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BIOCHEMICAL COMPOSITION OF VASCULAR SAP FROM THE INFLORESCENCE OF APPARENTLY HEALTHY AND ROOT (WILT) DISEASED COCONUT PALMS

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ABSTRACT

Vascular sap was collected from the inflorescences of apparently healthy and root (wilt) diseased coconut palms under aseptic conditions. The pH of the sap ranged from 6.8 to 7.5. Osmotic concentration was lower in the sap from the root (wilt) affected palms (540-620 m moles/kg) while apparently healthy palms showed higher values (790-850 m moles/kg). Direct analysis of the sap for the biochemical constituents, viz. sugars, protein, free amino acids, lipids and sterols showed discernible difference between the healthy and diseased palms. The major amino acids, sugars and organic acids were detected in the sap based on chromatographic studies. The possibility of utilising this data in preparing the media for culturing mycoplasma like organisms is discussed.

INTRODUCTION

Association of mycoplasma like organisms (MLOs) has been shown in root (wilt) disease of coconut (Solomon *et al.*, 1983) as is the case with lethal yellowing disease of coconut in Jamaica (Beakbane *et al.*, 1972). These organisms are restricted to the phloem sieve elements of sink areas such as rachilla of unopened inflorescence, apical meristem, petiole of developing leaves and root apices (Plavsic Benjac *et al.*, 1972; Solomon *et al.*, 1983). These fastidious organisms requiring special nutritional and environmental conditions are not readily amenable to cultivation in *in vitro* systems. Studies on the analysis of the sap were carried out by earlier workers to understand the biochemical/nutritional requirement of the organisms (Stemmer *et al.*, 1982). In this study, an attempt has been made to determine the composition of the vascular sap and assess the physical and chemical parameters so that a medium akin to the sap could be synthesised for the culturing of the organisms.

MATERIALS AND METHODS

Seven healthy coconut palms (*Cocos nucifera* L. var. West Coast Tall) and seven root (wilt) diseased palms with disease index 11-20, grown in the Institute campus were selected for the collection of the vascular sap. Unfermented sap

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was collected from the inflorescence under aseptic conditions as per the method standardised earlier (Rajagopal, *et al.*, unpublished).

Frozen sap thus collected, was immediately brought to laboratory, thawed and the volume measured. The pH and osmotic concentration of the sap was measured, using a Beckman pH meter and Wescor vapour pressure osmometer respectively. Fermentation of the sap was judged by odour and determination of pH.

Aliquots from the fresh sap were used for the analysis of the biochemical constituents. Protein was estimated from the sap by Folin-Dennis method (Lowry *et al.*, 1951) after precipitating the protein in trichloroacetic acid and dissolving in dilute alkali. Total sugars were estimated directly by the phenol-sulphuric acid method (Dubois *et al.*, 1951) and reducing sugars by the modified method of Nelson (1944). The levels of free amino acids in the sap were determined by the method of Moore and Stain (1948). Modified Sakaguchi's method was used to estimate arginine levels in the sap (Mcpherson, 1942) and phenols by the Folin-Ciocalteu method (Bray and Thorpe, 1954.)

For the extraction of total lipids, the sap was treated with chloroform: methanol mixture (3:1) twice, pooled and evaporated to dryness. The lipids were estimated gravimetrically. The extracts were saponified by adding 3 KM KOH at 80°C for 2 hours. Sterols, which come under nonpolar saponified lipids were extracted with petorleum ether, volume decreased by evaporation and estimated colorimetrically (Abell and Kendak, 1952).

The sap samples were fractioned through Dowex columns after concentrating them with insoluble polyvinyl pyrolidone (PVP). The resins – Dowex – 50 X_8 (H^+, 200-400 mesh) and Dowex–1 X_8 (Cl^{-1}, 200-400 mesh) – were used for fractionation in 1×6 cm columns by a modification of the method of Canvin and Beevers (1961). Three fractions were collected, comprising aminoacids (basic fraction), sugars (neutral fraction) and organic acids (acidic fraction). The fractions were lyophilised and aliquots of the water extracts were used for further chromatographic studies.

Cellulose coated plates (1 mm thick) were used for the detection of aminoacids with solvent system Butanol:acetic acid: water (4:1:1). Sugars and organic acids were detected by paper chromatography using Whatman No. 1 filter paper. The solvent systems used were Butanol:Acetic acid:Water (4:1:1) and Ethanol:Water: Ammonia (100:12:16) respectively. The spots in all cases were identified based on the R_f values of authentic standards.

