

H⁺-ATP_{ASE} - A BIOCHEMICAL MARKER FOR EARLY DETECTION OF ROOT (WILT) DISEASE IN COCONUT PALMS (*COCOS NUCIFERAL*)

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Abstract. - H⁺-ATPase, the key enzyme of secondary transport of ions, cell turgor changes and intracellular pH regulation showed maximum activity in leaf samples of healthy palms, whereas the palms of diseased area expressed a reduction in activity to a minimum level. Activity difference of H⁺-ATPase in healthy and diseased palms was further substantiated by detection of the enzyme by histochemical method. A decrease in activity of H⁺-ATPase was noticed in palms without any visual symptoms of the disease, otherwise called as apparently healthy, from the healthy palms of disease free area. This result indicates that, the palms of diseased area lacking visual symptoms could be identified biochemically by H⁺-ATPase activity. Physiological suppression of H⁺-ATPase in diseased palms was also supported by structural variations of leaf and root tissues observed under SEM.

1. Introduction

Root (wilt) disease of coconut palm has been a puzzling problem of coconut growers of Kerala, since the early phase of this century (Pillai et al 1973). Detection of resistant coconut palms from diseased area and application of specific control measures are two major issues faced by the farmers. In root (wilt) affected areas, the diseased palms show a centrifugal appearance of visual symptoms like ribbing, yellowing and marginal necrosis from the inner whorl of the crown foliage (Radha and Lal 1972). Those palms lacking any visual symptoms unevenly distributed in the diseased pockets and other neighbouring areas, could not be considered either as carrier or resistant of the disease. Efforts for the earlier diagnosis of the disease in such palms has been a subject of investigation for a long time but no conclusive results have been achieved so far (Solomon et al 1983; Sasikala et al 1998). So any attempt that can lead to early detection of the disease will be a substantial contribution with regard to proper planning of control measures for saving the palm. With this end in view, this study was undertaken to quantify the activity of H⁺-ATPase, a key enzyme having a pivotal role in plant metabolism, in leaf and root tissues of coconut palm of diseased and diseased free areas.

2. Materials and Methods Plant Material

Sound healthy coconut palms were identified from disease free areas of Thiruvananthapuram district and

diseased palms from highly infected areas of Alappuzha district, where the disease incidence is 71%. Coconut palms were randomly selected from the diseased and disease free areas. Since the leaves are arranged spirally in the crown, three types of leaves were identified from inner, middle and outer whorls of the palms. For the assay leaflets from terminal, middle and basal regions were collected and pooled. The data was subjected to statistical analysis. The age of the palm, position of the leaf and leaflets were kept constant for all the studies. Fresh roots were collected from all the sides of diseased and healthy palms and pooled for the enzyme assay.

Light and Scanning Electron Microscopy

Detection of H⁺-ATPase in leaf samples was done by lead salt method (Gahan 1984). Root and leaf samples for SEM were prepared following the method of Falk (1980). The coated specimen was observed in SEM (S-2400 Hitachi).

Isolation and Assay of H⁺-ATPase

All steps for the enzyme isolation were carried at 0-4°C (Nagao et al 1987). Leaflets were chopped into fine pieces and ground in extraction buffer containing 0.25 M Sucrose, 25mM Tris-MES and 3 mM EDTA (pH 7.8) using a waring blender. The homogenate was filtered with two layers of cheese cloth. The filtrate was centrifuged and the supernatant was used for the assay.

ATPase activity was measured by determining the inorganic phosphate released from the substrate following the method of Shimazaki and Kondo (1987). The reaction buffer contained 50 mM Tris-MES buffer (pH 6.8), 2 mM ATP, 5mM MgSO₄, 50 mM KCl and 1mM EDTA. Absorbance was measured at 750 nm. In control, the enzyme fraction was added after termination of the reaction.

3. Results and Discussion

Cocos nucifera - West Coast Tall variety was selected for the study and the results presented here are pertaining to this genotype. The age of the palm, position of the leaf and leaflets were kept constant for all the trials.

Detection of H⁺-ATPase in leaf tissues

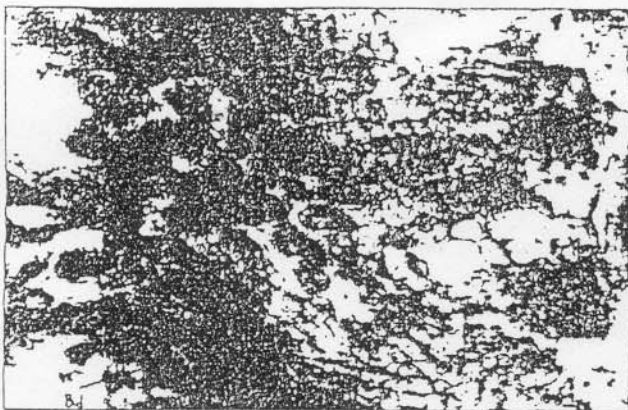


Fig.1. Photomicrograph of leaf cells of healthy palm showing black deposits of lead sulphide which inturn confirms the activity of H⁺-ATPase. Bd - Black deposit

