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SUPPRESSION OF PLASMA MEMBRANE H^+- ATPase IN ROOT (WILT) DISEASED COCONUT PALMS (*COCOS NUCIFERA* L.) IMPAIRS STOMATAL OPENING AND PHLOEM TRANSPORT

Mathews T Thelly, Sharmila L B and Mohankumar C
Plant Biochemistry Unit, Department of Botany,
University College, Thiruvananthapuram

Abstract

Plasma membrane H^+-ATPase, couples ATP hydrolysis to proton transport thereby establishing the driving force for solute transport into and out of plant cells. A sound depletion of H^+-ATPase activity was noticed in diseased palms from that of healthy ones. The suppression of plasma membrane H^+-ATPase in diseased palm was further substantiated by the impaired functioning of stomata and the disintegration of phloem and tracheary elements as observed under Scanning Electron Microscope (SEM). The results indicate the functional imbalance of electro chemical gradient created by H^+-ATPase in the leaf cells. Moreover, the undifferentiated shrunken leaf tissues of diseased palms clearly clarify the loss of turgidity and cell wall plasticity due to suppression of H^+-ATPase.

1. Introduction

The plasma membrane H^+-ATPase otherwise called as P type ATPase plays a significant role in many of the life functions of plant cells. By mediating ATP dependent H^+ extrusion to the cell exterior, plasma membrane H^+-ATPase sets up the driving force for solute transport in terms of an inwardly directed proton electrochemical gradient, at the plasma membrane (Sanders 1989, Briskin and Hanson 1984). Transport of solutes into and out of the cell involves secondary transporters, whose ability to function is directly dependent on the proton - motive force created by the plasma membrane H^+-ATPase. The proton gradient generated by the enzyme is the driving force for active nutrient transport and the pH changes resulting from proton pumping may be involved in growth control (Serrano 1989). At the level of whole plants, the loading of root xylem with inorganic nutrients and loading of leaf phloem with organic nutrients seem to depend on active transport processes driven by H^+-ATPase. In stem and leaves, immunodetection experiments show H^+-ATPase to be chiefly present in guard cells and phloem cells with high concentration in the companion cells of the phloem (Samuels *et al;* 1992; Stenz *et al;* 1993). It has been observed that in root (wilt) diseased coconut palms the activity of P-type ATPase is at a lower level than in healthy palms (Thelly and Mohankumar 2000). In this communication, we made an attempt to correlate the suppression of P-type ATPase with the mechanism of opening and closure of stomata and the phloem transport.

2. Materials and Methods

2.1. *Plant Material*

Sound healthy coconut palms were randomly selected from disease free areas of Thiruvananthapuram district. Diseased palms were identified from infected pockets of Alappuzha district. Leaf samples of tender, middle and peripheral whorls were collected and pooled for the assay and the mean value was taken. H^+-ATPase activity is defined as the liberation of 1 µmol Pi/min.

2.2. *Scanning Electron Microscopy (SEM)*

For the SEM preparation, leaf sample was taken from healthy and diseased palms at fixed time intervals, i.e., 7 a.m., 12.00 noon, 5 p.m and 10 p.m. Specimen was processed following the method of Falk (1980). Dehydrated specimen was coated and observed under SEM (S-2400 Hitachi)

2.3. *Atomic Spectroscopy (AS)*

Fresh leaf samples were dried and powdered for mineral analysis using AS. (Jones *et al;* 1991; Bharghava and Raghupathi 1993).

2.4. *Isolation and Assay of Plasma membrane H⁺-ATPase*

Plasma membrane H^+-ATPase was isolated from fresh leaf samples of tender, middle and peripheral whorls of healthy and diseased palms. All steps for enzyme isolation were carried out at 0-4°C. A known amount of tissue was ground in a chilled mortar and pestle using the extraction buffer with following composition : 0.25 M Sucrose, 3 mM EDTA and 25 mM Tris-HCl, pH 7.8 (Gallagher and Leonard 1982). The homogenate was centrifuged at 1,000 g for 5 min, followed by 30,000 g for 15 min. The supernatant was used as enzyme source.