RESULTS

The pH and osmotic concentration of the sap from diseased and healthy palms are presented in Figs.1 and 2. The average value of the pH of the sap from healthy palms was always less (6.1-6.4) than that from diseased palms (6.9-7.2). In general, the pH of the sap collected during night in both diseased and healthy palms was less as compared to the day collections. Similar trend was also observed in the case of lethal yellowing in Jamaica (Milburn and Zimmermann,

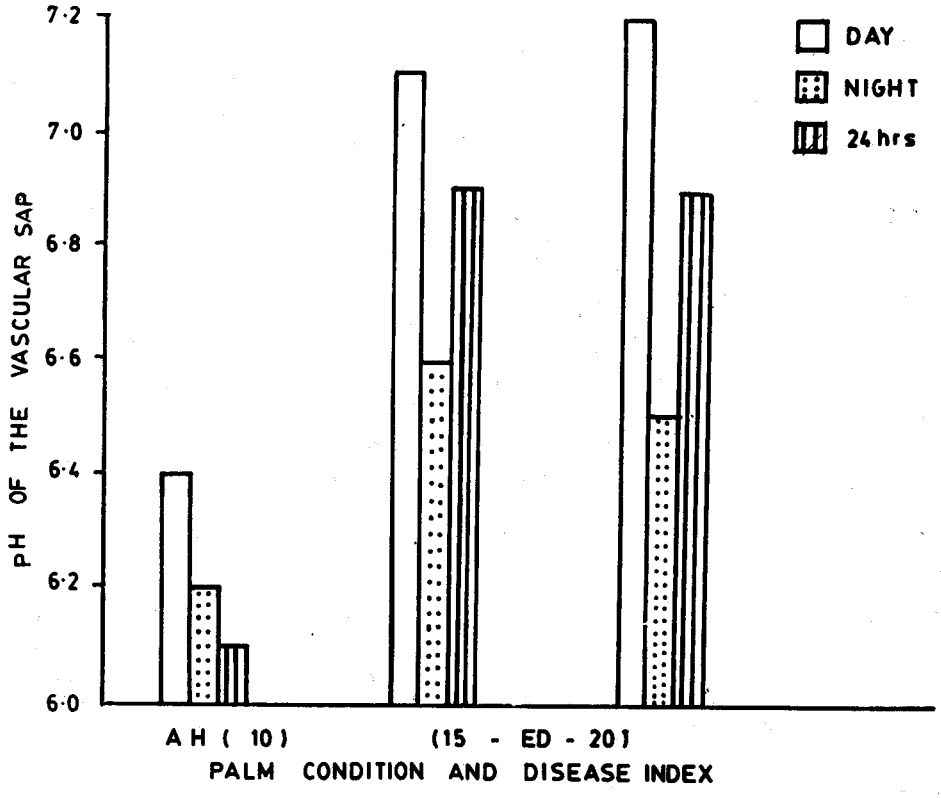

Fig. 1. pH of the vascular sap collected from the inflorescence of diseased and healthy coconut palms.

1977; Edengreen and Waters, 1982).

The osmotic concentration of the sap from healthy palms was higher than that from diseased palms both during day and night. (Fig. 2). Though the values for osmotic concentration of the sap from healthy palms differed between day and night, in diseased palms it was invariably higher during night.

Table I shows in detail the levels of the biochemical constituents present in the sap from healthy and diseased palms. Most of these constituents show marked differences between healthy and diseased palms. Thus proteins, phenols, reducing sugars, free amino acids, lipid components and sterols were found to be in

Table. I. *Biochemical constituents of vascular sap.*
(Values expressed as mg. g^{-} sap solids)

Constituents	Apparently healthy	Diseased
Total sugars	299.50	286.20
Reducing sugars	98.10	65.90
Proteins	4.90	2.50
Free aminoacids	45.90	18.30
Arginine*	0.41	0.68
Phenols	0.60	0.20
Lipids	0.83	0.57
Sterols*	0.40	0.22

*Values expressed as mg. ml^{-1} sap.

