2 isoforms are connected with structural defence against pathogen (Montalbini *et al.*, 1995). Thus, presence of PO_2 in apparently healthy palms and its disappearance from the diseased palms indicates its role in the resistance/susceptibility of coconut to RWD. The higher intensity of esterase in healthy palms indicates an increased rate of synthesis of membrane lipids, acting as a barrier to the infesting pathogens as reported in other crops.

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