Plant Physiol & Biochem Vol.18 (1), 21-25, 1991

Identification of Biochemical Constituents in the Phloem Sap from Root (Wilt) Diseased and Apparently Healthy Coconut palms

B. Chempakam*, V. Rajagopal* and A. Gopalam**
Central Plantation Crops Research Institute Regional Station, Kayamkulam-690533, Kerala and **National Academy on Agricultural Research and Management, Hyderabad.

The phloem sap from the inflorescence of root (wilt) diseased and apparently healthy coconut palms (*Cocos nucifera* L.) were analysed for individual amino acids, organic acids and sugars. The concentration of arginine, aspartic acid and tyrosine and of glucose and galactose were higher in the sap from the diseased palms than that from the apparently healthy palms. All the constituents showed only quantitative differences between the palms. The possibility of using this information for the *in vitro* culturing of mycoplasma-like organisms, which are implicated with the disease is discussed.

Key words **: *Cocos nucifera*,** inflorescence, phloem sap, mycoplasma-like organisms, sugars, Organic acids, Arginine,

INTRODUCTION

Root (Wilt) disease (RWD) of coconut palms affects about 32% of the total population of coconut, particularly in the Southern and Central districts of Kerala (1). Recent studies on the etiology of the disease have shown the association of mycoplasma-like organisms (MLOs) with the disease as revealed through the electron microscope (17). These organisms were also detected in the lethal yellowing disease (LY) of coconut in Jamaica (10). MLOs are restricted to the phloem sieve elements of sink areas such as rachilla of unopened inflorescences, developing petioles and apical meristems (17).

Mycoplasmas being phloem restricted, an understanding of the chemical constituents of the phloem sap may aid in formulating suitable synthetic media for the *in vitro* culturing of the organism. The biochemical composition of the phloem sap from LY-affected palms has been reported (18). Our earlier studies on RWD were confined to the method of sap collection and determination of the overall nature of the composition of the sap (3, 13). The present paper aims at identifying the individual constituents of the sap from the inflorescences of diseased palms so that a media akin to the sap could be prepared by the addition of adequate amounts of the nutrients necessary for the growth of the organism.

MATERIALS AND METHODS

Coconut palms (*Cocos nucifera* L. var. West Coast Tall) cultivated in the Institute Farm under a randomised block design with normal cultural and agronomic practices were used for the collection of the inflorescence sap. Using the Disease Index (DI) method of George and Radha (6), seven apparently healthy (DI 0 to 10) and seven diseased palms (DI 11 to 25, early stage of the disease) were selected for the experiments. The nature of the palms were confirmed by diagnostic tests (12).

The sap from the inflorescence of healthy and diseased palms were collected under asceptic conditions as per the method standardised earlier (13). Maximum precautions were taken to collect the sap in the unfermented condition. After conducting the fermentation tests viz. pH, osmotic concentration, sugar contents, besides colour and odour of the sap, the unfermented samples were preserved at 20°C in the deep freeze, while the fermented samples were

*Central Plantation Crops Research Institute, Kasaragod, Kerala. CPCRI. Contribution No. 768

discarded. Only five out of seven palms selected in each category could be taken up for the sap collection. Aliquots of unfermented sap were used for the fractionation and subsequent analysis.

Dowex-50 X 8 (H^+, 200-400 mesh) and Dowex-1 X 8 (Cl^-, 200-400 mesh) supplied by Sigma Chemicals Co., U.S.A. were used for fractionation. Ten ml of the sap was concentrated using soluble PVP and applied to the column. Three fractions viz. acidic, basic and neutral were collected following the method of Canvin and Beevers (2). Each fraction was lyophilized and the residue preserved for further analysis.

The basic fraction which constituted the amino acids were dissolved in 0.2 N HCl and applied to cellulose coated plates. Thin layer chromatography (TLC) was run using the solvent system butanol : acetic acid : water (4:1:1) in the first direction and phenol : water (3:1) in the second direction. After spraying with ninhydrin, the identified spots were compared with those of authentic standards. The intensity of the spots were measured after eluting with 80% ethanol containing copper sulphate at 570 nm using a Beckman Spectrophotometer (8).

The neutral fraction comprising sugars was separated by TLC on silica gel G plates using the solvent butanol : acetic acid : water (4:1:1). Individual components were identified and estimated by phenol-sulphuric acid method (4).