Fig.1. displays the activity of H⁺-ATPase in the leaf tissue of healthy palms. Due to ATPase action on exogenous substrate ATP, inorganic phosphate (iP) are released, that forms calcium phosphate. The calcium is subsequently replaced by lead ions which are then converted into lead sulphide. The black deposits visible in the figure are due to the formation of lead sulphide, which confirms the release of phosphate ions (Fig.1). For control, the leaf was blanched in boiling water bath for 20 min, to inactivate the enzyme. No black deposits formed confirming the release of iP_i due to the action of ATPase in the experimental samples. In the case of leaf sections of diseased palms the intensity of colour was limited which supports the decline of H⁺-ATPase activity (Fig 2) These histochemical observations were further substantiated by quantitative assay.



Fig 2. Leaf cells of diseased palm showing limited black deposits of lead sulphide

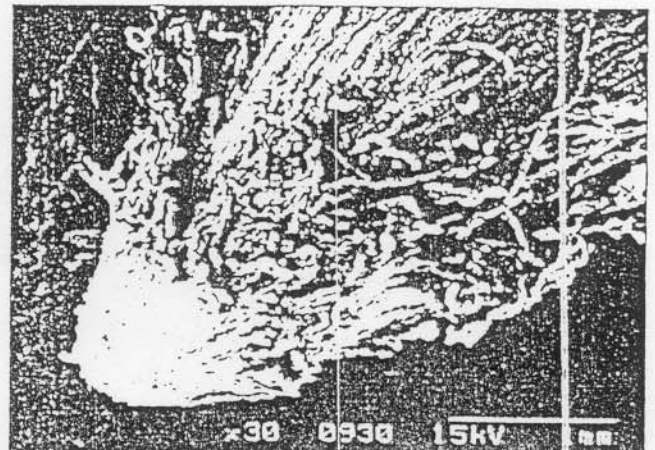


Fig.3 SEM micrograph of root tip of diseased palm - Surface view



Fig.4 SEM micrograph of root tip of diseased palm - Sectional view

Scanning Electrone Microscopy

Significant structural variations indicating the functional abnormality was observed in root and leaf tissues of diseased palms, when compared to healthy ones. In diseased palms, the terminal portion of the root showed a disorganised irregular rough surface with electron dense regions (Fig.3 and 4). The internal cells exhibited a disintegrated pattern without conspicuous cell boundary. Root tissues of healthy palms displayed a normal smooth surface with intact internal cells indicating the absorptive nature (Fig.5 and 6). Fig 7 demonstrates the occurrence of opened stomata in leaf samples of diseased palms collected at night against the closed stomata in healthy leaf. The mechanism of H^+ -ATPase in regulating the swelling and shrinkage of guard cells leading to the opening and closure of stomata are better understood (Keams and Assmann 1993). The unusual observation of opened stomata in leaf samples of diseased palms indicate the functional abnormality of H^+ -ATPase in leaf system. Histopathological studies on roots and stomatal mechanism in leaf samples of diseased palms were studied by Govindankutty and Vellaichamy (1983) and Rajagopal et al (1986) respectively. However SEM observations on disorganised root surface and abnormal stomatal nature in leaf samples of diseased palms have not been reported before.

Assay of H^+ -ATPase

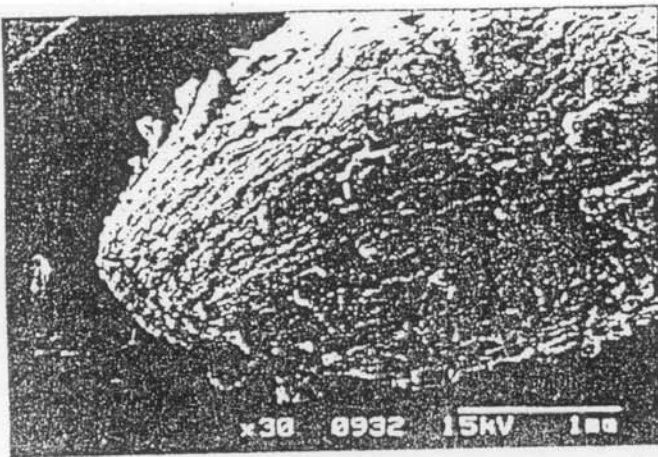


Fig.5. SEM micrograph of root tip of healthy palm - Surface view.



Fig 6. SEM micrograph of root tip of healthy palm - Sectional view

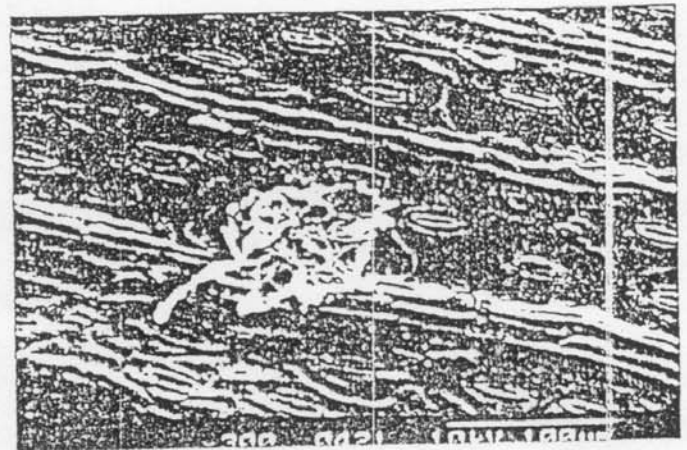


Fig.7. Electron micrograph of diseased leaf collected at night showing opened stomata. Os - Opened stomata.

