17. DETERMINATION OF THE MORPHOLOGIES OF PLANT VIRUSES IN THE PHILIPPINES BY ELECTRON MICROSCOPY

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Introduction

The viruses that attack plants are of many shapes; some are rod shaped, either rigid or flexuous, some are spherical-shaped, while others are bullet-shaped. In some cases, a few plant viruses assume a combination of any one of the above mentioned shapes, ranging from spherical to bacilliform or bullet-shaped.

These plant viruses, irrespective of the form they take, cause most frequently such diseases as mosaic, yellow, stunt and ringspots. Some of them can kill the plants they attack while others do not, but they can reduce yield considerably. Their modes of transmission from plant to plant under natural conditions are by insects, by fungi, by nematodes, and by mites, etc.

Differentiating one virus from another by the symptoms they induce on plants they attack is difficult to attain as one virus type can produce different symptom types on different plant species. On the other hand, two or more different viruses can produce indistinguishable symptoms on a single plant species.

Therefore, the use of electron microscope to establish viral morphology is indispensable on this regard because it can help tremendously in easing out the problem of virus control once the type of virus is established and can then be typed-grouped with other well-established and well-studied viruses whose control have already been worked out.

With the installation of a JEOL electron microscope at the Natural Science Research Center at the University of the Philippines at Diliman, Quezon City last year, it made possible the investigation of the morphologies of some viruses attacking crops in the Philippines.

Although there are about 137 plant virus diseases recorded in the Philippines this present investigation includes only 61 diseases, a few of which are unreported, examined under the electron microscope. Furthr investigations along this line are being conducted at present.

Materials and methods

1. Virus diseases investigated. Several different plants showing suspected virus

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infection which were obtained from crop groups such as fiber crops, field crops, fruits, legumes, ornamentals, pasture crops and vegetables were examined for the identification of their causal viruses under the electron microscope. Priorities were given to the diseases that do great damage to said crop groupings.

2. **Electron microscopic investigations.** For EM investigation of the viruses, at least four different methods of sample preparations were used. These were negative staining of crude sap, negative staining of purified virus suspension, shadowing of purified virus suspension and ultrathin sectioning.

(1) Negative staining of crude sap.—(Dip method). Crude sap for negative staining was prepared by first macerating virus-infected leaf tissue in a motar and pestle and crude sap was later extracted by pressing the macerated tissues between the thumb and forefinger. A drop of this crude sap was placed on a previously carbon-coated, collodium-supported electron microscope grid and stained with a drop of 2% phosphotungstic acid, pH 6.9, for a few seconds. Excess sap and PTA stain were removed from the grid by slightly touching the edge of the drop with absorbent blotting paper. When sufficiently dry, the grid was examined under the electron microscope.

(2) Negative staining of purified virus suspension.—A purified virus suspension was first prepared by using the alternate low- and high-speed centrifugation techniques as shown in Figure 1. The "Lourdes" centrifuge was used for low speed centrifugation while the Beckman Model L Ultracentrifuge was used for high-speed centrifugation. A drop from this purified sample was stained in the same manner as the crude sap before it was examined under the electron microscope.

(3) Shadowing of purified virus suspension.—A drop of the concentrated virus suspension was placed on a previously carbon-coated and collodium-supported grid. This sample was air dried after removing the excess suspension through absorption by a blotting paper. This air dried sample was then placed inside an evaporator unit and then shadowcast with a heavy metal (chromium) at 25° angle, then examined under the electron microscope.

(4) Ultrathin sectioning.—Before specimens were ultrathin-sectioned, they were first prepared by fixing separately small pieces of infected and healthy leaves in phosphate-buffered 5% glutaraldehyde at pH 6.9 for 2 hrs. These were then post-fixed in phosphate-buffered 1% osmium tetroxide for 2 hrs. After these treatments, the small leaf pieces were dehydrated in ethanol and then were finally embedded in Epon-Araldite. These embedded materials were then ready for ultrathin sectioning. Ultrathin sections were cut using a LKB ULTRATOME III, then double stained with uranyl acetate and lead citrate and examined under the electron microscope (JEM 100 Model U).

Results and discussions

Of the 57 plant virus diseases collected from 52 plant species in 46 genera and 15 families (Table 1) that were examined in the electron microscope, for the presence of viruses, only 30 plant viruses were successfully seen. These 30 plant viruses can be conveniently grouped into three: rigid rods, flexuous rods, and isometrics. No bacilliform or bullet-shaped and multiparticled plant viruses were observed, (Table 2). No attempt is made here, however, to group these viruses according to the one proposed by Harrison, *et al.* (1971). Twenty of these 30 plant viruses are flexuous rods, 7 are rigid rods and 3 are isometric. Majority of these plant viruses have aphids as their vectors, one or possibly 2 by whitefly, and probably one nematode-borne.

Aside from the aforementioned viruses a few Philippine plant viruses were reported by other workers, but which EM investigations were done in other countries. These are

Table 1. Host and diseases collected from various places in the Philippines.

