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INCIDENCE OF POTYVIRUS DISEASE IN OIL PALM NURSERY SEEDLINGS*

Oil palm seedlings raised in some nurseries in Karnataka State, India exhibited mosaic, mottling and ringspot symptoms indicative of virus infection. The incidence was in the range 0.001%-0.01% and was mostly observed in material imported from Costa Rica (ASD). Electron microscopic examination of leaf sap revealed flexuous filamentous particles, and ultra-thin sections of symptomatic leaves showed pin wheel inclusions and scrolls in mesophyll tissues, characteristic features of poty virus. This paper highlights the virus incidence in the introduced material and its implications for quarantine.

Keywords: cytoplasmic inclusion, flexuous filamentous particles, pin wheel, scrolls, quarantine

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INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.), a native of West Africa and the highest oil yielding crop, has been introduced into India in recent years for commercial cultivation. India's seed requirement to establish an oil palm area of 85 000 hectares is mainly met by importing seed from leading oil palm growing countries, notably Ivory Coast, Papua New Guinea and Costa Rica. The imported germplasm is raised and maintained in isolated nurseries for quarantine certification in individual states.

During 1994, the post-entry quarantine inspection team of the Government of India noted symptoms of virus infection in some seedlings of Costa Rican origin in the nurseries of Karnataka State in Bhadravati Reservoir Project (Shimoga district), Gorur (Hassan district) and Bheemanakolli (Mysore district). The seedlings were 8-10 months old in polybags placed in open fields.

The incidence of infection was 0.0001%-0.01%. Affected seedlings were randomly distributed in the nurseries. Preliminary EM examination of some symptomatic leaf samples revealed flexuous filamentous rod-shaped

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particles. Leaf extracts from diseased plants reacted positively with Potyvirus antisera such as those from Peanut stripe virus and Peanut mottle virus (Reddy *et al.*, 1996). This paper provides more information from critical examination of sap and ultrastructural studies of symptomatic plants.

MATERIALS AND METHODS

Source of Planting Material

Fully-opened leaves from both symptomatic and asymptomatic oil palm seedlings from different nurseries in the states of Karnataka and Andhra Pradesh *(Table 1)* were studied. The planting materials were from Costa Rica (ASD), Ivory Coast (IRHO), India (Palode) and Papua New Guinea.

Electron Microscopy

Leaf dip preparations were made by cutting off leaflets and drawing the cut ends over droplets of negative stains placed over carbon coated 300 mesh copper grids. The stains used were 2% potassium phosphotungstic acid (KPTA) pH 7.0, 2% aqueous uranyl acetate (UA), 2% methylamine tungstate (MT) and 5% ammonium molybdate (AM) pH 6.9.

TABLE 1. DETAILS OF OIL PALM SEEDLINGS SAMPLED AT KARNATAKA AND ANDHRA PRADESH

S. No.	Nursery	Source of planting material	Age of seedling (months)	No. of plants sampled	
				Healthy	Symptomatic
	KARNATAKA				
1.	BRP - Shimoga Dist.	Costa Rica	12-15	5	5
		IRHO	12-15	3	3
		Palode	30	–	1
2.	Gorur - Hassan Dist.	Costa Rica	12-15	5	5
		IRHO	12-15	–	2
3.	Bheemanakolli, Mysore Dist.	Costa Rica	12	5	–
4.	Mandya	IRHO	13	–	1
	ANDHRA PRADESH				
5.	Palmtech India Ltd., Rajamundry	Papua New Guinea	21	–	1
6.	Godrej Soaps Ltd., Pothapalli	Costa Rica	15	–	1
7.	Fats Foods and Fertilisers, Tadepalligudem	Costa Rica	12	–	2

Leaf extract was prepared by placing a piece of leaf 0.5 cm^2 on a microslide in 3-4 drops of distilled water and crushing it with another slide. The clear sap extract was removed with a Pasteur pipette and a droplet placed over a carbon-coated grid for 30-45 seconds. The grid was washed with several droplets of stain and drained by touching its edge with filter paper. KPTA, UA, MT and AM of the concentrations mentioned earlier were used to stain the leaf grindates. The air-dried grid was examined in a Carl Zeiss EM 109 Turbo Transmission Electron Microscope at an accelerating voltage of 50 KVA.

For ultra structural studies, leaf pieces of symptomatic and asymptomatic leaves of 0.5 cm^2 were sliced, fixed in 2% glutaraldehyde in 0.2M phosphate buffer pH 7.2, post-fixed in 2% osmium tetroxide, processed and embedded in Spurr's resin. Ultra thin sections of 600-700A° cut in a LKB IV ultratome were placed on uncoated 200 mesh copper grids, stained with UA and lead citrate (Reynolds, 1963) and examined under EM.

RESULTS

Symptom

The symptoms were chlorotic streaks, leaf mottling, alternating dark green and chlorotic areas exhibiting a mosaic appearance and ringspots of dark green areas surrounded by pale green rings (halo). At times, all the three symptoms - mottling, mosaic and ringspot - were evident on the same leaf (*Figures 1* and *2*). Symptomatic plants were generally stunted.

Dip Preparation

In the leaf dip preparations, virus particles could not be seen with all the four negative stains tried. Unlike in herbaceous plants, the cut ends of oil palm leaves do not exude sap and this may explain the non-detection of the virus in the samples.

