JICA/NPQS Occasional Paper No. 1

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To pay tribute in memory of late Mr. Tetsuo Suetsugu

Mr. Suetsugu was a JICA expert on Plant Pathology Inspection Technology at the National Plant Quarantine Services Project in Sri Lanka.

Since the project has started, he has made great efforts to establish and improve the Pathological work at the National Plant Quarantine Service of Sri Lanka, and his contributions to the project were invaluable.

Unfortunately, he passed away after a brief yet serious illness, at a moment his widely admired service at the N.P.Q.S. was at its best, making a great loss to the project.

Here we published a series of his achievements during his service to the project, and trust you may find this paper interesting and useful.

Yasuharu Ikegami
Team Leader
National Plant Quarantine Services Project
Japan International Cooperation Agency

March, 1998

OCCURRENCE OF PAPAYA RING SPOT POTYVIRUS STRAIN P IN SRI LANKA

W. G. S. Perera,* Tetsuo Suetsugu and Norihiko Saito*****

INTRODUCTION

Among the several viruses which caused severe damage to papaya cultivation in the world, only Papaya Ring Spot Potyvirus (PRSV) has been recorded in Sri Lanka (Shivanathan and de Silva, 1989). Papaya plants in wet and intermediate zones of Sri Lanka are widely infected with this virus while scattered plants with the infection of this virus can be seen in dry zone also.

Papaya ring spot virus belongs to potyvirus group. This virus is transmitted naturally by aphids and mechanically by inoculation of sap. However host range is limited to the plants in the families Caricae, Cucurbitaceae and Chenopodiaceae. Two strains of PRSV are recognised. They are strain P (designated PRSV-P) and strain W (designated PRSV-W). PRSV-P infects both papaya and cucurbits but PRSV-W infects only cucurbits but not papaya. Both strains cause lesions on *Chenopodium amaranticolor* and *C. quinoa.*

The PRSV-P is one of the most destructive viruses infecting to papaya plants. Infected plants show range of symptoms, which include mosaic, mottling, filiform leaves and spots on petioles, stem and spots or rings with water soaked appearance on fruits (Fig. 5). Infected plants show growth and yield reduction.

Though the occurrence of PRSV in Sri Lanka was reported, it had not been identified as strain P. Therefore we attempted to identify the strain of PRSV and its characteristics.

This is the first report of detailed study of PRSV-P in Sri Lanka.

ISOLATION OF VIRUS

Virus isolate: A sample of young leaves from papaya plants showing viral disease-like symptoms were collected from Gampaha District (Fig. 1). A young infected leaf was used for making the inoculum. The virus was isolated from single local lesion on *Chenopodium quinoa* and designated as NPQS-S. The isolate was propagated in papaya seedlings and the inoculated plants were maintained in greenhouse.

Symptoms: The diseased plant showed ring spot on leaves, and mosaic symptoms on the leaves with blister like patches of green tissue, distributed all over the yellowish green lamina. The younger leaves were chlorotic and reduced to filiform shape (Fig. 1). Elongated water soaked spots and streakes were visible on petiole and stem (Fig. 2). Clear watery like ring spots appeared on the fruit skin (Fig. 5).

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Mechanical inoculation: An infected papaya leaf was ground with 1/15 M potassium phosphate buffer 1:10 dilution (pH 7.1) and the plant sap was mechanically inoculated to three seedlings of test plants using carborundum (400 mesh) as the abrasive. The plants were maintained in a greenhouse and observed for more than three weeks after inoculation. Infection of the virus was confirmed by back inoculation to papaya seedlings.

HOST RANGE

Twenty two species of herbaceous plants belonging to seven families were used for host range study (Table 1).

Three plants from each species were mechanically inoculated with the isolate NPQS-S. The inoculated plants were maintained in a greenhouse and observed for more than three weeks after inoculation. Symptomless plants were back-inoculated to papaya and *Chenopodium quinoa.* The isolate infected *Carica papaya, Cucumis sativus, C. melo* and *Chenopodiaceae* plants. *Carica papaya* showed mosaic symptoms with ring spot (Fig. 3), distortion induced in leaves (Fig. 4) and numerous oily spots on the stem (Fig. 2) similar to symptoms seen in naturally infected *C. papaya. Cucumis sativus* and *C. melo* showed mosaic, while *Chenopodium amaranticolor, C. murale* and *C. quinoa* developed local lesions.

