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ANATOMICAL CHANGES IN THE TISSUES OF THE TENDER LEAVES OF COCONUT PALMS AFFECTED BY THE ROOT (WILT) DISEASE

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A sap transmissible virus has recently been isolated from coconut palms affected by the root (wilt) disease (Shanta and Menon, 1960). Preliminary studies on the anatomical changes of the tender leaves of affected palms revealed considerable variations in the different tissues, especially the mechanical and conducting systems, comparable with the external foliar symptoms. A brief account of it is reported hereunder.

Material and Methods

Comparative samples of tender leaves which had not developed chlorophyll, were collected from one healthy and two diseased trees in the advanced stage exhibiting typical symptoms of flaccidity of leaflets. All trees were of 25–30 years in age. The leaf samples were fixed in FAA, dehydrated and cleared by the alcohol-xylol series, embedded in paraffin after suitable infiltration, sectioned at 9 μ thickness and stained with hematoxylin and safranin (Esaw, 1944). 1% alcoholic hematein and the triple stain of hematein (1% aqueous), safranin (1% aqueous) and fast green (1% alcoholic) were also used, especially the latter, for staining longitudinal sections.

For making the observations, 24 sections were selected at random from 6 slides and a length of 3320 μ of an identical portion in each transection was examined. Comparative studies on the area of the vascular bundles, xylem and phloem tissues were done by measuring their maximum length and breadth.

Symptoms of the Disease

The leaves and leaflets of a diseased palm are generally stunted when compared to those of the healthy. The middle leaflets of the particular leaves sampled here were, on an average, 127·8 cm. and 116·0 cm. in length and 5·51 cm. and 4·24 cm. in breadth in the healthy and diseased respectively. The important symptoms generally associated with tender leaves of diseased palms are flaccidity and slight

paling of leaflets, the other symptoms of the disease, *viz.*, chlorosis, wilting and marginal necrosis, appearing later on as the leaves mature.

RESULTS

Since the main differences were observed in the epidermal, mechanical and conducting tissues, brief descriptions of these as they are found in healthy trees are given first.

The normal leaf is protected on both the abaxial and adaxial surfaces by a layer of cuticle, the one on the upper surface being slightly thicker (9 μ) than that on the lower surface (about 6 μ. Pl. I, Fig. 1). There is a thin layer of wax on the outer surface of the cuticle. Below the epidermis, there is a two-layered tissue of hypodermis on the upper surface and a single broken layer of storage tissue on the lower surface. Distributed on the inner side of the epidermis and lying in between the cells of the hypoderm are groups of fibres on both surfaces. These bundles, which give rigidity to the leaves, vary in size and often extend into the mesophyll, those on the upper surface being generally bigger in size. The fibres in each bundle have an average wall thickness of about 6 μ (Pl. I, Fig. 1).

There are about 20–25 vascular bundles running lengthwise on each side of the leaflet, of which five or six are big and the rest narrow. Besides these, there are a few diminutive vascular bundles distributed along the inner margin of the lower hypoderm (Pl. I, Fig. 1). Each normal vascular bundle is surrounded by a highly lignified 2–3 layered bundle sheath (Pl. I, Fig. 1). The structure of the vascular bundle conforms to the usual type, with the phloem lying towards the lower surface and xylem towards the upper. The metaxylem elements occur almost surrounded by the tissues of the phloem (Pl. I Fig. 1).

In the leaves of the diseased trees, considerable changes are noticed in the epidermal, mechanical and conducting tissues. Although little noticeable variation is observed in the thickness of the cuticle on the lower surface, the thickness of that on the upper surface is found to be considerably reduced in the diseased (Pl. I, Fig. 3) when compared to that of healthy (Pl. I, Fig. 2).

No variation is met with in the shape of the epidermal cells of the diseased material. However, considerable changes are observed in the number of these cells per unit area which result in the general stunting of the leaflets of the diseased palms. Detailed quantitative studies indicate that when compared to that of healthy, transverse divisions are accelerated and longitudinal divisions are curtailed in the upper epidermis of leaves of the diseased palm at the particular stage of development under study. A critical study of the development of the epidermis is being made and will be reported in due course.

