

PHENOL METABOLISM OF THE COCONUT PALMS (*COCOS NUCIFERA L.*) IN RELATION TO ROOT (WILT) DISEASE

K.V. JOSEPH AND N.P. JAYASANKAR

*Central Plantation Crops Research Institute, Regional Station,
Kayangulam, Krishnapuram 690 533 Kerala, India*

ABSTRACT

Enzymatic assay with the acetone powder preparations of the coconut roots revealed an increase in the levels of polyphenol oxidase and peroxidase with the incidence and increase in intensity of coconut root (wilt) disease. The levels of free phenols in the coconut roots were comparatively low in the root (wilt) affected palms compared to the apparently healthy palms. The levels of phenyl alanine ammonia lyase were also higher in the coconut palms cultivated in the root (wilt) affected palms compared to the palms cultivated in the disease free region. The results are suggestive of an accelerated phenol metabolism characteristic of root (wilt) disease.

INTRODUCTION

Earlier observations from this laboratory have indicated a fall in the total phenol content in roots of coconut with the incidence and increase in intensity of root (wilt) disease (Joseph and Jayasankar, 1973). The activities of phenol oxidising enzymes such as polyphenol oxidase (PPO) and peroxidase (PO) are comparatively high in diseased root samples compared to the healthy ones (Joseph *et al*, 1976). In plants, increased activity of phenylalanine ammonia-lyase-(PAL) has been shown by several workers due to varying factors such as infection, wound injury and environmental conditions (Biehn *et al*, 1968; Camm and Towers, 1973; Engelsma, 1972; Zucker, 1971; Yamamoto *et al*, 1977).

In coconut root (wilt) disease, the casual agent is still unknown, an attempt has been made to elucidate the level of PAL, PPO and PO activity for a comparison between the healthy samples from Kasaragod and diseased samples from Kayangulam. The results are discussed in this paper.

MATERIALS AND METHODS

Newly emerging root samples without any apparent injuries were collected from diseased palms and kept in the freezer for 24 to 48 hrs. prior to enzyme preparation. Healthy root samples were similarly collected from Kasaragod, a disease free tract, and frozen samples were brought to Kayangulam by keeping it in an ice box.

Acetone powder was prepared from each sample in the same way as described in the earlier paper (Joseph *et al*, 1976). Approximately 15 g of sliced samples were macerated in a Waring blender with 250 ml of cold citrate-phosphate buffer pH 7.0. The extract was filtered through cheese cloth to remove the debris and the extract was added to double the volume of chilled acetone with gentle stirring. The precipitate was collected by using a Buchner funnel and dried in a vacuum desiccator kept at 0°C. All operations were carried out in a cold room maintained at 0°—4°C. The acetone powder was used as crude enzyme. The polyphenol oxidase activity was determined by the method of Matta and Dimond (1963) and Peroxidase activity by the Pyrogallol test (Colowick and Kaplan, 1955).

PAL activity was determined by the method described by Subba Rao and Towers (1970). 2.0 ml of tris-chloride buffer pH 8.8 (0.05 M), 2.0 ml of L-Phenylalanine (0.01 M) and 1 ml of enzyme (250 mg acetone powder dissolved in 10.0 ml tris-chloride buffer pH 8.8) with a total volume of 5.0 ml was incubated at 38°C in a water bath for 4 hrs. The reaction was stopped by the addition of 0.5 ml of 1 N.Hcl and the cinnamic acid was extracted with diethyl-ether. The ether layer was taken and traces of water were removed by adding 0.5 gm anhydrous sodium sulphate and the ether extract was evaporated to dryness by using a hair drier. The residue was dissolved in 5.0 ml of 0.05 N.NaoH and the cinnamic acid content was determined spectrophotometrically at 268 m in Beckman Du-2 Model Spectrophotometer. Assay mixture with 0.5 ml of 1 N.Hcl incubated at 38°C was the control.

RESULTS AND DISCUSSION

Increased content of phenolic compounds in plants have been reported by several workers due to infection and other environ-

mental conditions (Kiralay and Farkas, 1962; Camm and Towers, 1973; Engelsma, 1972; Zucker, 1971; Yamamoto, 1977). But Seevers and Daly (1970) reported that there is no significant change in the total phenolics in healthy or inoculated, resistant or susceptible plants at any stage of disease development. On the contrary Joseph and Jayaksankar (1973) reported a fall in the total phenol content in roots with the incidence of Coconut Root (wilt) disease. The fall is highly significant between healthy and diseased (Table 1).

Table I Total phenolic content in healthy and diseased coconut root samples

Condition of the palm	No. of samples analysed	Mean phenolic content (mg/100 g dry wt.)
Healthy	36	130.08
Diseased	21	59.95

Explanation for such change is given by Joseph *et al* (1976). Activity of the phenol oxidising enzymes increased with incidence of the disease (Table II).

Table II Polyphenol oxidase (PPO) and peroxidase activity (PO) in coconut root samples.