For the identification of organic acids, methyl esters of the acidic fraction was used. The chloroform extracts of the esters (4 µl) were injected into a Gas chromatograph (Model 5730, Hewlett Packard) with a flame ionisation detector at 250°C. The column used was Carbowax-20 programmed from 90°C. Nitrogen served as the carrier gas with a flow rate of 45 ml min^{-1}. Out of the seven peaks, two major peaks were identified by preparative TLC as lactic and citric acids. The concentration of the organic acid was calculated based on the retention time and area under each peak (7).

The experiments on sap collection were carried out during 1983 and 1984. Analysis of the pooled lyophilised samples was undertaken in 1985. GLC analysis for organic acids were conducted at the National Research Centre for Spices, Calicut.

RESULTS AND DISCUSSION

The major amino acids identified in the apparently healthy and diseased sap samples were arginine, aspartic acid, methionine, phenyl alanine and glycine (Figure 1 and Table 1). The concentrations of cystine, leucine, glutamic acid and serine were less in the sap of diseased palms than that from the apparently healthy ones. The contents of aspartic acid and tyrosine were high in the sap from diseased palm, which accounted for more than 50% of the total amino acids present. Even though the individual amino acids showed variations, the total content was less in the diseased sap as reported already (3). Such variations

Table 1. Composition of amino acids, organic acids and sugars identified in the phoem sap from the inflorescences of apparently healthy and root (wilt) diseased coconut palms. Values are expressed as mg. g-1 sap solids.

	Compounds identified	App. healthy	Diseased
A)	Amino acids (average of 5 palms) :		
	Cystine + Cysteine	0.25	0.20
	Arginine	0.19	0.28
	Serine	0.13	0.09
	Glycine	0.11	0.09
	Aspartic acid	0.19	0.25
	Glutamic acid	0.12	0.06
	Leucine	0.14	0.12
	Methionine	0.17	0.13
	Phenylalanine	0.27	0.24
	Tyrosine	0.06	0.23
B)	Organic acids (average of 5 palms) :		
	Oxalic acid	1.54	1.03
	Malic acid	1.29	1.02
	Fumaric + succinic acids	0.41	0.29
	Maleic acid	0.90	0.64
	Malonic acid	75.07	82.05
	Citric acid	11.73	12.93
	Lactic acid	13.67	23.70
C)	Sugar (average of 4 palms) :		
	Sucrose	135.50	128.30
	Glucose	25.50	39.40
	Galactose	18.10	28.90
	Mannose	11.50	9.50
	Lactose	16.30	14.70
	Raffinose	20.40	17.50

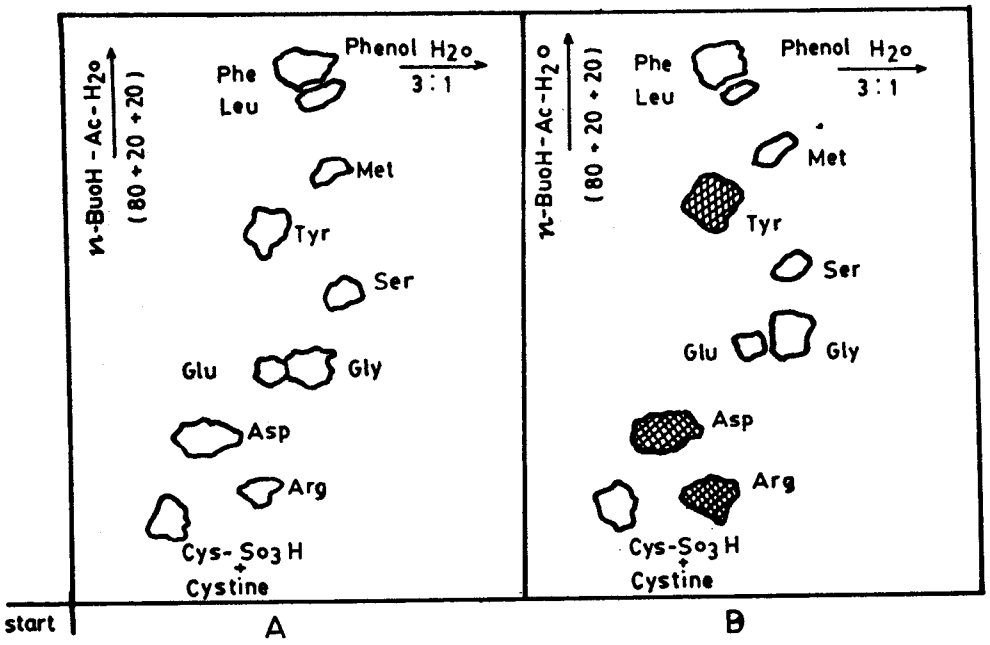

Fig. 1. Two dimensional chromatographic seperation of aminoacids in the basic fraction of phloem sap from coconut inflorescences. A - Apparently Healthy, B - Root (wilt) diseased palms. Visualization by Ninhydrin. Shaded spots indicate higher concentration.

in the contents of amino acids were also reported from the trunk sap of LY-affected coconut palms (18).