The stomata, distributed on the lower surface of the leaf, are slightly narrower in the diseased material than in the healthy which correspond to the general stunting of the epidermal cells in this plane

and which results in a parallel increase in the percentage distribution of stomata per unit area in the diseased leaflets (Pl. I, Figs. 4, 5). This is interesting in view of the fact that the rate of trahspiration per unit area in the diseased leaves is about one and a half times higher than that in the healthy (unpublished data).

The rate of deposit of suberin in the hypodermal cells is also found to be considerably affected as seen from the comparatively thinner walls of this layer in the diseased (Pl. I, Figs. 2,3; Pl. II, Figs. 6, 7). When stained with Sudan IV, these cell walls absorbed very little stain in the diseased in contrast to the brightly stained, corresponding tissues of the healthy.

One of the important changes observed in the diseased leaves is in the sclerenchymatous fibres occurring in the hypoderm on both surfaces. While the wall thickness of these fibres in the healthy leaf is about 6 μ (Pl. II, Fig. 8), that of the diseased is only about 3 μ (Pl. II, Fig. 9) which brings about the loss of rigidity in the leaflets of the diseased palm.

A similar change is observed in the sheaths around the vascular bundles also. Although the bundle sheaths in both healthy and diseased are 2–3 layered, the lignification of these cells is considerably affected in the diseased as evidenced by the rate of absorption of safranin by them. In the healthy the sclerenchymatous cells are thick-walled (about 9 μ; Pl. II, Fig. 10) and bright red whereas in the diseased, practically no safranin is absorbed by these, which appear highly thin-walled (Pl. II, Fig. 11).

As described earlier, measurements of xylem and phloem tissues were made by taking their maximum length and breadth. Thus it was found that the average area occupied by xylem in the bigger bundles in the diseased leaf was 130 μ × 110 μ as against an area of 166 μ × 119 μ in a healthy one. The vessels were also found to be slightly smaller in size in the diseased tissues.

Considerable changes are met with in the phloem of the main vascular bundles of the diseased leaves. In an average area of 324 μ × 160 μ occupied by phloem per field of observation, the number of cells were 191 in the healthy leaves. The corresponding number in the diseased one was 230 confined to an area of 300 μ × 160 μ occupied by phloem per field of observation, indicating proliferation of this tissue.

Discussion

The root (wilt) disease of coconut of South India, the aetiology of which is still unknown but which is suspected to be of a virus origin, brings about considerable changes in the epidermal, mechanical and conducting tissues of the tender leaves. The different internal changes are correlated with the various external manifestations of foliar symptoms. Thus the general reduction in size of the epidermal cells accompanied

by the changes in the rate of their multiplication result in the general stunting and downward curling of leaflets of affected trees. Loss of water brought about by increased transpiration and reduced absorption by the damaged root system resulting in general wilting is another important outcome of the disease. Increased transpiration seems to be facilitated by the thinness of the cuticle, increased percentage distribution of stomata per unit area and by the low amount of suberin deposited on the walls of the hypodermal cells. The thin cuticle of the diseased leaves might also render leaf surface less resistant to the mechanical penetration of secondary parasites.

Flaccidity of leaves of affected palms is one of the primary and important symptoms of the disease. The reduced wall thickness of all types of sclerenchymatous tissue, which normally helps to maintain the rigidity of leaves, seems to be one of the factors contributing to this flaccidity.

Changes in the conducting tissues are suggestive of the impaired translocation brought about by the disease. The proliferation of phloem, evident in the diseased palms, is comparable to the phloem proliferation associated with many plant virus diseases.

Further critical studies on the conducting tissues are in progress and will be reported in due course.

Summary

The external symptoms in tender leaves of coconut palms affected by the root (wilt) disease are correlated with various anatomical changes in the epidermal, mechanical and conducting tissues. In spite of the apparent stunting of leaves, an evident change in the rate of division of the upper epidermal cells in the longitudinal and transverse planes takes place resulting in slight downward curling of these. The comparative thinness of the cuticle on the upper epidermal surface and the increased percentage distribution of stomata per unit area contribute towards increased water loss. Failure of development of all types of sclerenchymatous tissues and phloem proliferation are the other important changes that occur in leaves of affected palms.

Acknowledgements

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EXPLANATION OF PLATES I & II

PLATE I

FIGS. 1-5

FIG. 1. Transection of a healthy coconut palm showing the general structure of leaf tissues. *B*, Bundle of fibres. *BS*, Bundle sheath. *C*, Cuticle, *D*, Diminutive vascular bundle. *P*, Phloem, *X*, Xylem (× 130).