Condition of the palm	No. of samples analysed	Enzyme activity (OD mg of protein/5 min)	
		PPO	PO
Healthy	30	5.2 (2.1 — 8.0)	4.7 (1.0 — 7.0)
Diseased	29	12.4 (9.5 — 20.0)	11.8 (8.0 — 23.1)

The activities of the polyphenol oxidase and peroxidase are highly significant between apparently healthy and diseased. Decrease in the total phenol content may be due to higher oxidation.

The conversion of L-Phenylalanine to *trans* cinnamic acid is catalysed by the enzyme Phenylalanine ammonia-lyase (PAL) which provides phenylpropanoid skeleton for the various phenolic compounds. This enzyme is widely distributed in plants and its activity is altered due to various factors. In *Glycine max* an initial increase in total phenols with an increased activity of PAL has been shown by Biehn *et al* (1968) when inoculated with *Helminthosporium carbonum*. According to Green *et al* (1975) the activation of PAL and subsequent increase in phenol content are general responses associated with resistant plants. Increased activity of PAL has been shown in potato when inoculated with *Eriwinia caratovora* and in sweet potato when there is a cut injury. (Gupta and Tripathi, 1978; Tanaka *et al*, 1977). Studies on PAL in coconut roots revealed that the activity of the enzyme is increased with the onset of the disease. The results obtained are shown in Table III.

Table III. Specific activity of Phenylalanine Ammonia - Lyase in coconut roots.

Condition of the palm	No. of samples analysed	PAL Activity (ug/mg of protein/4 hr.)
Healthy (Kasaragod)	14	0.9 (0.62 — 1.23)
Diseased	25	17.9 (2.05 — 49.28)

The activity of the enzyme in diseased samples is highly significant. The results are suggestive of an accelerated phenol metabolism characteristic of root (wilt) disease. Investigations are in progress to evaluate correlation, if any, between the Coconut Root PAL and foliar symptoms of disease.

REFERENCES

- BIEHN, W.L., KUC, J., AND WILLIAMS, E.B. 1968. Accumulation of phenols in resistant plant—fungi interactions. *Phytopathology* 58 : 1255—1260.

- CAMM, E.L., AND TOWERS, G.H.N. 1973. Is cinnamic acid 4-hydroxylase of potato a light induced enzyme? *Can. J. Bot.* **51** : 824—825.
- COLOWICK, S.P., AND KAPLAN, N.O. 1955. The Pyrogallol test. *Methods in Enzymology* Vol. II. p. 773.
- ENGELSMA, G. 1972. Possible Role of Divalent Manganese ions in production of phenylalanine Ammonia-Lyase. *Plant Physiol.* **50** : 599—602.
- GREEN, N.E., HADWIGER, L.A., AND GRAHAM, S.O. 1975. Phenylalanine Ammonia—Lyase, Tyrosine Ammonia Lyase in wheat inoculated with *Erysiphe graminis* f. sp. *tritici*. *Phytopathology* **65** : 1071—1074.
- GUPTA, R.P., AND TRIPATHI, R.K. 1976. Phenylalanine Ammonia Lyase and phenol in tubers in Kurfi Dewa in Relation to resistance against bacterial soft rot. *Indian J. Expt. Biol.* **14** : 365—366.
- JOSEPH, K.V., AND JAYASANKAR, N.P. 1973. Polyphenol content in coconut roots in relation to root (wilt) disease. *J. Plant. Crops* **1** (supplement). 99—101.
- JOSEPH, K.V., POTTY, V.P., AND JAYASANKAR, N.P. 1976. Increase in polyphenol oxidase and peroxidase with higher intensities of coconut root (wilt) disease. *J. Plant. Crops.* **4** (1) : 4—6.
- KIRALY, Z., AND FARKAS, G.L. 1962. Relation between phenol metabolism and stem rust resistance in wheat. *Phytopathology* **52** : 657—664.
- MATTA, A., AND DIMOND, A.E. 1963. Symptoms of Fusarium wilt in relation to quantity of fungus and enzyme activity in tomato stems. *Phytopathology* **53** : 574—578.
- PAUL, M., SEEVERS AND DALY, J.M. 1970. Studies on Wheat Stem Resistance at Sr. 6 Locus. 1. Role of Phenolic Compounds. *Phytopathology* **60** : 1322—1328.
- SUBBA RAO, P.V., AND TOWERS, G.H.N. 1970. L-Phenylalanine Ammonia-Lyase (*Ustilago hordei*). *Methods in Enzymology*. Vol. XVII 581—585.
- YAMAMOTO, H., HOKIN, H., TANI, T., AND KADOTA, G. 1977. Phenylalanine Ammonia-Lyase in relation to crown rust resistance of Oat Leaves. *Phytopath. Z.* **90** : 203—211.
- YOSHIYOUKI, T., KAZUNOBU, M., AND URITANI, I. 1977. Some investigations on inactivation of Phenylalanine Ammonia—Lyase in cut-injured sweet potato root tissue. *Plant and Cell Physiol.* **18** : 1209—1216.
- ZUCKER, M. 1971. Induction of Phenylalanine Ammonia-Lyase in *Xanthium* leaf disc. *Plant Physiol.* **47** : 442—444.