Transmission by aphids: Aphid species reared on *Eupatorium orderatum* (common weed of Compositae family) were used in vector transmission test. After starvation for 2-3 hrs, aphids were allowed acquisition feedings for 10 min. on a tender papaya leaf infected with isolate NPQS-S. Five aphids fed with isolate were placed on a healthy papaya seedling at 3-5 leaves stage. After 24 hrs. the insects were killed by spraying insecticide. Transmission was detected by comparing with the control three plants fed with similar manner with healthy aphids. The inoculated plants together with control plants were kept in the greenhouse and observed for symptoms. After one week, symptoms appeared in 2 seedlings (Fig. 6).

STABILITY IN SAP

Infected papaya leaves were ground in 1/15 M potassium phosphate buffer (pH 6.98, 1/10 w/v dilution). The sap was centrifuged at 5,000 rpm at 18°C for 10 min. and the supernatant was used as the inoculum. C. papaya seedlings at 2-3 leaves stage, was used as the test plants.

Thermal inactivation point test was done by 5ml of sap with each small test tube, exposing to the different temperatures in a water bath for 10 min. Immediately after heat treatment, all tubes were placed in ice water at 10°C till the inoculation was done. Dilution end point test was done by using the supernatant in same buffer dilution series. For storage stability test, the supernatant was stored at 18°C. From these tests it was found that the thermal inactivation point was 53-54°C, dilution end point lies between 10^{-3} – 10^{-4} dilution, and the longevity in papaya sap lost in 24 to 48 hr. at 18°C (Table 2).

VIRUS PARTICLES AND SEROLOGICAL RELATIONSHIP

The infected papaya leaves were directly ground and stained with 2% phosphotungstic acid (PTA), pH 7.0 and observed under electron microscope. The isolate reacted with P strain Papaya Ring Spot Potyvirus antiserum (supplied by Dr. T. Maoka) by serologically specific electron microscopy (SSEM) (Fig. 9), flexuous rod shaped particles about 700-800nm length were detected by measuring the electron micrographs (Fig. 8).

DISCUSSION

Papaya is very important, popular and widely cultivated in Sri Lanka. It grows relatively easily from seed.

Papaya Ring Spot Potyvirus is one of the most destructive diseases of papaya, and widespread in Sri Lanka. Two types of Papaya Ring Spot Potyvirus are recognized, PRSV-P and PRSV-W. Both types cause local lesions on *Chenopodium amaranticolor* and *C. quinoa.* PRSV is rapidly spread naturally by several aphid species in non-persistent manner. Many cucurbits are susceptible to PRSV-P, but they are not important alternative hosts, because the dominant strain in cucurbits is PRSV-W. Stability of this virus is similar to PRSV. There is no record of seed transmission.

Papaya leaf distortion mosaic virus was discovered in Japan, which gives similar symptoms on papaya. It is a member of potyvirus group, but the hostrange is narrower than PRSV-P.

From the results of the host range study, symptomatology, aphid transmission, particle morphology, virus stability, serology and electron microscopy, the Sri Lankan isolate is identified as P-Strain of Papaya Ring Spot Potyvirus (PRSV-P).

This is the first record of PRSV-P in papaya in Sri Lanka.

Authors wish to thank Dr. M. H. J. P. Fernando, Director, Seeds Certification and Plant Protection Centre, Department of Agriculture, Dr. S. M. C. Subasinghe, Head of National Plant Quarantine Service and JICA Sri Lanka office for providing the opportunity for this study. We sincerely express our profound thanks to Dr. T. Maoka for supplying antiserum of Papaya Ring Spot Potyvirus P Strain. We would also like to thank Dr. A. Kawai, Yokohama Plant Protection Station, MAFF, Japan and Mr. R. S. Y. de Silva, Head of Division of Plant Pathology, National Plant Quarantine Service, for their useful suggestions.

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Table 1. Host Range and Symptomatology

Plant family/Species	Symptoms	
	NPQS-S	PRSV-P
Amarantaceae		
Gomphrena globosa	–	–
Caricaceae		
Carica papaya	DM	DM
Chenopodiaceae		
Chenopodium amaranticolor	L	L
C. murale	L	
C. quinoa	L	L
Beta vulgaris	–	–
Cruciferae		
Raphanus sativus	–	–
Cucurbitaceae		
Cucumis sativus (cv. "Gabajes")	M	M
C. melo (cv. "Cantaloup")	M	M (cv. "Kuromon")
Cucurbita maxima (cv. "Ebisu")	–	–
C. pepo (cv. "Diner")	–	–
Luffa acutangula	–	–
Luffa (wild type)	–	
Zucchini marrow	–	
Solanaceae		
Nicotiana benthamina	–	–
N. debuneyi	–	
N. glauca	–	
N. glutinosa	–	–
N. rustica	–	
N. tabacum (cv. "White burley")	–	– (cv. "Bright yellow")
Petunia hybrida	–	–
Phaseolus vulgaris	–	–

NPQS-S: Isolated in Sri Lanka (Perera, et al.)
PRSV-P: Isolated in Japan (Maoka, et al.)
DM: Distortion and Mosaic, L: Local lesion, M:Mosaic,
–: No infection.

