A Serological Test for the Detection of the Root (Wilt) Disease of Coconut

J. J. SOLOMON, M. SASIKALA and P. SHANTA

Central Plantation Crops Research Institute, Regional Station, Kayamkulam, Krishnapuram 690 533, Kerala, India

Abstract

Samples drawn from healthy and diseased palms of varying intensities and growing in different soil types were tested with root (wilt) antiserum by the cross absorption technique. All the 108 healthy samples from various parts of India gave negative reaction, while 157 out of 161 diseased samples gave positive reaction. The 44 of the 150 apparently healthy samples (with no visual symptoms) tested, which gave positive reaction, will be observed for the development of symptoms to assess the sensitivity and diagnostic value of this test.

Introduction

The root (wilt) disease, whose causative agent is yet to be determined, is diagnosed only on the basis of visual symptoms. Radha and Lal (1972) concluded that flaccidity or ribbing of leaves is the best diagnostic visual foliar symptom of the root (wilt) disease. Visual symptoms, be it due to pathogen or nutritional deficiency/imbalances or any other factor, are the culmination of derangement in the host metabolism reflected after the actual disorder has set in. Hence, for evolving a suitable diagnostic test, the changes in the host metabolism consequent to the disease were studied and a colour test was developed (Joseph and Shanta, 1963). However, this colour test, based on the differential dehydrogenase activity of leaf tissue developed after testing a considerable number of palms of all age groups, failed to yield consistent and conclusive results when applied to healthy samples from disease free area. The scope of a sero-diagnostic test was also reduced with the healthy and diseased grindates reacting against normal and root (wilt) antiserum (Shanta *et al.*, 1975).

The present paper deals with the development of a serological test for the early detection of the disease before the visual expression of the symptoms.

Materials and Methods

Purification

Fifty gram tender leaves from unopened spindles (spear) of root (wilt) disease affected coconut palms belonging to the West Coast Tall cultivar was homogenized in 3 volumes of *M*/15 Sorensen's phosphate buffer containing 0.005 *M* EDTA and 1% mercaptoethanol. It was filtered through muslin cloth and the filtrate clarified by low speed centrifugation at 5000g for 10 min. To the supernatant, polyethylene glycol 6000 and sodium chloride were added to give 4% and 0.1 *M* final concentrations, respectively. The sap was left in the refrigerator overnight allowing a precipitate to be formed. The precipitate was collected by centrifuging at 10,000g for 15 min and the pellet dissolved in *M*/15 phosphate buffer containing 1% Triton X-100. The undissolved particles were removed by centrifugation at 5,000g for 10 min and the supernatant was centrifuged at 1,05,000g for 60 min. The pellet dissolved in *M*/15 phosphate buffer was cleared of larger aggregates by low speed centrifugation at 5000 g for 10 min. The resulting suspension was subjected to two cycles of differential centrifugation and the final glossy pellet was dissolved in distilled water. The concentration and purity of the suspension was determined spectrophotometrically before using it as an antigen.

Preparation of Antisera

White female rabbits previously bled for normal serum were injected intramuscularly at weekly intervals with partially purified preparation emulsified with Freund's incomplete adjuvant. Four weekly intramuscular injections were followed by a booster dose with double the concentration of the emulsion given on 50th day from the start of immunization. The bleeding and collection of antisera were started a week after the 4th injection and they were continued at weekly intervals for eight succeeding weeks.

Serological Tests

Double diffusion tests were carried out with microslides (7.5 × 5.0 cm) precoated with 0.5% formvar. They were layered with 2.5 ml of melted oxoid Ionagar No. 2 in phosphate buffered saline (pH 7.4) containing 0.22% sodium azide as preservative. Blocks of agar were removed with a pressure pipette to give a four member well pattern 4 mm in diameter and spaced at 6 mm from the centre well. Extracts of tender leaves from unopened spindle (spear) ground in 0.005 *M* phosphate buffered saline (pH 7.4) (1:1 w/v) were used as antigens in the tests unless otherwise specified.

Results

The partially purified preparation used as antigen for immunization had maximum absorption at 260 nm and minimum at 280 nm. The

260-280 ratio was 1.3. Work on further fractionation and characterization of the above fraction is in progress. The antiserum prepared against this partially purified fraction produced on testing two precipitin lines against diseased and a single precipitin line against healthy extracts indicating the presence of host specific antibodies To have a disease specified antiserum, free from host antibodies, cross absorption was done by direct mixing of healthy sap with the antiserum (cf. Noordam, 1973). Cross absorption was incomplete when healthy sap was mixed in quantities upto four volumes (Table 1). This was evident from a weak non-specific