Assay : Plasma membrane H^+-ATPase assay was done following the procedure of Shimazaki and Kondo (1987). The release of inorganic phosphate was detected colorimetrically at 750 nm.

2.5. *pH Specificity*

pH specificity of H^+-ATPase was determined by changing the pH of the assay buffer from 2-13. The activity of the enzyme at each pH was calculated. A stock solution of 0.5 mM vanadate was used for the inhibition study.

3. Results and Discussion

The impaired stomatal mechanism in leaf samples of root (wilt) affected palms was confirmed by SEM. The leaf samples were collected and fixed from healthy and diseased palms at different time, at 5 hours intervals, i.e., 7 a.m., 12 noon., 5 p.m. and 10 p.m. Healthy and diseased palms were identified from disease free areas of Thiruvananthapuram and Alappuzha districts respectively. It could be noticed that in leaf samples of healthy palms, the stomatal behaviour towards the light and dark periods are in normal condition. At morning 7 a.m., a few stomata are in opened state and some are about to open indicating the active physiology. At noon, the stomata are completely opened, showing the peak pace of photosynthesis, (Fig 1)

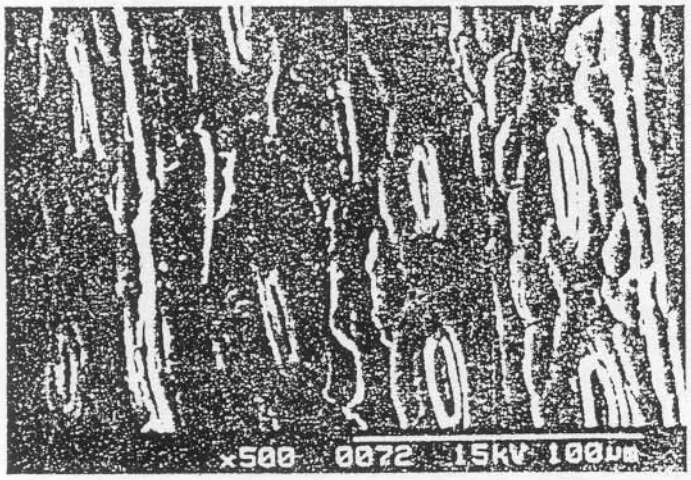

Fig 1. The surface view of leaf tissue of healthy palm collected at noon displaying opened stomata under Scanning Electron Micrograph

and subsequently they get closed at night. These observations regarding the mechanism of regulating the swelling and shrinking of guard cells and leading to the opening and closure of stomata clearly support the active phase of plasma membrane H^+ ATPase in leaf tissues. It is well established that stomatal opening is induced by increased turgor pressure as a result of K^+ and anions influx energized by H^+-ATPase. The guard cells accumulate large quantities of K^+ and synthesize or take up anions. The resulting increase in osmotic pressure draws water in and the cells swell. Because of the physical constraints, swelling of the cells open the stomatal aperture. Stomatal closing is triggered by effectors such as, a decrease in light, or the presence of abscisic acid, which is induced by water stress and requires the efflux of K^+, and anions from the guard cells. Water thus flows out, the cell becomes less turgid and the stomata close (Kearns and Assmann, 1993). In the case of leaf samples of diseased palms collected uniformly by keeping the time intervals as in healthy palms, it could be seen that the stomata are permanently in partially opened condition throughout the time, except at noon (Fig. 2). This observation clearly substantiates the functional imbalance of H^+-ATPase in the leaf samples

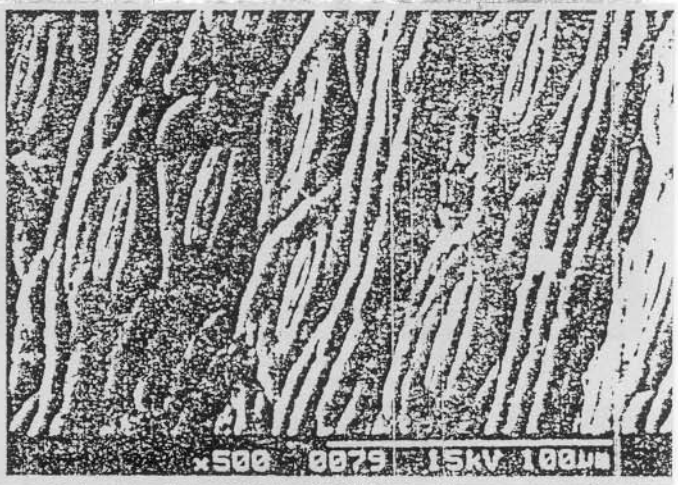

Fig 2 Partially opened stomata on leaf surface of diseased palm collected at night

