Indicator plants: Tools for Detecting Papaya Ring Spot Potyvirus and Cucumber Mosaic Cucumovirus

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ABSTRACT

Papaya ring spot potyvirus (PRSV) and Cucumber mosaic cucumovirus (CMV) cause considerable yield losses in important horticultural crops. Therefore, diagnosis of disease at early stage is essential to avoid outbreaks and to control prevalence. In the case of viral diseases, host range studies are still important in diagnosis. Electron microscopy, serological tests and PCR are the most prominent techniques for detecting viruses. However, scientists in many laboratories still rely on biological tests such as inducing symptoms on indicator plants.

In the present study, symptoms were observed on a range of indicator plants three weeks after inoculation. Ten different indicator plant species along with the natural host, Papaya, were inoculated with the PRVS. None of the indicator plants gave clear symptoms except *Chenopodium quinoa*, which showed local lesions, gave clear symptoms. However, all the inoculated papaya plants exhibited typical PRSV symptoms. Indirect ELISA test was done to check the presence of the virus in those plants, which showed visual symptoms. Eighty percent of papaya plants and 50% of *Chenopodium quinoa plants* gave positive value in the indirect ELISA test. Similarly, ten different indicator plant species were inoculated with the CMV and five of them gave clear visual symptoms. However, ELISA test with a monoclonal antibody against CMV produced negative results in some of the indicator plants that gave visual symptoms.

Out of ten plant species studied for PRSV, *Chenopodium quinoa* was shown as only potential indicator plant. For CMV, five potential indicator plants *Capsicum annum*, *Datura stramonium*, *Solanum melongena*, *Chenopodium quinoa* and *Gomphrena globosa* were identified.

Keywords: Diagnostic Code, Papaya Ring Spot, Cucumber Mosaic, *Chenopodium quinoa*

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INTRODUCTION

Papaya (*Carica papaya* L.) is widely grown in tropical and sub tropical regions in the world. It is mainly grown in India, Australia, Hawaii, Sri Lanka and Burma. Papaya fruit is rich in vitamin A, B_1 and B_2, and contains valuable proteolytic enzyme papain, that helps in digesting protein rich food. It offers considerable promise as a commercial crop for both local and export purposes.

Among the several viruses which cause damage to papaya cultivation in the world, papaya ringspot potyvirus (PRSV) has been recorded in Sri Lanka (Shivanathan and de Silva, 1989) for a long time. Recently papaya mosaic virus (PMV) was reported to occur in Sri Lanka (Jeyanandarajah and Krishnarajah, 2000). Papaya plants in wet and intermediate zone of Sri Lanka are widely infected with PRSV where as in dry zone it can be seen in scattered locations. The highest virus incidence was recorded in Kandy district and the lowest has been reported from Hambantota.

Cucumber mosaic virus (CMV) is geographically one of the most widespread viruses, infecting more than 770 plant species belonging to 85 families (Francki, 1979; Douine *et al*., 1979). It is destructive on *Capsicum annum*, *C.frutescens*, *Lycopersicom esculentum*, *Musa* spp., *Solanum melongena* and *Cucurbita* spp. Twenty-six species of aphids are known to transmit the virus in a nonpersistant manner in nature. Cucumber mosaic virus exists as number of allied strains and some of them produce symptoms very different from those characteristic of the type strain. In tomato cucumber mosaic virus produces "Fern Leaf" symptom where the lamina of the leaf is reduced or absent.

PRSV and CMV have become major limiting factors for growing papaya and many horticultural crops in Sri Lanka.

These two viruses are mechanically transmissible to a range of herbaceous plants. However, herbaceous indicator plant test is only useful for detecting those agents transmitted by rub inoculation.

This study was done to find out diagnostic code of indicator plants for papaya ring spot virus and cucumber mosaic virus. The Enzyme linked Immunosorbent Assay (ELISA) technique is highly sensitive diagnostic method for detection of plant viruses, bacteria and possible other plant pathogens. Viruses are often difficult to detect in plants transported internationally. One successful detection method is to inoculate them into indicator hosts. It is low cost, simple and applicable method to country like Sri Lanka.

The objectives of this study were to determine the symptoms of viral infection on different host plants, and to develop of a biological detection test to diagnose PRSV and CMV.

Symptamatology

a) ***Papaya ring spot potyvirus*** **-**

A destructive disease caused by Papaya Ring Spot Virus is a major obstacle in wide scale planting of papaya trees. It causes mosaic, vein clearing, mottling, blister like patches and distortion of leaves (Fig.1). There appears elongate water soaked spots and streaks on petiole and stem, clear watery like ring spots on fruit skin (Fig. 2).