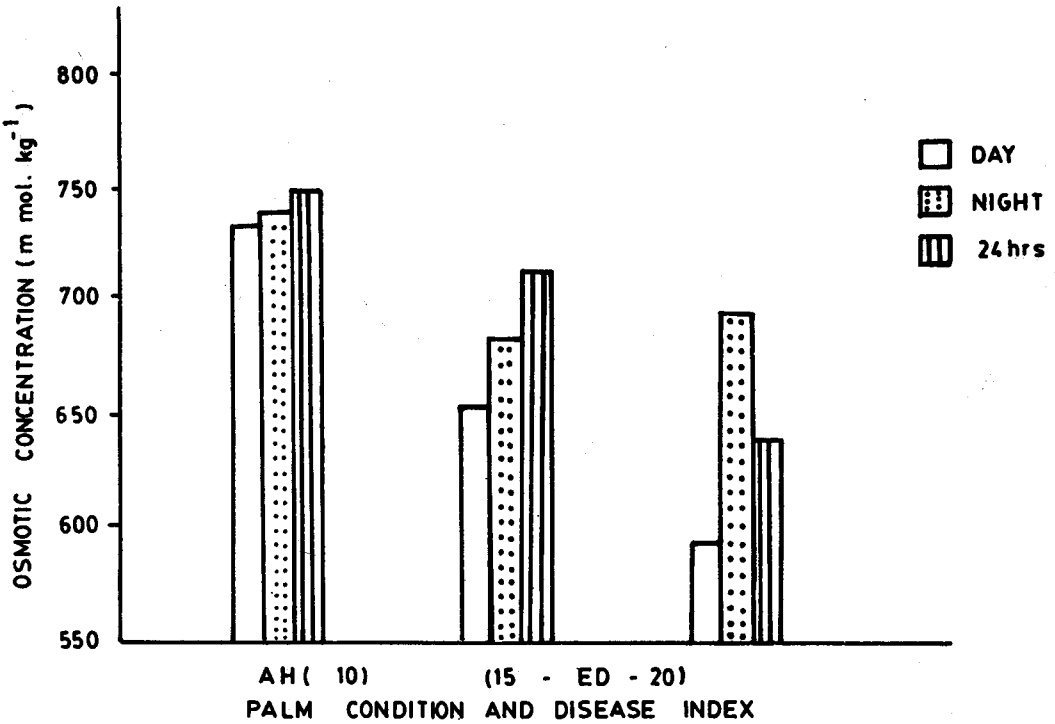

Fig. 2. *Osmotic concentration of the vascular sap collected from the inflorescence of diseased and healthy coconut palms.

lesser quantity in the sap of diseased palms as compared to healthy. Interestingly, arginine levels were found to be more in the sap from diseased palms even though the total free amino acids were less.

The following individual aminoacids, organic acids and sugars could be detected in the sap from both healthy and diseased palms. The amino acids were: Cysteine, Cystine, Arginine, Serine, Alanine, Tyrosine, Glutamic acid, Aspartic acid, Methionine and Isoleucine. The organic acids were Malic acid, Fumaric acid, Succinic acid, Maleic acid and Lactic acid and sugars were Sucrose, Glucose, Fructose, Raffinose, Galactose and Lactose. On comparison, no qualitative difference was seen between healthy and diseased palms. The quantification of these parameters is underway to arrive at a detailed account of the composition.

DISCUSSION

The lower osmotic pressure in the sap collected from diseased palms as compared to healthy indicates the concentration of the constituents like sugar fractions, organic and aminoacids which contribute to solute concentration. This solute concentration is dependent on xylem pressure potential which showed difference between healthy and diseased (Rajagopal *et al*, unpublished). Similar trend in osmotic concentration was seen in lethal yellowing disease too (Edengreen and Waters, 1982).

The lower content of the major biochemical constituents in the sap from diseased palms might be possibly due to the higher rate of utilisation by MLOs associated with the disease, for their growth and multiplication. This lower solute concentration is reflected in the lower osmolarity of the sap from diseased palms. These observations assume significance in the light of the findings on the culturing of the citrus stubborn organism (*Spiroplasma citri*) which could be achieved by enhancing the osmolarity of the culture media with compounds like sorbitol and other sugars (Saglio *et al.*, 1971).

Mycoplasma like organisms do not possess a cell wall, unlike all other prokaryotes. Mostly, lipids especially glycolipids, phospholipids and lipopolysaccharides and proteins constitute the cell membrane (Razin, 1975; Kahane and Marchesi, 1973; Smith *et al.*, 1976). Since these organisms are phloem limited, the lipid and protein components, of the diseased sap may be utilised for constituting the plasma membrane, thereby resulting in the presence of lower quantities in the sap. In addition, the mycoplasmas and spiroplasmas have an absolute growth requirement of sterols (Stemmer *et al.*, 1982) in forming the lipid bilayer of the cell membrane (Rotham and Lenard, 1977). In the present case, the lower content of the sterols in the sap can be explained on the basis of higher utilisation by the MLOs for their cell membrane growth.

In contrast, arginine levels were high in the sap from diseased palms, as reported earlier (Pillai and Shanta, 1968). Arginine dihydrolase pathway has been proposed as the major energy source for the growth of the organism, in some non-fermentative mycoplasmas (Schmike *et al.*, 1966) which has been confirmed by other workers too (Razin, 1969; Fenske and Kenny, 1976). In this case, the presence, of higher levels of arginine in the sap from diseased palms might provide a constant energy supply in the form of ATP, for the growth of MLOs, by entering the dihydrolase pathway. Further studies to substantiate the hypothesis are in progress.

Thus, the present study reveals an overall picture of the nature and composition of the vascular sap, which with further quantification will help in formulating appropriate media for *in vitro* culturing of MLOs associated with the disease.

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