Arginine content of the diseased sap was about 70% more than that of the healthy. Earlier observations also showed higher content of arginine in the tender leaf (spindle or cabbage leaf) and also the middle leaf of RWD affected palms (unpublished). Accumulation of amino acids was also seen in the tender leaf of the diseased palms (11). Relatively high arginine content in the diseased tissues might be attributed to its role as an energy source for the growth of MLOs (19), besides its requirements for active transport of amino acids (15). Thus on the basis of arginine levels, the MLOs of RWD appear to be of the arginine utilising strain, though more confirmation is needed.

Besides arginine, aspartic acid and tyrosine were found to be in higher concentration in the sap from the diseased as.compared with that from the healthy palms (Table 1). Lower concentration of glumatic acid, as also reported in other MLO diseases (14) shows that it is continuously channelled for arginine synthesis.

There was no qualitative difference in the sap from apparently healthy and diseased palms in the organic acids, but quantitatively they differed (Fig 2 and Table 1). Seven organic acids were identified of which malonic acid constituted about 75 to 80% of the total content of organic acids. The contents of malonic acid and lactic acid were high in the diseased sap. The low concentration of a majority of the Krebs cycle acids in the sap of diseased palms indicated that the cycle was active in the MLOs observed in the affected palms. High lactate might lead to enhanced synthesis of fatty acid precursors for constituting the protein-lipid bilayer of the mycoplasma membranes.

Among the six sugars identified, sucrose constituted the major component in both the healthy and diseased samples (Fig. 3, Table 1). Qualitatively there was no difference among the palms of the two catego-

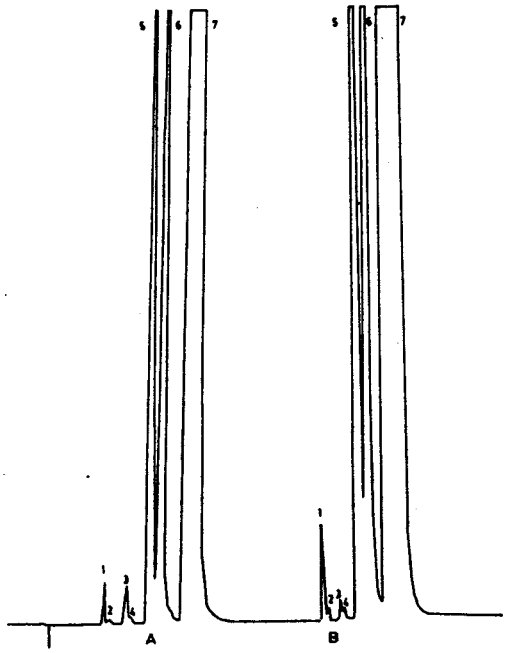

Fig. 2. GLC profile of organic acids in the acid fraction of phloem sap from coconut inflorescences. A - Apparently healthy. B - Root (Wilt) diseased palms. Peak identity : 1. Oxalic acid, 2. Malic acid, 3. Fumaric + Succinic acid, 4. Maleic acid, 5. Lactic acid, 6. Citric acid and 7. Malonic acid. Column and GLC conditions are described under Materials and Methods.

ries. Sap from diseased palms had significantly higher content of glucose and galactose. Stemmer *et al.* (18) found higher sucrose content in the trunk sap of LY-diseased coconut palms. The metabolizable sugars were shown to be incorporated into the glycolipids which constitute the major portion of the cell membrane (9, 16).

The study thus revealed the biochemical composition of the phloem sap which might have favoured the growth of MLOs in the diseased palms. These constituents could be used to formulate the appropriate growth medium for mycoplasmas. The concentration and nature of the various constituents are reflected in the solute concentration, pH and osmolarity of the sap in the diseased palms (3). The present