TABLE 1. Reaction of antiserum at various degrees of cross absorption

Volume of healthy sap added (in ml)	Reaction against		Titer of antiserum
	Healthy palms	Diseased palms	
0	++++	++++	1/1024
3	++	+++	1/128
4	+	++	1/32
5	–	–	1/8

reaction of the cross-absorbed antiserum against healthy samples. A decrease in the titer of the antiserum at various volumes of added healthy sap was also noticed leading to the total absence of reaction against both healthy and diseased at the antigen excess level. Obviously, the disease specific antibodies were lost along with that of host antibodies at this level. This was overcome by adopting the technique of van Regenmortel (1976), by which the centre antiserum well was first charged and equilibrated with healthy sap. The antigen was allowed to diffuse and from a concentration gradient in the gel. The excess undiffused antigen was removed, and the well was recharged with antiserum and the peripheral wells with the antigens to be tested. In the test, the root (wilt) antiserum formed a single precipitin line against the diseased samples midway between the antigen and antiserum reservoir. No precipitin reaction was evident against any of the healthy samples tested from different parts of India. Of the 105 apparently healthy looking palms tested from the diseased tract, 44 reacted to the antiserum. A gradation as to the intensity of reaction with the severity of the disease was also evident, the intensity being highly pronounced in the early stages of the disease followed by a drop with the advancement of the disease. Healthy and diseased palms growing in different soil types—laterite, alluvial, clay, and sandy loam—were also tested against the antiserum. In all the tests, irrespective of the soil type involved, the antiserum reacted only against diseased samples and not against any of the healthy samples (Table 2). Palms of different age groups also could be diagnosed as to their health conditions by this test,

At the same time, none of the 108 healthy palms and as many as 158 samples out of 161 diseased palms sampled reacted to the antiserum.

TABLE 2. Relative precipitin reaction of root (wilt) antiserum against healthy and diseased palms from different soil types

Condition of palm and soil type	No. of samples tested	No. of samples reacted
Healthy – Laterite	40	nil
,, – Alluvial	30	nil
,, – Sandy loam	8	nil
,, – Clay	20	nil
Diseased – Laterite	32	31
,, – Sandy loam	121	118
Diseased – Reclaimed sandy loam	8	8

Discussion

The coconut sap, is an unsuitable medium for tests which involve direct mixing of the reactants because of its high tannin content. Direct addition of sap to antiserum before the removal of host antibodies resulted in non-specific reaction against healthy palms at lower levels of sap added and total absence of reaction against both healthy and diseased at the antigen excess level. This could be due to the non-specific binding and precipitation brought about by the tannins in the clarified sap resulting in a low titered antiserum (Table 1). Such non-specific reactions between serum proteins and leaf and root coconut tissue extracts have been reported earlier also (Shanta *et al.*, 1975). The intra-gel cross absorption plate method of van Regenmortel (1967) was found more suitable, as it was capable of completely cross-absorbing the host antibodies in the gel, permitting only the specific antibodies to diffuse through the barrier and react with disease-specific antigens. The antiserum, being specific in its reaction, react only with the diseased samples irrespective of the age of the tested palm. The versatility of this test was further evident from the fact that diseased and healthy palms growing in different soil types also could be easily identified with certainty. The reaction of some of the apparently healthy palms (in the diseased tract) with the antiserum could be due to the presence of the aetiological agent at a sub-clinical level. However, this can be confirmed only if these palms are observed after a period of incubation.

Acknowledgements

We thank Dr. K.V. Ahmed Bavappa, Director, for his interest and encouragement.

References

Joseph, T. and Shanta, P. (1963). Studies on a colour test for the root (wilt) disease of coconut. *Proc. 50th Session Indian Sci. Congress.*

Noordam, D. (1973). Identification of Plant Viruses : Methods and Experiments (ed. D. Noordam). pp. 117. Centre for Agricultural Publishing and Documentation, Wageningen, The Netherlands.

Radha, K. and Lal, S.B. (1972). Diagnostic symptoms of root (wilt) disease of coconut. *Indian J. Agric. Sci.* 42 : 410-413.

Regenmortel, van, M.H.V. (1967). Serological studies on naturally occurring strains and chemically induced mutants of tobacco mosaic virus. *Virology.* 31 : 467-480.

Shanta, P., Hariharasubramanian, V., and Gopinathan Pillai, N. (1975). Possible association of tobacco mosaic virus with the root (wilt) disease of coconut. *J. Plantation Crops* 3 : 77-80.

Discussions

Ninan : I would like to know if you have extended these studies to the hybrids and Dwarfs ? It would be interesting to study the varietal differences, if any, specially since the Dwarfs in general, and the Green Dwarf in particular, are reported to be resistant to wilt ?

Solomon : The serological studies are mainly done with WCT samples. Of course, we have tested the varieties also, but we do not have enough data to draw conclusions on this aspect at present.

Sane : Your results suggest that in the diseased palms, the intensity of a certain proteins reacting with your antisera is higher. Would it not be possible to examine protein patterns on the gels (gel electrophoresis) and identify the protein associated with the diseased palm ? Knowing the protein, it should be possible to identify the organism responsible for the production of this protein. Gel electrophoresis is a much simpler and quicker procedure.

Solomon : Yes. The protein pattern in healthy and diseased palms could be studied by polyacrylamide gel electrophoresis, but I wonder how far this would help in identifying the causal organism responsible for the disease, as the technique will indicate only the presence or absence of a particular protein.

Chenulu : Have you made any serological tests other than gel diffusion test ? It so, what was the result ?

Solomon : Yes. I have done slide agglutination test, tube precipitin, immun-electrophoresis, and immuno-osmophoresis tests. The first two tests, though simple, gave highly nonspecific reaction, especially in coconuts, as the latter contains large amounts of tannins. The other two tests gave results identical to what we have been getting in double diffusion plate method.