ALTERATIONS IN THE LEAF PROTEIN CONTENT OF COCONUT AFFECTED BY ROOT (WILT) DISEASE

G. PADMAJA*, B. SUMATHYKUTTY AMMA, CHACKO MATHEW, P.T.N. NAMBIAR AND R. SNEHI DWIVEDI**

Central Plantation Crops Research Institute, Regional Station, Kayangulam, Krishnapuram-690533, India.

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SUMMARY

Changes in the protein content of leaves from healthy and root wilt affected coconut palms have been studied. Alkali—extractable, water—extractable and ethanol—extractable protein fractions were found to be higher in the healthy palms when compared to the diseased palms. A preferential extraction of proteins at low pH values could be obtained in the middle and outer leaves from apparently healthy and diseased palms. An increase of 53.7% and 24.9% over the protein values in the healthy palms was noticed in the middle and outer leaves respectively of the apparently healthy palms while the increase was 42.8% and 14% in the respective leaves of diseased palms. The implications of the protein changes in root (wilt) disease have been discussed.

INTRODUCTION

Root (wilt) disease of coconut (*Cocos nucifera Linn*) is a complex disease, the causative factors of which have not yet been fully uuderstood. However it is believed to be caused by the combined effect of pathogens, nutrient imbalance and water stress. Physiological and biochemical derangements have also been noticed in the diseased palm. Accumulation of free aminoacids especially aspartic acid, threonine, asparagine and glutamine has been reported in the case of the diseased palm (Pillai and Shanta, 1965). An increase in the non-protein nitrogen in the diseased palm with a concomitant decrease in the water-soluble and protein nitrogen fractions has also been observed (Thomas Varkey, *et al.*, 1969). No further reports are available on the nitrogen metabolism of root (wilt) diseased coconut palms. Qualitative and quantitative protein changes are known to occur in plants as they

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Present Address: *Central Tuber Crops Research Institute, Sreekariyam, Trivandrum-695017.

**Central Soil and Salinity Research Institute, Karnal, Haryana.

get diseased (Uritani, 1971). In the present study, an attempt has therefore been made to understand the alterations occuring in the proteins in the root (wilt) affected coconut palm.

MATERIALS AND METHODS

The determinations were carried out in the leaf samples from 10 healthy, 10 apparently healthy and 10 root (wilt) diseased West Coast Tall variety of coconut palms. The plams were of 18-25 age group and growing in the farm areas of CPCRI, Kasaragod (healthy area) and CPCRI, Kayangulam (diseased area). Leaf samples belonging to four categories viz. spindle leaf, first fully opened leaf, middle leaf and outer leaf were collected from these palms. From each leaf, six leaflets were collected from both sides of the rib. Mid ribs were removed from the leaflets and middle portion of the leaflets were cleaned and sliced to pieces of 1 cm length. The sliced tissues were pooled and 1 gm was taken from this as the representative sample. Five different protein fractions viz. the alkali-extractable protein, water-extractable protein, trichloroacetic acid (TCA)—extractable protein, trichloroacetic acid (TCA)—precipitable protein and ethanol—extractable protein were estimated in the leaf samples. The various extracts for protein determination were prepared as below :—

1 g each of the finely cut tissues from healthy, (H) apparently healthy (AH) and diseased (D) palms were weighed into clean dry mortars and macerated well with a little acid—washed sand. Extracting solvents were added to each of the four leaves from the three categories of palms as follows :

1. 25 ml of 0.1 N sodium hydroxide
2. 25 ml of double-distilled water
3. 25 ml of TCA (2%). (out of the five concentrations from 1% to 5% tried, 2% TCA was found to extract more protein)
4. 25 ml of 0.1 N sodium hydroxide. To 5 ml of this, 5 ml of 10% TCA was added. The precipitate obtained by centrifugation at 3000 rpm for 30 minutes was washed with double distilled water and recentrifuged. The precipitate was then dissolved in 0.01 N sodium hydroxide and used for protein determination.
5. 25 ml of ethyl alcohol (40%). (Out of the various concentrations from 10% to 90% tried, 40% ethanol extracted more protein and so used in the study).

The extracts were filtered through Buchner funnel using whatman No. 1 filter paper and filtrate collected in stoppered flasks of 50 ml capacity. Protein contents of the extracts were determined in an aliquot from each by the method of Lowry *et al.* (1951). The protein content was calculated from a standard curve prepared using bovine serum albumin and the values are expressed on a dry weight basis.