of diseased palms. It is interesting to note that stomata o leaf samples of diseased palms at noon are closed; which can be interpreted as the influence of light factor in closin the stomata due to the flaccid nature of guard cells b evaporation rather than ATPase interference (Fig. 3). Th amount of K^+ quantified by Atomic Spectroscopy showe a value of 18 mg/g in leaf samples of both healthy an diseased palms. The persistent occurrence of partiall opened stomata in diseased palms, at light and dark period indicates the immobilization of K^+ in the guard cell confirming the depletion of H^+-ATPase activity in lea tissues. Normally opening and closing of stomata in lea tissue can be correlated with influx and efflux of K regulated by H^+-ATPase.

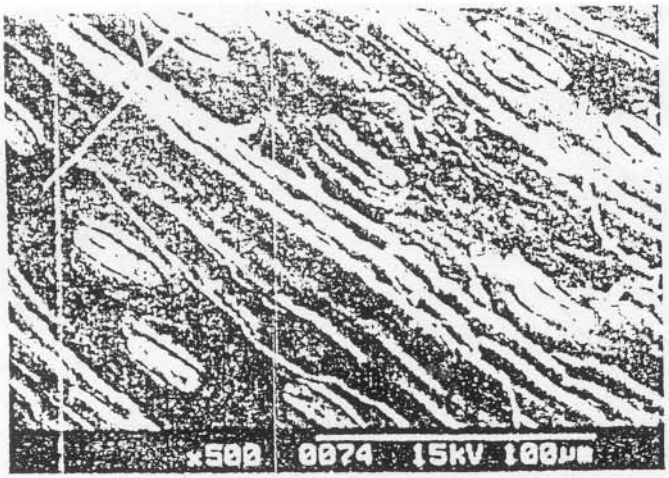

Fig 3 Scanning Electron Micrograph showing completely closed stomata of diseased palm collected at noon.

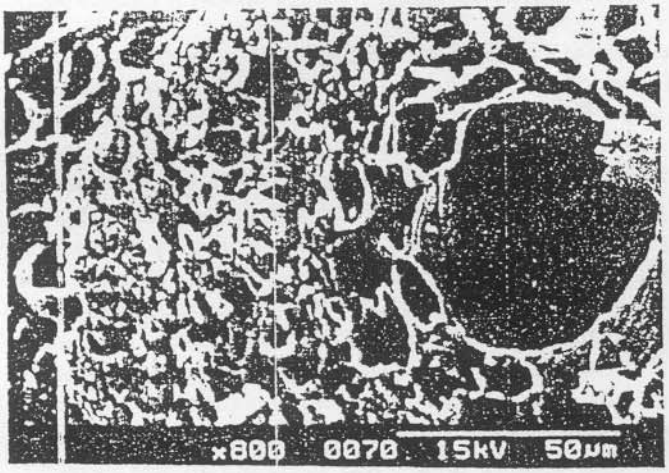

Fig 4. Structure of vascular element of leaf tissue of healthy palm showing normal structure of xylem (x) and phloem (p).

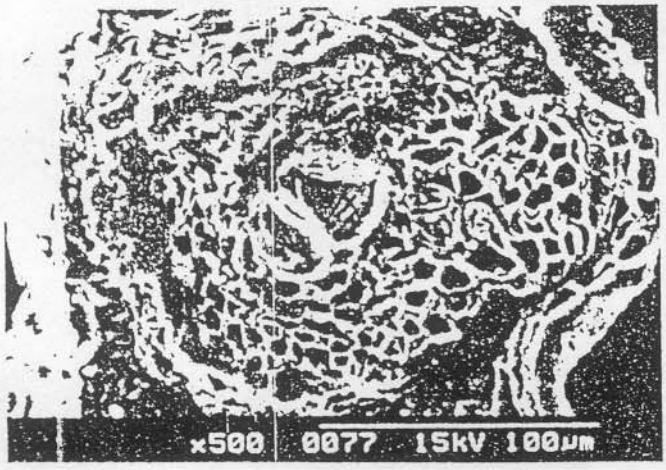

Fig 5. The histological nature of vascular elements of diseased palms showing undifferentiated phloem (p) tissue and deformed xylem (x) cells