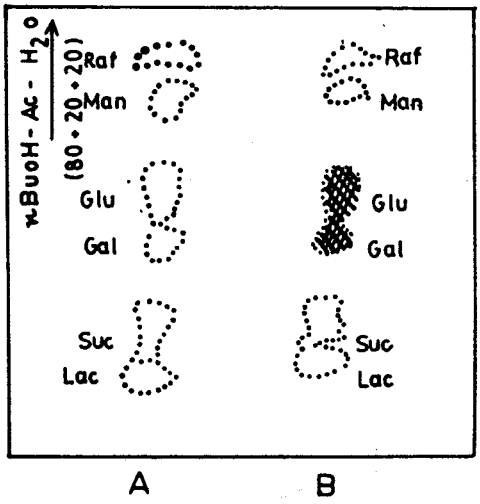

Fig. 3. Thin Layer Chromatographic seperation of sugars in the neutral fraction of phloem sap from coconut inflorescence. A - Apparently healthy and B - Root (wilt) diseased palms. Visualization by Aniline Pthalate reagent. Shaded spots indicate higher concentration.

data highlights the significance of some of the constituents like arginine, lactic acid, glucose and galactose, which along with the steroids reported earlier, might be playing a key role in the proliferation of MLOs in coconut of RWD palms. It may be worthwhile in studies related to culturing of MLOs to incorporate these essential constituents in different proportions, as suggested by Eden-Green and Waters (5) in LY disease of coconut palms.

ACKNOWLEDGEMENTS

We thank Dr. N.P. Jayasankar, for providing the facilities and Dr. J.J. Solomon, the Project Leader on MLO diseases for valuable suggestions.

REFERENCES

1 Anonymous 1986 Tech Bull No 14, CPCRI, Kasaragod, India

2 Canvin OT, Beevers H 1961 Sucrose synthesis from acetate in the germinating castor bean : Kinetics and pathway. J. Biol Chem 236 : 988-995

3 Chempakam B, Rajagopal V 1989 Biochemical composition of vascular sap from the inflorescence of

apparently healthy and root (wilt) diseased coconut palms. J Plantn Crops 16 (Supplement) : 41-46

4 **Dubois M, Gilles K, Hamilton JK, Rebers PA, Smith F** 1951 A colorimetric method for the determination of sugars. Nature **168** : 167-174

5 **Eden-Green SJ, Waters H** 1982. Collection and properties of phloem sap from healthy and lethal yellowing diseased coconut palms in Jamaica. Phytopathology **72** : 667-672

6 **George MV, Radha K** 1973 Computation of disease index of root (wilt) disease of coconut. Indian J Agric Sci **43** : 366-370

7 **Harver WR, Hale RW, Ikeds RM** 1970 Determination of organic acids in plants and food products. Tob. Sci **14** : 141-143

8 **Moore S, Stein WH** 1948 Photometric method for use in chromatography of amino acids. J Biol Chem **176** : 367-388

9 **Plackett P, Shaw SJ** 1967 Glucolipids from Mycoplasma laidlawii and Streptococus M.G. Biochem J **104** : 61c–62c

10 **Plasvic-Benjac B, Hunt P, Maramorosch K** 1972. Mycoplasma like bodies associated with lethal yellowing disease of coconut palms. Phytopathology **62** : 298-299

11 **Pillai NG** 1964 Biochemical investigation in the coconut palm. A preliminary study on the amino acids in the coconut palm tissues. Indian Cocon J **26** : 109-113

12 **Rajagopal V, Sasikala M, Sumathykutty Amma B, Chempakam, B, Rawther TSS** 1988 Early diagnostic tests on the root (wilt) disease of coconut in India. Philipp J Cocon Studies XIII (2) : 31-35

13 **Rajagopal V, Chempakam B, Cecil SR, Kamalakshy Amma PG** 1989 Studies on the phloem sap collection from root (wilt) diseased and apparently healthy coconut palms. Plant Physiol and Biochem **16** (1) : 52-56

14 **Razin S, Gotterfield L, Rottem S** 1968 Amino acid transport in mycoplasma. J. Bacteriology **95** : 1685-1691

15 **Schmike RT, Barlile MF** 1963 Arginine metabolism in Pleuropneumonia like organisms isolated from mammalian cell culture. Journal of Bacteriology **86** : 195-206

16 **Shaw N, Smith PF, Koostra WL** 1968 The lipid composition of Mycoplasma laidlawii Strain B. Biochem J **107** : 329-333

17 **Solomon JJ, Govindankutty MP, Nienhaus F** 1983 Association of mycoplasma like organisms with the coconut root (wilt) disease in India. Z. Pflanzenk **90** : 295-297

18 **Stemmer WPC, Archer DB, Daniels MJ, Davies AMC, Eden-Green SJ** 1982 Effects of lethal yellowing on the composition of phloem sap from coconut palms in Jamaica. Phytopathology **72** : 672-675

19 **Townsend R** 1976 Arginine metabolism by Spiroplasma citri. J General Microbiol **94** : 417-420