Family/Species	Disease type
Apiaceae	
Apium graveolens L. var. *dulce* DC. (Celery)	mosaic
Petroselinum crispum Mill. Nym. ex Airy-Shaw (Parsley)	mosaic
Asteraceae	
Chrysanthemum coronarium L. (Chrysanthemum)	yellow mosaic
Dahlia variabilis (Willd.) ex Hook (Dahlia)	mosaic
Elephantopus mollis HBK (Elephantopus)	mosaic
Gerbera jamesonii Bolus Hook. (African daisy)	mosaic
Helianthus annuus L. (Sunflower)	mosaic
Helichrysum bracteatum (Vent.) Andr. (Everlasting)	mosaic
Lactuca sativa L. (Lettuce)	mosaic
Sultan spp.	mosaic
Traepealum spp.	mosaic
Zinnia elegans L. (Zinnia)	mosaic
Brassicaceae	
Brassica oleracea var. *botrytis* L. (Brocolli, Cauliflower)	mosaic
Brassica oleracea var. *capitata* L. (Cabbage)	mosaic
Brassica pekinensis (Lour.) Rupr. (Pechay)	mosaic
Raphanus sativus L. (Radish)	mosaic
Cucurbitaceae	
Citrullus vulgaris L. (Watermelon)	mosaic
Cucumis melo L. (Muskmelon)	mosaic
Cucumis sativus L. (Cucumber)	mosaic
Cucurbita maxima Dcne. (Squash)	mosaic
Lagenaria leucantha Duch. Rusby. (Upo, Calabash, White-flowered gourd)	mosaic
Momordica charantia L. (Ampalaya)	little leaf
Malvaceae	
Abelmoschus esculentus L. Maench. (Okra)	little leaf
Meliaceae	
Sandoricum koetjape (Brum. f.) Merr. (Santol)	enation
Moraceae	
Morus alba L. (Mulberry)	mosaic
Musaceae	
Musa sapientum (L.) Kuntz. (Banana)	bunchy-top
Musa textilis Nee. (Abaca)	bunchy-top
Myrtaceae	
Psidium guajava L. (Guava)	vein net
Oleaceae	
Jasminun sambac L. (Jasmine or Sampaguita)	yellow ringspot mosaic
Papilionaceae	
Arachis hypogaea L. (Peanut)	mottle rosette
Calopogonium muconoides Desv. (Calopogonium)	yellow mosaic

Family/Species	Disease type
Centrosema pubescens Benth. (Centrosema)	mosaic
Cassia podocarpa L. (Cassia)	mosaic
Dolichos axillaris L. (Dolichos)	yellow mosaic
Desmodium procumbens L. (Desmodium)	yellow mosaic
Glycine max (L.) Merr. (Soybean)	mosaic
Phaseolus aureus Roxb. (Mungbean)	yellow mosaic
Pueraria phaseoloides Roxb. Benth. (Tropical kudzu)	yellow mosaic
Vigna sinensis (Torner) Savi X *Vigna sesquipedalis* (L.) Fruwith. (Bush sitao)	mosaic
Vigna unguiculata (L.) Walp. (Cowpea)	little leaf
Poaceae (Compositae)	
Digitaria sanguinalis non (L.) Scop. (Back.) (Hairy crab-grass)	mosaic
Sorghum vulgare Pers. (Sorghum)	red stripe
Saccharum officinarum L. (Sugarcane)	mosaic
Zea mays L. (Corn)	mosaic
Roseceae	
Rosa spp.	mosaic
Solanaceae	
Capsicum frutescens L. (Pepper)	mosaic
Lycopersicon esculentum Mill. (Tomato)	mosaic yellow leaf curl
Nicotiana tabacum L. (Tobacco)	mosaic
Solanum melongena L. (Eggplant)	mosaic
Solanum tuberosum L. (Irish potato)	leaf roll
Vitaceae	
Vitis vinifera L. (Grapevine)	fan leaf
Total: Families 15	Diseases 57
Genera 46	Species 52