The figures 4 and 5 demonstrate the scanned surface view of vascular elements of leaf samples of healthy and diseased palms respectively. It is clear that the leaf tissues of healthy palms, have active xylem vessels with scalariform thickening circumscribed by other tracheary elements(Fig. 4). The phloem cells appeared normal in size and shape. In diseased palms, the leaf samples show inconspicuous structure of vascular elements with deformed vessels and disintegrated phloem and allied cells (Fig. 5). It is already known that the enrichment of ATPase at the phloem of leaf vein, supports the role of the enzyme in phloem loading and transport (Parets-Soler *et al;* 1990). The H^+-ATPase dependent electrochemical gradient also confers the energy necessary for the transport of organic compounds (Morsomme and Boutry, 2000). Transmembrane steps of sucrose movement from the mesophyll cells to phloem and to storage cells energized by H^+-ATPase was confirmed (Lemonie, 2000). The coagulated phloem mass observed in the leaf cells of diseased palms clearly indicates its non functional tendency due to the inactivation of H^+-ATPase (Fig. 5).

The flaccid nature of leaf cells of diseased palm compared to healthy palm are visible in figures 6 and 7. The internal differentiation of tissues in leaf samples of

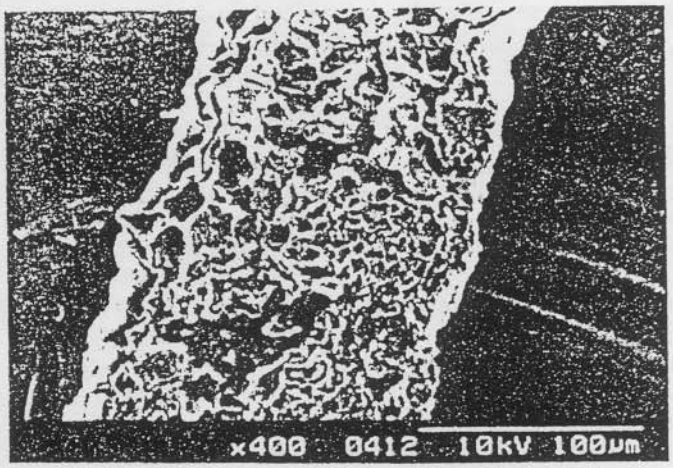

Fig 6. Internal structure of leaf tissue under Scanning Electron Micrograph showing shrinkage of cells.

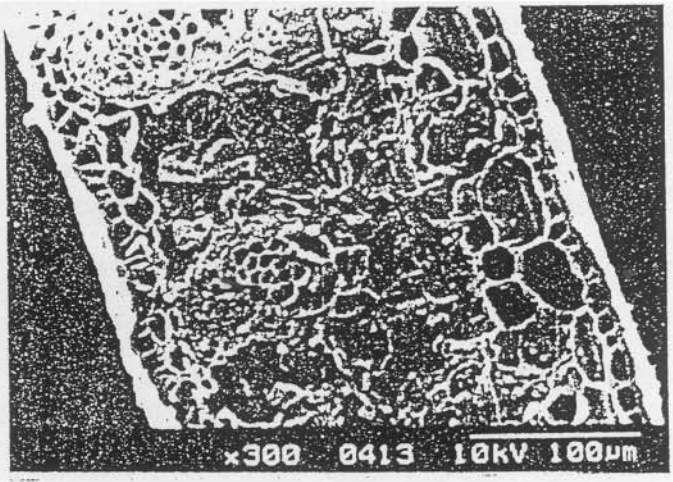

Fig 7 A healthy leaf under Scanning Electron Micrograph showing normal internal structure.