Fig.1: Papaya Ring Spot Virus-Mottling, blister like patches and distortion of leaves

Fig. 2: Papaya Ring Spot Virus-Clear watery like ring spots on fruit skin.

b) ***Cucumber mosaic cucumovirus*** –

The first characteristic symptom of the disease appear about 10 days after inoculation and consist of a spindling appearance of young leaves in the terminal bud (Fig.3). These leaves twist round in a corkscrew fashion, the young leaves, which in a normal plant start to unfold at an early stage, remain folded, curve downwards or curl up in spirals.

Fig.3: Cucumber mosaic cucumovirus-Symptom of the disease appearance of young leaves.

METHODOLOGY

Research Site

The experiment was conducted at National Plant Quarantine Service, Katunayake from October 2002 to February 2003.

Sample collection

***Papaya Rring Spot Potyvirus* (PRSV)**

Sample of papaya leaves showing symptoms of papaya ring spot virus was collected from the field of Plant Virus Indexing Centre Homagama.

***Cucumber Mosaic Cucumovirus* (CMV)**

Sample of cucumber leaves showing symptoms of Cucumber mosaic virus was collected from the vegetable fields in Matale District.

Host range Study

Preparation of Host plants (indicator plants) for the experiment

Pots (8.5 cm diameter and 11cm height in size) filled with soil were sterilized at 90 ^{o}C and 15 psi for one hour in an autoclave.

Seeding and Indicator – inoculation test

Ten indicator plant species for PRSV and 11 plant species for CMV were tested. Five pots containing four indicator plants from each plant species were used in each experiment (Tables 1 and 2). Plants in four pots were inoculated and the other was not inoculated and kept as control. Inoculum was prepared by grinding sample tissues (1g/ml) in 0.07M phosphate buffer at pH 7.2. Sap was filtered through a muslin cloth and was immediately inoculated to host and indicator seedlings by rubbing on the leaves with previously dusted 400-mesh carborandum. After inoculation, plants were rinsed with water and kept in an insect-proof greenhouse. The test plants were regularly observed for local and systemic symptoms.

Table 1. Indicator plants used for the detection of Papaya Ring spot Virus (PRSV)

Family	Indicator Plants
Caricaceae	*Carica papaya*
Chenopodiacea	*Chenopodium amaranticolor*
Chenopodiacea	*Chenopodium quina*
Cucurbitaceae	*Cucumis melo*
	Cucumis sativus (Cantaloupe)
Amarantaceae	*Gomphrena globosa*
Solanaceae	*Datura stramonium*
	Nicotiana glutinosa
	Nicotiana tobacum cv samsum
	Nicotiana debneyii

Table 2. Indicator Plants used for the detection of Cucumber Mosaic Virus (CMV)

Family	Indicator Plants Name
Solanaceae	*Capsicum annum*
Chenopodiaceae	*Chenopodium quinoa*
Cucurbitaceae	*Cucumis sativus*
Solanaceae	*Datura stramonium*
Amaranthaceae	*Gomphrena globosa*
Solanaceae	*Nicotina debneyii*
	N. tobacum cv xanthi
	Nicotina glutinosa
	Nicotiana tobacum cv white burley
Amaranthaceae	*Physalis floridana*
Solanaceae	*Solanum melongena*

Confirmation test for detection of PSRV and CMV

Indirect ELISA (Enzyme Linked Immunosorbent Assay)

Confirmation test for the pathogen was done using the viral regent set-indirect ELISA, alkaline phosphatase label Agdia ^{R} and the manufacture recommended standard protocol of the kit was followed. This test was carried out one month after inoculation with diseased samples of indicator plants according to the previous assay. Samples were taken only from the plants showing symptoms.

RESULTS AND DISCUSSION

Host range Study for PRSV

Inoculation on various families Caricaceae, Chenopodiacea, Amaranthaceae, Solanaceae, and Cucurbitaceae, indicates that the PRSV can only infect papaya plants and

Chenopodium quinoa under the given conditions (Table 3, Figures 4 and 5). Perera et al., (1998), reported that *Chenopodium amaranticolor*, *Chenopodium quinoa*, develop local lesions where as *Cucumis melo* gave mosaic symptoms upon PRSV infection. However, in the present study, attempt to transmit the virus to Cucurbit plants was unsuccessful.

