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Seed Transmissibility of Papaya Ringspot Virus*

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The seed transmissibility of papaya ringspot virus (PRV) in 'Cavite Papaya' strain under Philippine conditions was established. Two out of 1,355 (0.15%) seedlings grown from seeds taken from PRV-infected fruits showed symptoms that closely resembled those of papaya ringspot (PRS). The same symptoms were observed when sap from the infected seedlings was mechanically inoculated into healthy seedlings. This was further confirmed by aphid transmission test, indicator host technique, serology and electron microscopy.

Even a low level of seed transmissibility of PRV can have a tremendous impact because the disease can spread very rapidly. Implications of this finding on the papaya industry were discussed.

Key words: Papaya ringspot virus, seed transmissibility, growing-on test.

Papaya (*Carica papaya* L.) is one of the most versatile fruit crops in the Philippines because of its varied uses. It is consumed both as fresh and processed fruit and it has medical and industrial uses. Its export potential is great although the country is yet to take advantage of this potential.

In the past, papaya diseases were not considered major production contraints in the country. However, the discovery of papaya ringspot (PRS) believed to be caused by papaya ringspot virus (PRV) in some areas in Silang, Cavite in 1982 (Opina, 1984) marked the beginning of the decline of the papaya industry in Cavite and the neighboring provinces. Due to the explosive nature of PRS epidemic (Magdalita, 1988), production in Southern Tagalog dropped drastically from 36,000 MT in 1981 to 10,000 MT in 1987 (MAF, 1982 and 1988). The disease has now spread to the four regions in Luzon (Manila Bulletin, 1989) and has become the number one problem of papaya growers in Luzon.

The exact origin of PRS and the circumstances behind its introduction into the country are not clear. It was probably introduced through infected plant materials (Opina, 1986). However, the disease was already known to be present in many parts of the world (Lindner et al, 1945; Herold and Weibel, 1962; Conover, 1964; Story and Halliwell, 1969; Sanchez De Luque and Martinez-Lopez, 1976; Wang et al, 1978) long before its discovery in Silang, Cavite in 1982.

The virus is known to be readily transmitted through sap and by several species of aphids (Jensen, 1949; Conover, 1964; Zettler et al, 1968; Opina, 1986). Published reports (Hendrix and Matsuura, 1947; Capoor and Varma, 1956; Ishii and Holtzmann, 1963; Wey et al, 1978; Wang et al, 1978; Deomano and Pua, 1986; Opina, 1986) claimed that PRV is not seed transmissible. However, since seed transmissibility of plant viruses is governed by many factors such as crop variety, virus strain, temperature, time of infection, etc. (Matthews, 1970; Gibbs and Harrison, 1976) it was deemed necessary to verify the seed transmissibility of PRV under Philippine conditions using different detection methods.

The knowledge gained from the study is important in the formulation of appropriate control measures that would prevent the spread of PRS to geographically isolated areas in the country where the disease is not yet present.

MATERIALS AND METHODS

Growing-on Test

Papaya fruits ('Cavite Strain') heavily infected with PRV were obtained from Silang, Cavite. The seeds were extracted, washed thoroughly under running water, air-dried for one week and stored in a desiccator jar and kept in an air-conditioned room until use. Seeds from healthy fruits were used as control. They were obtained from Davao Experiment Station, Bureau of Plant Industry, Bago

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Oshiro, Davao City, where the disease is not present. The seeds were germinated in sterilized soil contained in 12.7 x 20.3 cm polyethylene bags. They were kept in an insect-proof nethouse. Two seedlings were maintained per polybag. They were sprayed with chlorobenzilate (Akar) at the rate of 0.1 mL per 500 mL of water to control mites (*Tetranychus kansawai*).

A total of 1,355 seedlings were observed for three months for PRS symptoms. Seedlings that exhibited symptoms similar to PRS were maintained in screencages and used in subsequent tests.