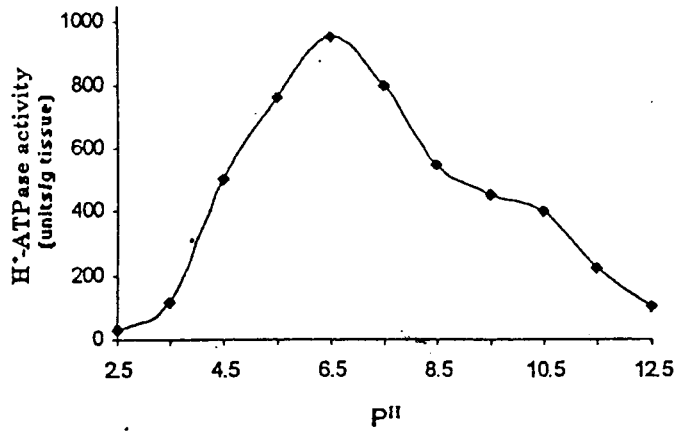

Fig 8 : Activity of Plasma membrane H$^+$-ATPase showing pH specificity

diseased palm were not apparent and showed a thickness of 150μ, whereas in healthy palm the internal differentiation was obvious, with a thickness of 300μ. Under healthy condition, plasma membrane H$^+$-ATPase in plants, pump H$^+$ across the plasma membrane from the cytoplasm to the apoplast. Acidification of the cell wall is thought to induce cell wall plasticity, thus allowing turgor-driven cell wall expansion. Alkalinization of the cytoplasm triggers cell division. In short, H$^+$-ATPase can maintain an electrochemical gradient of H$^+$ that develops across the plasma membrane which will finally create an acidification phase in the cell wall regions and an alkalinization in the cytoplasm. Thus H$^+$-ATPase in oneway preserve the turgidity of cell walls. Flaccid and the

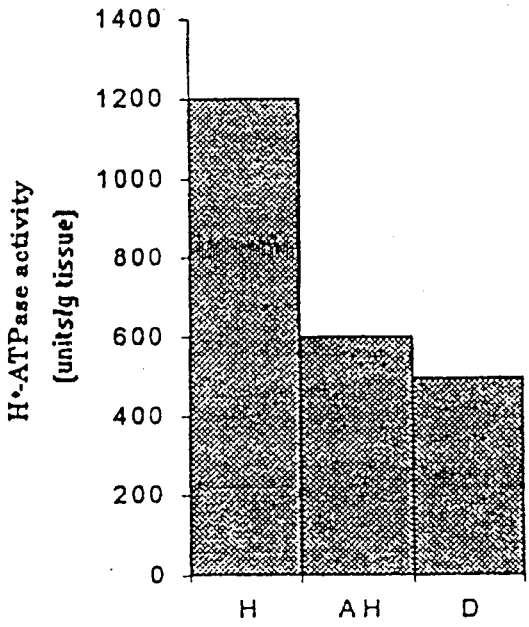

Fig 9 : Functional diversity of Plasma membrane H$^+$-ATPase in healthy (H), apparently healthy (AH) and diseased (D) palms.

shrunken cells of leaf tissues of diseased palm as could be seen in figure 6 can be interpreted in two ways. One is the excess loss of water through the partially opened stomata and the other is the loss of plasticity of cell wall due to the depletion of H$^+$-ATPase activity.

The pH specificity of P-type ATPase compared to mitochondrial and vacuolar ATPase was detected and presented in figure 8. Plasma membrane H$^+$-ATPase showed a high activity at the range of pH 6-7 with a maximum at pH 6.5. At this pH maxima the enzyme was substantialhy inhibited in the presence of sodium vanadate, the specific inhibitor of plasma membrane H$^+$-ATPase. The level of ATPase depletion in early and acute diseased palm of infected areas compared to healthy are demonstrated in figure 9. The above data clearly showed an unambiguous correlation of suppression of plasma membrane H$^+$-ATPase activity in diseased palm with functional abnormalities occurring in stomata and phloem cells. Molecular studies on plasma membrane H+-ATPase gene expression on coconut palms, both healthy and diseased, are warranted to establish the functional imbalance of the enzyme, whether natural or induced.

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