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Central Plantation Crops Research Institute,
Regional Station, Kayangulam, Krishnapuram, India

A Serological Appraisal of the Connection of the Tobacco Mosaic Virus Coconut Isolate with the Root (Wilt) Disease of Coconut

By

J. J. SOLOMON and M. SASIKALA

With one figure

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The probability of a sap transmissible virus as the aetiological agent of the root (wilt) disease of coconut was suggested based on transmission studies (NAGARAJ and MENON 1956). SUMMANWAR *et al.* 1969 and SUMMANWAR, RAYCHAUDHURI and JAGDISHCHANDRA (1971) reported the isolation of a rod shaped virus capable of infecting *Chenopodium amaranticolor* and *Nicotiana tabacum* cv Xanthi from root (wilt) diseased coconut tissues. The rod shaped virus measuring 320 nm length and 24—35 nm width was identified as a strain of TMV (SUMMANWAR *et al.* 1971). The virus was reported to be infectious only in purified preparations and the non-infectious nature of the crude sap was attributed to the presence of viral inhibitors, notably tannins in the coconut tissue. GUPTA *et al.* (1975) surmised that the inhibitory effect of tannins could be reversed and infectivity restored by supplementing known tannin inhibitors to the crude sap. SHANTA *et al.* (1975) verified the inhibitory role of coconut sap and concluded that if TMV is present even in low amounts it could be detected by infectivity tests and the particles identified in negatively stained preparations.

This paper is to further critically evaluate the association of TMV-Coconut isolate with the root (wilt) disease by Serology.

Materials and Methods

TMV-coconut isolate (hereafter referred to as TMV) kindly supplied by Dr. A. S. SUMMANWAR, Virus Pathologist, Indian Agricultural Research Institute, New Delhi, was propagated on *Nicotiana tabacum* cv. xanthi. The virus was purified from tobacco leaves showing severe mosaic symptoms by the polyethylene glycol-differential centrifugation method with the non-ionic detergent Triton-x-100 (GOODING and HEBERT 1967, HARIHARA-SUBRAMANIAN, ZAITLIN and SIEGEL 1970). The final pellet was suspended in double distilled water and the concentration and purity of the suspension determined spectrophotometrically before being used as antigen. Tender leaves from unopened spindles of root (wilt) diseased WCT coconut palms similarly processed were also used as antigen for immunisation.

Preparation of antisera

White rabbits previously bled for normal sera were injected intramuscularly at weekly intervals with purified preparations emulsified with Freund's incomplete adjuvant. The animal was bled at weekly intervals starting a week after the fourth injection. A booster dose with double the concentration of antigen was administered intramuscularly on the 50th day.

Serological test

For double diffusion tests microslides (3″x1″) precoated with 0.5 % formvar were layered with 2.5 ml of melted 0.85 % Oxoid Ionagar No. 2 in 0.005 M phosphate buffered saline pH 7.2 containing 0.22 % sodium azide. Chunks of agar were removed with a pressure pipette to give a four member well pattern surrounding a center antiserum depot. The wells with a diameter of 6 mm were spaced 4 mm from the central well. After the addition of the reactants the slides were incubated in moist Petri plates at 25 °C for 2 days. Tissue extracts prepared in 1 : 1 vol. of 0.005 M phosphate buffered saline pH 7.2 were used as crude antigen. In all the serological tests both purified preparations and crude tissue grindates were tested unless otherwise stated. Immunoelectrophoresis were run for 30 min at 220 V in 1 % Oxoid Ionagar No. 2 gel prepared in phosphate buffer pH 7.4 as described by MATHEWS (1967).

Immuno-osmophoresis were run for 10 min at 220 V in 1 % Oxoid Ionagar No. 2 gel prepared in 0.01 M Tris-Succinate buffer pH 6.75 (JOHN 1965).