Confirmatory Tests

Mechanical transmission. Leaf samples were taken from seedlings showing PRS symptoms from growing-on test. These were macerated in 0.01 M phosphate buffer (pH 7.0) in a 1:1 ratio using sterilized mortar and pestle. Crude extract was obtained by straining the ground tissues in nylon cloth. Approximately 1% celite and one drop of mercaptoethanol were added to the extract to serve as abrasive and inactivator of virus inhibitors, respectively. The extract was then rubbed gently onto the leaves of ten healthy papaya seedlings. The inoculated leaves were washed with distilled water to remove the celite and possible toxic or inhibitory substances present in the extract. Inoculated seedlings were kept in insect-proof screencages and observed for two months. For the positive control, the same procedure was followed except that the crude sap was obtained from PRV-infected plants maintained in the screenhouse.

Aphid transmission. Wingless aphids (*Myzus persicae* Sulz.), reared on raddish plants (*Raphanus sativus* L.), were starved for 30 min at room temperature by placing them on petri plates lined with moist filter paper. They were allowed 30-min acquisition access period on seedlings that exhibited PRS symptoms in the growing-on test. They were then transferred to 8 healthy papaya seedlings (10 aphids per seedling) and aphids were given an overnight inoculation access period before they were killed. For the control, the same procedures were followed except that the aphids were allowed to feed on healthy papaya seedlings. All test seedlings were kept in insect-proof cages and observed for two months.

Indicator host. Two-week-old seedlings of *Chenopodium quinoa* were transplanted in plastic cups containing sterilized soil. Three weeks after transplanting, *C. quinoa* leaves were inoculated separately with crude sap derived from (a) leaves of papaya seedlings showing PRS symptoms in the growing-on test, (b) leaves of PRV-infected plants maintained in the screenhouse, and (c) healthy papaya leaves obtained from Davao. The inoculated plants were incubated in insect-proof screencages and observed daily for the appearance of local lesions.

Serology. Crude sap was extracted from papaya seedlings with PRS symptoms following the procedure described in mechanical transmission. Crude sap from healthy papaya leaves and purified PRV were included to serve as negative and positive controls, respectively. The procedure of Espino et al (1989) on the double antibody sandwich enzyme-linked immunosorbent assay (ELISA) using both polyclonal and monoclonal antibodies was followed to detect the presence of PRV in the sample.

Electron microscopy. The presence of PRV in infected seedlings was determined with the use of an electron microscope following the leaf dip method of Green (1984). A drop of 2% potassium phosphotungstic acid (PTA) was placed on the collodion-coated copper grids (400 mesh). One end of the freshly cut leaf sample (2 x 3 mm) was dipped into the PTA solution and excess stain was drained with strips of filter paper. Grids were examined with the aid of JEOL JEM 100 U electron microscope and photomicrographs of the virus particles were taken.

RESULTS AND DISCUSSION

Growing-on Test

Of 1,355 papaya seedlings grown and observed for three months inside an insect-proof nethouse two seedlings developed symptoms which closely resembled the PRS. Initial symptoms on the two seedlings were observed six weeks after emergence. The other seedling in each of the two polybags showed the same symptoms two to three weeks later. The growth of infected seedlings were more stunted than the companion seedlings that were infected later (Fig. 1). Their leaves were chlorotic, mottled, dis-