Table 1 represents the distribution of H^+ -ATPase activity in tender, mature and old leaves collected from inner, middle and outer whorls of healthy and diseased palms. Each value is the mean activity of five determination and is expressed in units/g tissue. In healthy palms, a slow pace of reduction of H^+ -ATPase activity was noticed and this depended on the physiological maturity on the leaf. A

prominent depletion in H^+ -ATPase activity was seen in the leaf samples of diseased palms from that of healthy palms. They showed a sharp decline from tender to mature leaves and slowed down to minimum level in the old leaves (Table 1). Considering the maturity of the leaf, a slow pace reduction of H^+ -ATPase from tender to old leaf in healthy palms shows the physiological activeness of tender leaf. A sound decrease in activity of H^+ -ATPase from tender to peripheral leaf could be possible due to impaired metabolism of diseased palm. But it was found that the tender leaf of both healthy and diseased palms showed high level of H^+ -ATPase activity. Root samples of diseased palm showed a reduction of H^+ -ATPase activity as in leaf samples (Table 2). Waning in activity observed in diseased palms from mature to old leaf can be correlated with visual symptoms of root (wilt) - ribbing, yellowing and marginal necrosis.

Table 1 Distribution of H⁺-ATPase activity (units/g tissue) in three stages of leaf samples - tender, mature, old based on their maturity

Healthy palms			Diseased palms		
Tender	Mature	Old	Tender	Mature	Old
1747	1612	1395	1400	352	52
1890	1725	1245	1326	201	47
2776	2650	2185	1250	273	109
2005	1891	1622	1365	481	120
1581	1318	1029	1032	340	42.5
1579	1211	989	1312	328	81
1603	1348	1216	1280	210	48
1688	1298	1110	1210	320	52
1998	1768	1348	1340	280	56
1687	1269	1188	1386	345	63

Table 2 H⁺-ATPase activity in root samples of healthy and diseased palms

Healthy palms H ⁺ -ATPase Activity (Units/g tissue)	Diseased palms H ⁺ -ATPase Activity (Units/g tissue)
212.8	95.6
190.6	81.6
184.5	62.1
180.3	65.7
124.2	60.6
188.1	71.8
191.5	69.4
185.6	66.1
201.7	63.3
183.3	62.7
256.1	66.4
225.6	56.1
199.6	58.6
212.3	64.5
160.8	66.6

Table 3 H⁺-ATPase activity of leaf samples of healthy, apparently healthy and diseased palms

Healthy Area	Diseased Area	
Healthy palms Total activity	Apparently healthy palms Total activity	Diseased palms Total activity
1584	876	601
1620	764	525
2537	762	544
1839	923	655
1309	686	472
1260	820	574
1389	745	513
1365	765	527
1705	810	559
1381	866	598

H⁺-ATPase activity of leaf samples of coconut palms distributed in diseased and disease free areas are expressed in Table 3. The palms of diseased area are categorised into two. Palms with visual symptoms of disease and without visual symptoms. The later palm look apparently healthy. Activity of each palm is the mean value of tender, mature and old leaf samples. Sound variation in activity of H⁺-ATPase was noticed between palms of diseased and disease free areas. Moreover, healthy and apparently healthy palms expressed prominent difference in H⁺-ATPase activity, which confirms that palms lacking visual symptoms of the disease are susceptible to the disease.

Conclusion

From these observations, it would be possible to consider the activity difference of H⁺-ATPase as a parameter for distinguishing apparently healthy palms and palms having the early phase of the disease from diseased area, where the disease incidence is high. Thereby it will help to select resistant and susceptible palms from the plantation and also promote active control measures at the early phase of disease.

4. References

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IDENTIFICATION OF A NEW CYTOPLASMIC MALE STERILE SOURCE IN RICE AND DEVELOPMENT OF RICE IDEOTYPES

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Abstract : Wild Abortive cytoplasm is the major source of cytotsterility used in hybrid rice. Identification and use of additional source of cytoserility suitable to humid tropics will solve the defects of WA source experienced in tropical situation. Cytoplasm of Vytilla 3, a pokkali rice variety was identified as a new source for cytoplasmic male sterility. Ideotype breeding in rice will offer great opportunities to enhance the yield potential. Among the 22 segregating generations obtained from the crosses of 12 genetically diverse parents, seven lines were high yielding with quality characters preferred by keralites. Two lines (Karthika/Bhadra and PK3355-5-1-4/Kachsiung Sen Yu 338) were identified as having ideotype features proposed by IRRRI.