FIGS. 2 and 3. Transections of the leaf of healthy (2) and diseased (3) coconut palms, showing the normal thick layer of cuticle and the suberized walls of the hypodermal cells in the healthy; and the thin layer of cuticle in the diseased. (Note the invisibility of the nonstained hypodermal cell walls in the diseased.)
C, Cuticle, *SW*, Suberized cell walls of the hypodermal cells, (× 720).

FIGS. 4 and 5. Peelings of the lower epidermis of the leaf of healthy (4) and diseased (5) coconut palms. *S*, Stomata, (× 130).

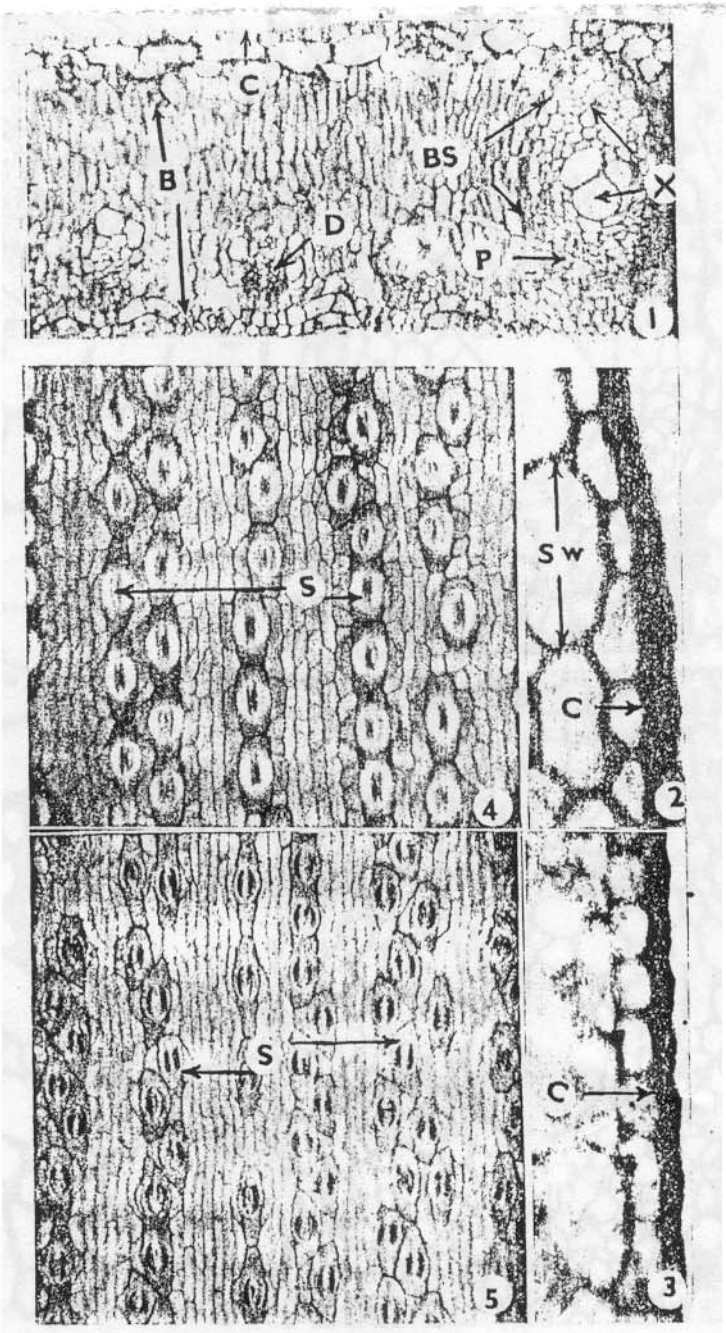
PLATE II

FIGS. 6–11

FIGS. 6 and 7. Peelings of the uper epidermis of the leaf of healthy (6) and diseased (7) coconut palms, showing the difference in staining of the hypodermal cell walls. The stained walls of the hypodermal cells are visible underneath the epidermal layer in the healthy whereas they are invisible due to non-staining in the diseased. *E*, Epidermal cell wall. *H*, Hypodermal Cell wall, (× 130).

FIGS. 8 and 9. Transections showing the difference in wall thickness of the fibres in the bundle of fibres in the leaf of healthy (8) and diseased (9) coconut palms. *W*, Wall of the fibre,(× 600).