Table 3. Host range and symptomatology for PRSV (3 weeks after inoculation) and ELISA test results

Botanical name	Symptoms *		ELISA ** +/-
	Local	Systemic	
Carica papaya	CS	LD,S,M	+
Cucumis melo	ns	ns	-
Cucumis sativus	ns	ns	-
Nicotiana debnyii	ns	ns	-
Nicotiana glutinosa	ns	ns	-
N. tobaccum (cv Samsum)	ns	ns	-
Gomprena globosa	ns	ns	-
Chenopodium quinoa	LL	ns	+
Chenopodium amaranticolor	ns	ns	-
Datura stramonium	ns	ns	-

* **Key to symptoms**
LD-Leaf distortion, S-Stunting, M-Mosaic CS-Chlorotic spot ns-No discernable symptoms compared to the check, LL-Local lesion

** ELISA --- + positive
- negative

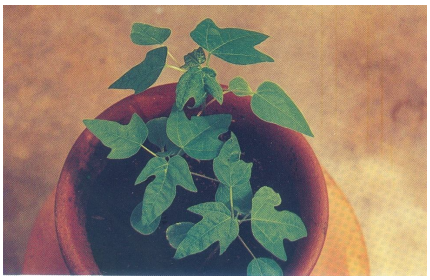

Fig. 4: local and systemic Symptoms on papaya seedlings

Fig. 5: Local lesion on *Chenopodium quinoa*

Local lesion produced in *Chenopodium quinoa* was positive to the indirect ELISA test (Table 3).It was reported by Green and Kim, (1994) that in most cases, efficiency of sap transmission ranged from 60 to 80 %. In this study sap transmission to papaya seedling was 80% successful because out of 20 seedlings 16 were given positive reaction to indirect ELISA. Other tested indicator plants showed sap transmission intensity of 0 % except *Chenopodium quinoa*, which was 50% (Table 4).

Table 4. Transmission of PRSV to indicator plants

Botanical name	No. of plant inoculated	No. of infected plants	% Infection
Carica papaya	20	16	80
Cucumis melo	20	0	0
Cucumis sativus	20	0	0
Nicotiana debnyii	20	0	0
Nicotiana glutinosa	20	0	0

N. tobaccum (cv Samsum)	20	0	0
Gomprena globosa	20	0	0
Chenopodium quinoa	20	10	50
Chenopodium amaranticolor	20	0	0
Datura stramonium	20	0	0

Host range Study for CMV

Discernable Symptoms were observed three weeks after inoculation on *Capsicum annum, Solanum melongena, Datura stramonium, Gomphrena globosa* and *Chenopodium quinoa* (Table 5). Appearance of local lesions on seedlings of some indicator plants was observed on leaves 21 days after inoculation (Figures 6 and 7).

Table 5. Host range and symptamatology for CMV (3 weeks after inoculation) and ELISA test results.

Botanical Name	Symptoms*		ELISA** +/-
	Local	Systemic	
Nicotiana tobaccum cv White burley	ns	ns	-
Capsicum annum	ns	LC,M	-
Nicotiana glutinosa	ns	ns	-
N. tobaccum cv xanthi	ns	ns	-
N.debneyii	ns	ns	-
Datura stramonium	ns	M	-
Solanum melongena	ns	Mot,LC	-
Chenopodium quinoa	LL	ns	-
Gomphrena globosa	LL	ns	-
Physalis floridana	ns	ns	-
Cucumis sativus	ns	ns	-

***Key to symptoms**
LC-Leaf distortion
M-Mosaic
Mot.-Mottling
LL-local lesion
ns-No discernable symptoms compared to the check

** ELISA --- + positive
- negative

Attempt to transmit the virus to *Nicotiana* spp. was unsuccessful. Green and Kim, (1994) reported that, *N. tobaccum* var.'white burly' developed pale green colour spot on inoculated leaves 2 to 3 days after inoculation as well as systemic infection first, to show as a slight curling of the veins followed by mild general mottle. According to them *Nicotiana glutinosa*, had necrosis, mottling and occasionally dark green blister on leaves, where as whole plant was stunted. However, the present study did not support above observations on *Nicotiana* spp. The negative reaction to indirect ELISA was observed with monoclonal antiserum (Table 5).

Fig. 6: Local lesion on *Gomphrena globosa*

Fig. 7: Local lesion on *Chenopodium quinoa*

However, reason for failure to transmit PRSV and CMV to indicator plants was not clearly understood. There are several reasons affecting mechanical inoculation. Among the other factors influencing mechanical transmission, the most important factor is the physiological state of plant at time of inoculation. Salazar (1999) indicated that some inhibition may bind to viral particle, but the mechanism of their action is not cleared. PRSV has a narrow host range, restricted to families like Caricaceae, Chenopodiacea and Cucurbitaceae. Therefore, its infection also may be restricted to a family or genus of plants. In practices, host range studies are still important compound of plant virus diagnosis in many laboratories and with standardization, they could become more useful.

CONCLUSIONS

Out of the ten indicator plant species studied *Chenopodium quinoa* was the only potential indicator for PRSV. Out of eleven indicator plant species studied *Capsicum annum, Solanum melomgena, Datura stramonium, Gomphrena globosa* and *Chenopodium quinoa* were the five potential indicators for CMV.

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