Table 2. Description of Papaya Virus

Item	NPQS-S	PRSV-CMI	PRSV-J
Particles	flexuous rod-shaped 700 x 800nm	flexuous rod-shaped 700 x 800nm	flexuous rod-shaped 760 x 800nm
Stability			
heating	53-54°C 10 min.	54-56°C 10 min.	54-55°C 10 min.
dilution	$10^{-3} - 10^{-4}$	10^{-3}	$10^{-3} - 10^{-4}$
storage	24-48h at RT	8h at RT	8h at RT

NPQS-S: Isolated in Sri Lanka (Perera, et al.)
PRSV-CMI: Report of CMI (Purcifull, et al., 1984)
PRSV-J: Report of Disease of Tropical Fruit Trees (Imada, 1995)

Fig. 1 Papaya plant naturally infected with Papaya Ring Spot Potyvirus P strain, showing symptoms of leaf distortion and malformation.

Fig. 2 Water soaked spots on stem.

Fig. 3 Mosaic and ring spot induced in papaya leaf.

Fig. 4 Distortion induced in papaya leaf, showing lobes reduced to veins and small portion of laminar tissue.

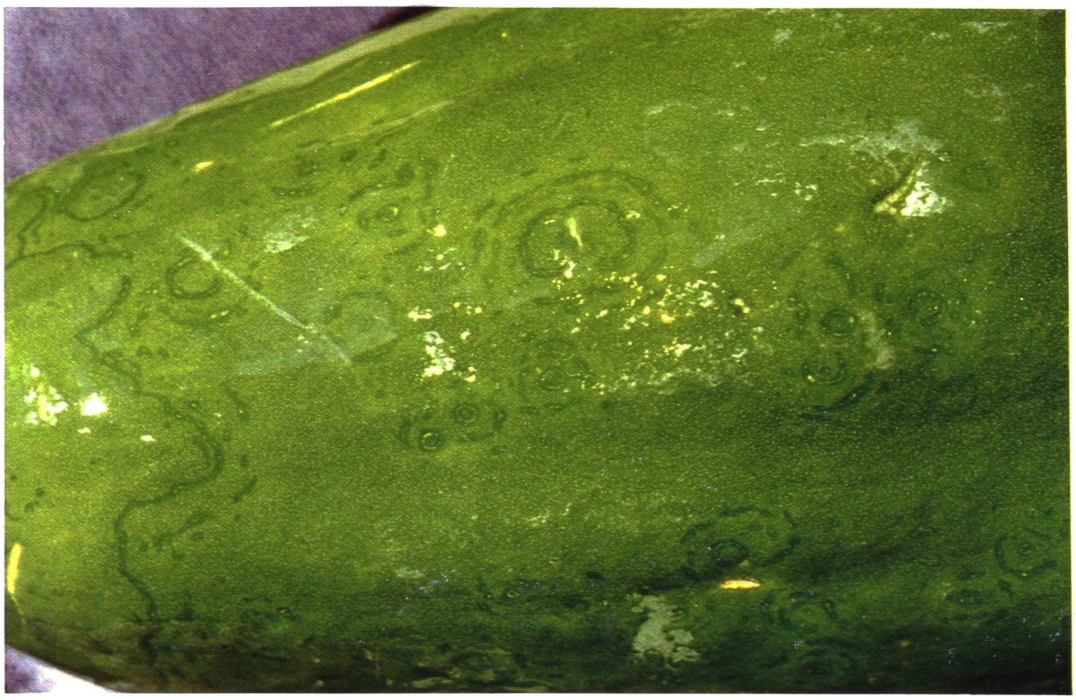
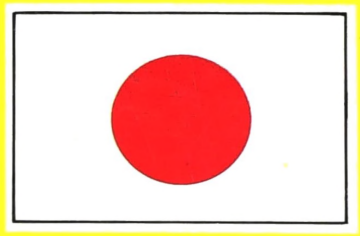


Fig. 5 Ring spot in papaya fruit of naturally infected plant.

Fig. 6 Mosaic symptoms in papaya seedling after virus transmission by aphids.

Fig. 7 Local lesions induced in *Chenopodium amaranticolor.*



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