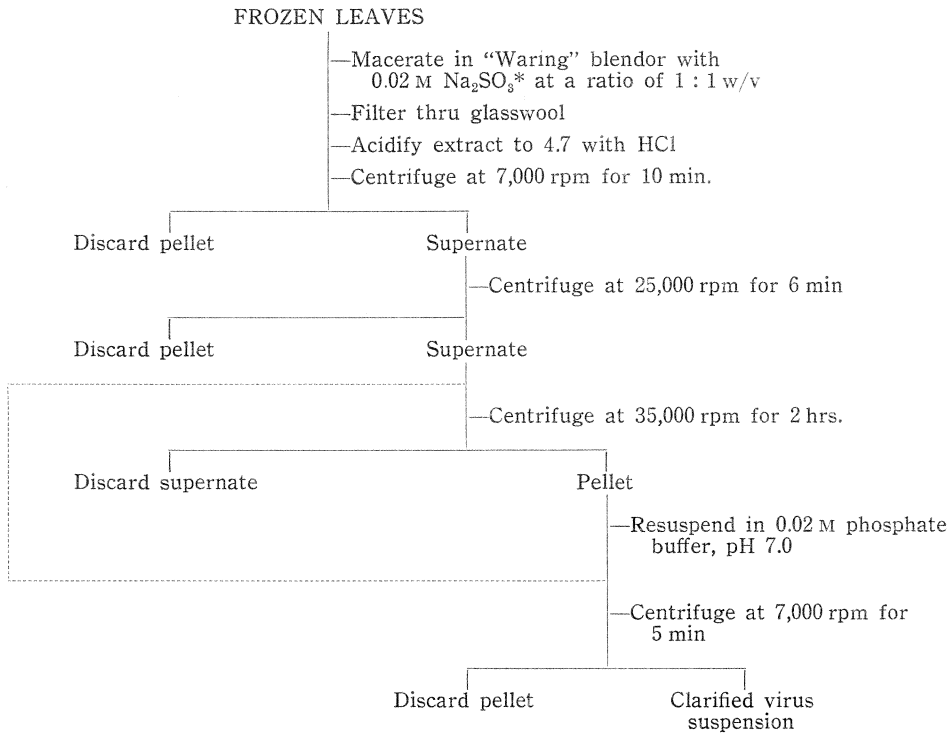
the rice tungro virus, rice orange leaf virus, and abaca mosaic virus. The first 2 viruses are isometric and leafhopper-borne, while the last is a flexuous rod and aphid-borne virus. This electron microscopic investigation further shows that some viruses, although transmitted by different invertebrates and inducing different symptom-types in different plant species, can have particles of similar dimensions as in the case of the isometric viruses and some flexuous rod viruses.

In this investigation some plant viruses are recorded for the first time. These are cowpea little-leaf virus, mungbean yellow mosaic virus, sampaguita yellow ringspot mosaic virus and tomato yellow leafcurl virus.

Table 2. Plant virus detected in 30 kinds of diseased plants by electron microscope.

Host plant	Disease type	Virus morphology*	Size (nm)	Mode of transmission
Calopogonium	yellow mosaic	RR	300–315	aphid, sap
Cowpea	little leaf	RR	330	aphid, sap, seed
Dolichos	yellow mosaic	RR	350	vector unknown
Muskmelon	mosaic	RR	270–300	aphid, sap, seed
Tobacco	mosaic	RR	300	aphid, beetles, sap
Tomato	mosaic	RR	300	aphid, beetles, sap
Upo	mosaic	RR	300–400	vector unknown
Desmodium	yellow mosaic	FR	550–600	vector unknown
Elephantopus	mosaic	FR	610	aphid, sap
Kudzu	yellow mosaic	FR	550	vector unknown
Pepper	mosaic	FR	450	aphid, sap
Cowpea	mosaic	FR	650–700	aphid, sap, seed
Sampaguita	yellow ringspot mosaic	FR	700	vector unknown, grafting
Abaca	mosaic	FR	680	aphid, sap
Ampalaya	little leaf	FR	750	vector unknown
Carabao grass	mosaic	FR	750	vector unknown
Cassia	mosaic	FR	700–750	aphid, sap, seed
Centrosema	mosaic	FR	680–760	aphid, sap, seed
Cucumber	mosaic	FR	750	aphid, sap, seed
Peanut	mosaic	FR	670–740	aphid, sap, seed
Pechay	mosaic	FR	750	aphid, sap
Mulberry	mosaic	FR	750	vector unknown
Sorghum	red stripe	FR	700–750	aphid, sap, seed
Soybean	mosaic	FR	730	aphid, sap, seed
Sugarcane	mosaic	FR	730–750	aphid, sap, seed
Tomato	yellow leaf	FR	830	white-fly
Watermelon	mosaic	FR	700–724	aphid, sap, seed
Grapevine	fan-leaf	IP	28–30	nematode, sap, grafting
Mungbean	yellow mosaic	IP	28–30	aphid, sap, seed
Peanut	rosette	IP	30	aphid, sap, seed

* RR: Rigid rod
FR: Flexuous rod
IP : Isometric polyhedron



* Other chemicals used in place of Na_2SO_3 are chloroform-butanol mixture, phosphate buffer, borate buffer. Other treatments are hot water and distilled water.

Fig. 1. Standard purification scheme for plant viruses

References

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Discussion

Y. Komuro, Japan: In Table 2, you use the term of "Plant viruses", I would like to know the meaning of the term, a little more precisely.

Answer: "Plant viruses"—It means the viruses that cause particular plant virus disease types.

K. Tomaru, Japan: By your description of TMV in your Table 2, mode of transmission of TMV is aphid, beetle and sap. Does this description is from your own experimental result?

Answer: No. It is from other references. However, TMV don't have a specific vector-type.

K. C. Ling, IRRI: Can your H strain of sugarcane mosaic attack rice plants?

Answer: We have not tried inoculating rice plants with SCMV yet.

M. D. Mishra, India: 1. Tomato yellow leaf virus is shown to be transmitted by white flies in your list. What are the evidences you have?

2. Do you have *Bemisia tabaci* in your country or some other species?

Answer: 1. There is a published report in 1971 about this particular disease.

2. Yes.