Leaf Grindates

Among the stains, ammonium molybdate was found to be the best for detecting virus

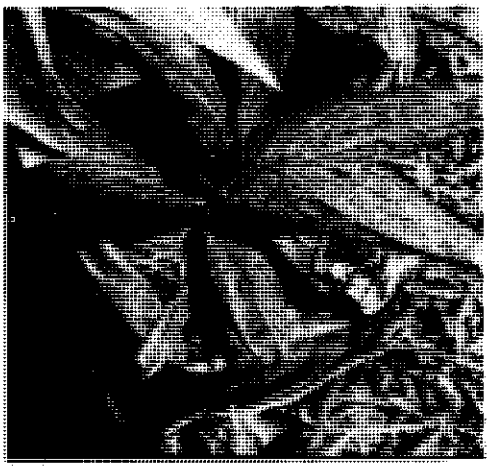

Figure 1. Oil palm seedlings showing mosaic and mottling symptoms.

Figure 2. Leaf showing ringspot symptoms.

particles in oil palm grindates. The oil palm sap which is rich in mucilage, coagulated with KPTA, UA and MT entrapping the virus particles in the conglomerate and made identification of the virus difficult. However, the virus particles were seen in sap stained with ammonium molybdate.

Flexuous filamentous particles of 720nm-900nm were observed in seedlings with mosaic, mottling and ringspot symptoms (*Figure 3*). The particles were seen in clusters, at times encircled by a membrane. Viral particles were not

Figure 3. Flexuous filamentous particles of virus in sap.

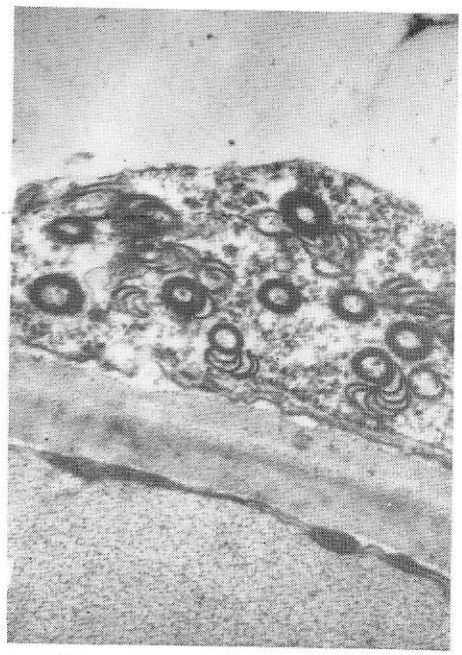

Figure 4. Ultra thin section of symptomatic leaf showing pin wheel inclusion in cytoplasm of mesophyll tissue.

healthy plants from three different Karnataka State nor plants from Pradesh with yellowing and mottling

particles were found in all 10 seedlings origin sampled - five each from nurseries exhibiting mosaic, ringspot symptoms. The virus was in six symptomatic plants from Coasts - three from BRP, two from from Mandya. Flexuous tubular also detected in a stray 2.5 year from Palode growing adjacent to plants in BRP nursery.

Study

inclusion were observed in of symptomatic leaves. The in the form of pin wheel and cytoplasm of mesophyll tissues. scrolls are characteristic fea- (*Figure 4*).

DISCUSSION

morphology of the filamentous and the characteristic pin identified the virus as a Poty virus. Based on the morphology of cytoplasmic inclusion, Potyviridae is broadly grouped into four subdivisions (Edwardson and Christie, 1991). The virus identified had both pin wheel and scrolls and resembled, in morphology, the viruses in sub-division I. Hence it was tentatively assigned to sub-division I of Potyviridae.

The virus particles observed did not resemble the rod-shaped particles observed in healthy and stressed coconut palms (Randles, 1975) nor those observed in Cadang-Cadang infected palms (Plavsic *et al.*, 1972). Plumb *et al.* (1978) reported the presence of three types of virus particles (one having isometric particles of 32 nm and the other two with filamentous particles of 607nm and 788nm length) in oil palm without any specific symptoms from Bisianumu in Papua New Guinea. However, there has been no further report to confirm its identity. Poty virus has been reported from California to occur in the Mexican fan palm (*Washingtonia robusta*) inducing mosaic symptoms (Mayhew and Tidwell, 1978). The infected palm was believed to be grown from seed imported from Mexico as no virus-like disease had hitherto been reported on other palm species in California. Rod-shaped virus causing mosaic symptoms in *Cocos nucifera, Corypha elata* and *Livingstonia*

Figure 5. Scrolls in the cytoplasm of mesophyll tissue.

rotundifolia has also been reported from Malaysia and the Philippines (Chase and Broschat, 1993). In India, no confirmed virus disease has been reported from coconut, arecanut, oil palm or any other cultivated and wild palm species so far. Hence, occurrence of this virus in imported oil palm material is of significance from the quarantine point of view. Although the damage and economic loss the virus can cause are not known at present, introduction of a new palm disease can pose a problem for oil palm and other palm species in the future. As members of Potyviridae are known to be transmitted through sap, aphid, fungi, mites and seed and are known to cause severe economic losses in other crops, effective quarantine measures are needed to check the introduction and spread of the disease in India.

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