Results

Double diffusion test

TMV antiserum produced three distinct precipitin lines — one thick line formed by the merger of two lines and a sharp thin precipitin line close to the thicker one, against the crude and purified TMV-both antigens degraded with sodium dodecyl sulphate (SDS). Degradation was done by the addition of SDS to the antigens to give a final concentration of 0.1% and incubating at 70 °C for 15 min. The intact preparations of the same antigens when tested formed a single precipitin arc close to or adjoining the antigen depot. No precipitin lines were evident against crude and partially purified root (wilt) antigens (Fig. 1 A). Since the root (wilt) antiserum was prepared against a partially purified preparation, to eliminate the anticipated reaction against the normal host components especially the fraction I proteins, it was tested by the intragel cross absorption plate method (SOLOMON *et al.* 1976). The center well (antiserum well) was repeatedly charged with healthy sap, allowing it to dif-

Table 1
Relative precipitin reaction of TMV and root (wilt) antisera against TMV and coconut antigens in double diffusion tests

Source and nature of sample		No. of samples	Number of samples reacting against	
			TMV antiserum (percentage)	root (wilt) antiserum (percentage)
Kasaragod	healthy	25	— (0)	— (0)
Mohitnagar	healthy	20	— (0)	— (0)
Hirehalli	healthy	20	— (0)	— (0)
Kayangulam	diseased (crude)	75	— (0)	75 (100)
Kayangulam	diseased (purified)	28	— (0)	28 (100)
	TMV — crude	20	20 (100)	— (0)
	TMV — purified	12	12 (100)	— (0)

Figures in parenthesis indicate percentage of reaction.

fuse and form a concentration gradient of the antigen in the agar gel. The excess antigen was removed after 48 h of diffusion and the well charged with the root (wilt) antiserum and the peripheral wells with the test antigens. The root (wilt) antigens formed a single precipitin line midway between the antigen and antiserum reservoirs against its homologous antiserum. No precipitin line was formed against the TMV antigens and healthy coconut sap (Fig. 1 B). Table 1 presents the results of this test with the two antisera against healthy coconut grindates, root (wilt) preparations and TMV antigens.

Immunoelectrophoresis

TMV antigens, both crude and purified separated by differential electrophoretic mobility formed four arcs against the TMV antiserum. No reaction

Table 2
Reaction of TMV and root (wilt) antisera against TMV and coconut antigens in immuno-electrophoresis

Source and nature of sample		Precipitin reaction against	
		TMV antiserum	root (wilt) antiserum
Kasaragod	healthy	—	—
Mohitnagar	healthy	—	—
Hirehalli	healthy	—	—
Kayangulam	diseased (crude)	—	+
Kayangulam	diseased (purified)	—	+
	TMV (crude)	+	—
	TMV (purified)	+	—