Results and Discussion

The results of the study are presented in table I. The alkali-extractable, water—extractable and ethanol-extractable protein fractions were found to be higher in the healthy palms when compared to the diseased palms. In the healthy palms, there was an increase of 34.2%, 15.9%, 19.0% and 10.1% in the alkali-extractable fractions in the spindle-leaf, first-fully opened leaf, middle leaf and outer leaf respectively as compared to those of diseased palms. The increase in the water extractable protein and ethanol-extractable protein was 59.0%, 50.2%, 49.5% and and 47.8% for the former in the four leaves respectively and 21.6%, 42 8%, 4.1% & 12.4% for the latter in the respective leaves. The protein contents progressively increased in the first three whorls of leaf with the maturity of the leaf while no steady pattern could be obtained in the outer leaves which were senescent. The senescent leaves undoubtedly have impaired metabolism where there will be imbalanced synthesis and degradation. The diseased tissues have been reported to synthesise the proteins at a slow rate as compared to the actively metabolising healthy tissue (Uritani, 1971). The low protein values met with in the diseased palm may therefore be the net effect of a decelerated protein synthesis and accelerated protein breakdown. A steady decline in the protein values has been reported in the *Luffa cylindrica* fruits after inoculation with *Pythium aphanidermatum* (Sharma and Seema Nahab, 1975). Carbodydrates are believed to play a decisive role in protein synthesis and growth. Glucose has been found to be essential for the nitrogen assimilation by *Chlorella* (Kandler and Ernst, 1955) and also for the protein synthesis by *Scopulariopsis brevicaulis* (Mac Millan 1965). A decrease in the carbohydrate fraction in the root (wilt) diseased coconut palm has been noted earlier (Chacko Mathew, 1977). This may also be adversely affecting the protein synthesis by the diseased palm. Ethanol (40%) was found to extract proteins even more than what alkali and water could extract. The tertiary and quaternary structure of the proteins organised mainly by the hydrophobic forces may necessitate the presence of an organic solvent like ethanol to rupture these forces. Once the hydrophobic bonds are broken, the proteins readily dissolve in water or alkali. This is further supported by the fact that 40% ethanol which contained 60% water extracted more proteins than the higher concentrations. In contrast to the alkali-extractable, water-extractable and ethanol-extractable protein fractions, the TCA-extractable and TCA-precipitable protein fractions showed a decrease in the diseased palm in the first two whorls of leaf and an increase in the middle and outer leaves. A preferential extraction of more proteins at low pH values of 2.8 could be obtained in the leaves of tobacco and cow pea after inoculation with Tobacco Necrosis Virus (Coutts, 1978). It is likely that the diseased palm may be containing more of basic proteins of the histone/protamine type which will have a preferential solubility at low pH. Acidic amino acids and their amides have been found to accumulate in the

Table I. Different fractions of proteins in the leaves of healthy, apparently healthy and root (wilt) diseased coconut palms†

(Mean value taken from 10 palms)

Type of leaf	Alkali-extractable protein			Water-extractable protein			TCA-extractable protein			TCA-precipitable protein			Ethanol-extractable protein		
	mg/g dry wt.			mg/g dry wt.			mg/g dry wt.			mg/g dry wt.			mg/g dry wt.		
	H	AH	D	H	AH	D	H	AH	D	H	AH	D	H	AH	D
Spindle Leaf	*	a		*	a		a	**	a	a			a	a	
	101.46	81.13	66.71	111.68	54.95	45.08	73.91	28.32	50.50	19.62	12.27	17.26	95.94	79.18	75.11
First fully opened leaf	a	a		*	a		a	a		*	*		*	a	
	155.88	145.21	130.18	181.66	99.14	90.42	113.30	63.95	85.04	49.49	25.05	34.65	207.4	147.13	120.11
Middle leaf	**	a		*	a		**	**		a	**		a	a	
	166.31	151.10	134.61	195.43	95.22	98.54	123.84	205.68	172.6	59.06	81.0	66.11	248.14	236.22	237.7
Outer leaf	a	**		*	a		a	a		*	a		a	a	
	138.94	164.27	124.88	187.05	106.83	97.66	139.29	142.36	111.4	31.09	58.23	50.60	259.46	252.85	221.37

H—Healthy palm, AH—Apparently healthy palm, D– Diseased palm

†—Healthy and apparently healthy palms compared with diseased palms for finding out statistical significance.

*—Significant at 5% level

**—Significant at 1% level

a—non-significant

diseased palm (Pillai and Shanta, 1965). The present finding is therefore in agreement with this previous report suggesting the probable non-participation of the acidic amino acids in protein synthesis by the diseased palm. The basic proteins in Tobacco Mosaic Virus infected tobacco leaves were found to have specific roles in the viral infection (Gianinazzi, *et al.*, 1977.) The basic proteins in coconut leaves may also be possibly associated either directly or indirectly with the wilting mechanism. The studies therefore conclude that altered protein metabolism can also be one of the factors responsible for the vast decay and wilting of the root (wilt) diseased coconut palm.

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