FIGS. 10 and 11. Transections of the leaf of healthy (10) and diseased (11) coconut palms showing the vascular bundles with varying features. *B'S*, Bundle sheath—two to three-layered, highly thick-walled in healthy and thin-walled in diseased. *P*, Phloem, *X*, Xylem, (× 450).



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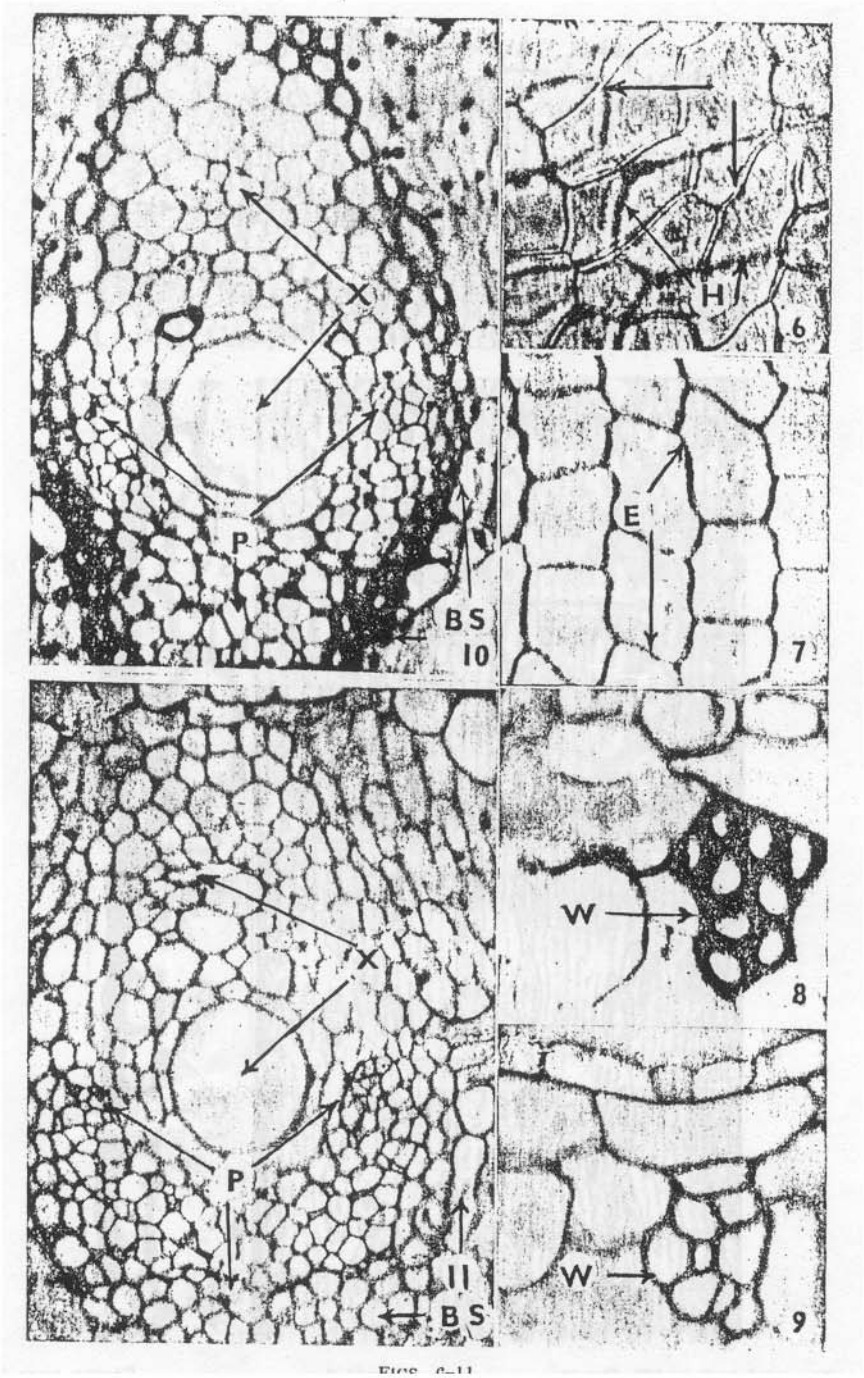

Figs. 6–11