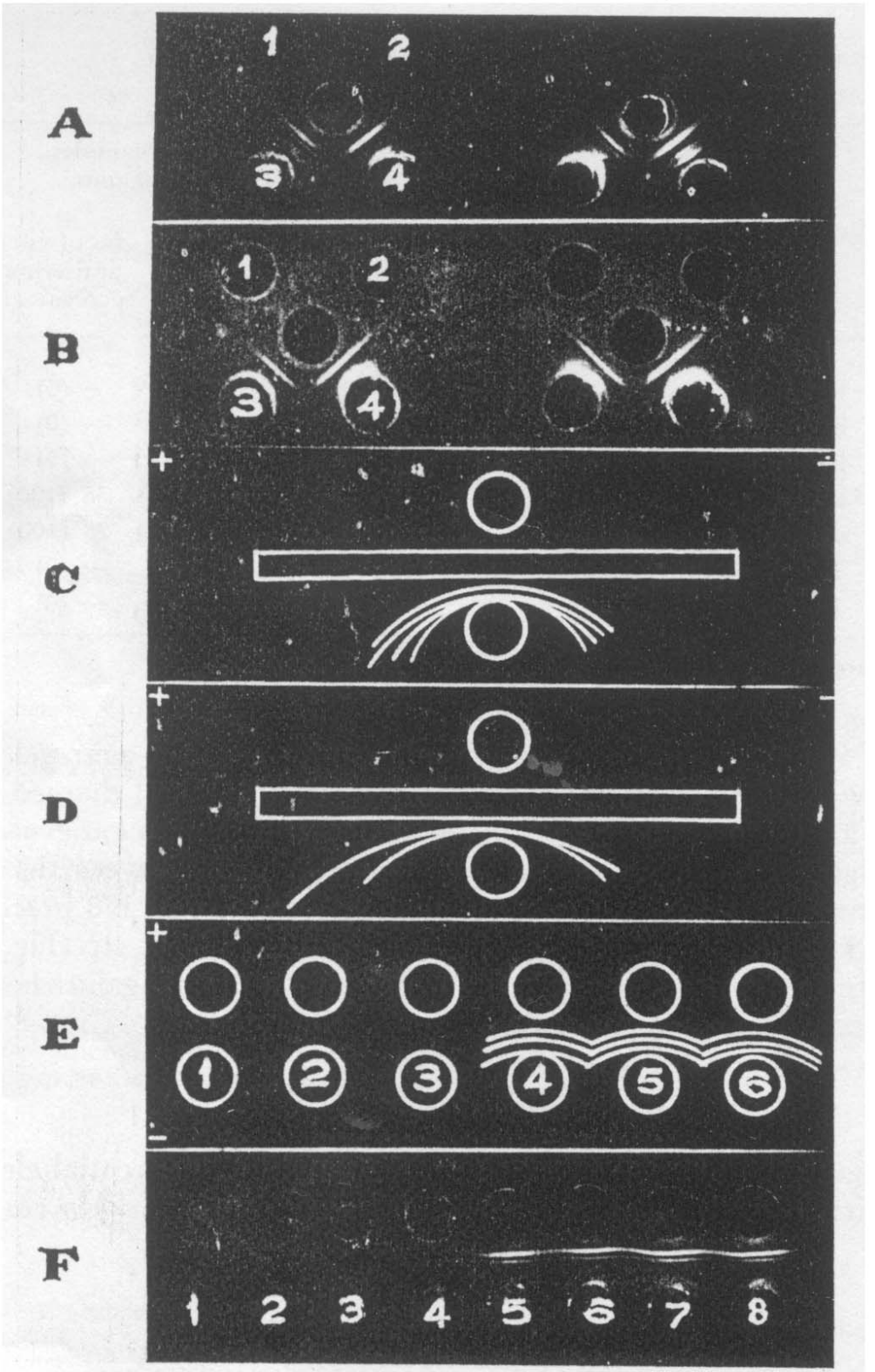

Fig. 1. A—F. Evaluation of TMV and root (wilt) antisera against homologous and heterologous antigens by Double Diffusion (DD), Immuno-electrophoresis (IE), and Immuno, osmophoresis (IO) methods. A. (DD) Centre well — TMV antiserum. 1. crude sap; 2. partially purified — root (wilt) antigen; 3. TMV — crude sap; 4. TMV — purified. B. (DD) Centre well — root (wilt) antiserum. 1. TMV — crude; 2. TMV — purified; 3. root (wilt) crude; 4. partially purified. C. (IE) Trough: TMV antiserum. Upper well: root (wilt)antigen; lower well: TMV antigen. D. (IE) Trough: root (wilt) antiserum. Upper well: TMV antigen; lower well: root (wilt) antigen. E. (IO) Upper wells: TMV antiserum; lower wells: 1—3 root (wilt) antigen, 4—6 TMV antigens. F. (IO) Upper wells: root (wilt) antiserum; lower wells: 1—4 TMV antigens, 5—8 root (wilt) antigens

was evident against the root (wilt) antigens (Fig. 1 C). The root (wilt) antiserum formed two lines one overlapping the other and extending further towards anode against its homologous antigens. No precipitin reaction was formed against the TMV antigens (Fig. 1 D). It is evident from Table 2 that both TMV and root (wilt) antigens reacted only with their homologous antiserum and not against the heterologous antiserum.

Immunoosmophoresis

Purified and crude TMV when used as intact forms produced a single precipitin arc adjoining the perimeter of the antigen reservoir against the TMV antiserum indicating, very little migration of the antigen. Degraded TMV formed three lines midway between the two reservoirs. No reaction was evident against healthy, diseased grindate and partially purified root (wilt) disease antigens (Fig. 1 E). Root (wilt) antiserum formed two precipitin lines one midway between the two reservoirs and another close to the antiserum reservoir against the root (wilt) antigens. No precipitin lines were formed against healthy coconut sap and the TMV antigens (Fig. 1 F).

Discussion

Coconut sap by virtue of its high tannin content is an unsuitable medium for slide agglutination, tube precipitin and ring interphase tests producing non-specific reactions against the normal serum (SHANTA *et al.* 1975). It is evident from the present study that systems which involve diffusion in a gel selectively excludes and compartmantalize the components which otherwise give non-specific reactions when the reactants are directly mixed with each other. In the double diffusion test the TMV antiserum reacted only with the purified and crude extract of TMV. No reaction was formed against the partially purified root (wilt) disease preparations as well as tissue extracts of diseased samples and healthy samples from Kasaragod, a known healthy area in the west coast, Mohitnagar and Hirehalli both healthy areas from North-Eastern India and Karnataka State respectively. On the contrary, the root (wilt) antiserum reacted against the partially purified and crude extracts of root (wilt) disease preparations and not against the TMV-antigens. It is evident from Table 1 that the root (wilt) antiserum also did not exhibit any reaction against healthy samples from Mohitnagar, Hirehalli and Kasaragod. The data obtained from the double diffusion plate method were further corroborated with the results of immuno-electrophoresis and immuno-osmophoresis where the antigens are further separated and sieved off in the gel by their differential electrophoretic mobility and rules out any substances interfering with the diffusion and precipitin reaction. In both the tests the TMV antiserum reacted only with the TMV antigens and not against root (wilt) and healthy coconut antigens. Similarly the root (wilt) antiserum reacted only against the root (wilt) antigens and not against the TMV antigens. The present

investigation provides ample serological evidences to show that TMV-Coconut isolate is not associated with the root (wilt) disease of coconut.

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Summary

Highly sensitive and antiserum specific serological tests like double-diffusion plate method, immuno-electrophoresis and immuno-osmophoresis were used to determine the association of TMV-Coconut isolate with the root (wilt) disease of coconut. In all the three tests TMV antiserum reacted only against the TMV antigens. No reaction was evident against healthy coconut samples and root (wilt) disease antigens. Similarly the root (wilt) antiserum reacted only against the root (wilt) diseased samples and not against the TMV-antigens and healthy coconut samples. This specific reaction of each antiserum against its homologous antigens and no reaction against the heterologous antigens clearly proves that the TMV-Coconut isolate is not associated with the root (wilt) disease of coconut.

Zusammenfassung

Serologische Untersuchungen über die Rolle des Kokosnußstammes des Tabakmosaikvirus in der Wurzelkrankheit der Kokosnuß

Mit empfindlichen und spezifischen Methoden (Doppeldiffusion, Immunelektrophorese und Immunosmophorese) wurde die Rolle des Kokosnußstammes des Tabakmosaikvirus in der Wurzel(Welke-)Krankheit der Kokosnuß untersucht. Mit allen Methoden reagierten TMV-Antiseren nur mit TMV-Antigenen. Mit Proben gesunder Kokosnußpflanzen und wurzelkranker Pflanzen wurden keine Reaktionen gefunden. Antiseren wurzelkranker Pflanzen reagierten nur mit Proben wurzelkranker Pflanzen und nicht mit TMV-Antigenen. Diese Ergebnisse führen zum Schluß, daß der Kokosnußstamm des TMV an der Wurzel(Welke-)Krankheit der Kokosnuß nicht beteiligt ist.

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Authors' address: Central Plantation Crops Research Institute, Regional Station, Kayangulam, Krishnapuram 690 533, Kerala (India).