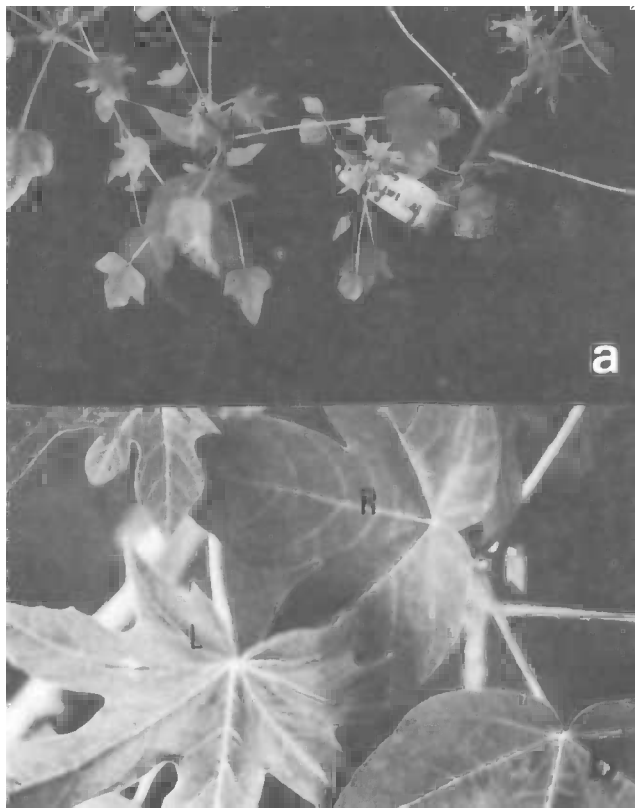

Fig. 1. PRS-infected seedlings derived from seeds extracted from infected fruits: a. Typical symptoms of PRS:Chlorosis, stunted growth, reduced and thickened laminae; b. Comparison of infected (L) and healthy (R) seedlings.

torted and thickened. They also had reduced leaf laminae. Later on, oily streaks developed on the stem of infected seedlings.

The manner by which the companion seedlings were infected is not certain. The most plausible explanation is through root grafting between the infected and healthy plants since the two seedlings were growing in the same container. According to Hunter (1958), a root from originally infected seedling can probably grow under or over one from the adjacent seedling at an early stage and is later forced to unite by pressure exerted through increase in root diameter and resistance of the soil or the container. Presumably, the virus had been transmitted to healthy seedlings through the connecting roots. Infected seedlings were not uprooted to determine the occurrence of root graft because the plants were used in further tests.

Confirmatory Tests

Mechanical and aphid transmission studies proved that infectious entities were present in the seedlings with virus-like symptoms in growing-on test. Six out of ten seedlings mechanically inoculated with sap from seedlings with virus-like symptoms developed symptoms typical of PRS 16 days after inoculation (Fig. 2a), while two out of eight seedlings were infected when *M. persicae* was used as the vector.

Chlorotic lesions developed on *C. quinoa* leaves inoculated with sap from seedlings with virus-like symptoms (Fig. 2b). When sap from these leaves with lesions were tested using ELISA, relatively higher absorbance readings were observed compared with the negative controls (Table 1), indicating the possible presence of PRV in the chlorotic lesions. While Yeh and Gonsalves (1984), reported that PRV can induce local lesions in *C. quinoa,* Opina (1986) and Ramos (1987), on the otherhand, failed to induce local lesions on *C. quinoa*. These conflicting earlier observations are probably due to differences in virus strain, environmental factors and *C. quinoa* strain used in the study.

Table 1. Absorbance values of samples tested against PRV polyclonal antiserum (PAS) and monoclonal antibodies derived from two cell lines P 4-25-8 and P 17-3-2.

Sample[2]	Absorbance Values at 410 nm[1]		
	PAS	P 4-25-8	P 17-3-2
A	0.218	0.288	0.208
B	0.213	0.288	0.363
C	0.238	0.243	0.178
D	0.090	0.087	0.080
E	0.095	0.050	0.065
F	0.085	0.070	0.100
G	1.295	1.138	0.808

[1]Each figure is the mean of two trials with two replicates per trial.
[2](A) papaya seedlings with virus-like symptoms fromgrowing-on tests; (B) papaya seedlings inoculated with sap from A; (C) *C. quinoa* inoculated with sap from A; (D) healthy papaya; (E) fetal calf serum; (F) phosphate buffered saline; (G) purified PRV.

The results of ELISA tests using polyclonal and monoclonal antibodies also indicated the presence of PRV in the sap of papaya seedlings with virus-like symptoms as shown by the higher absorbance readings at 410 nm compared with the negative controls (Table 1).

Electron microscopic examination of leaf samples from infected papaya seedlings using the leaf dip method revealed the presence of flexuous or filamentous virus particles (Fig. 2c) which resembled that of PRV based on the description of particle structure by Herold and Weibel (1962).

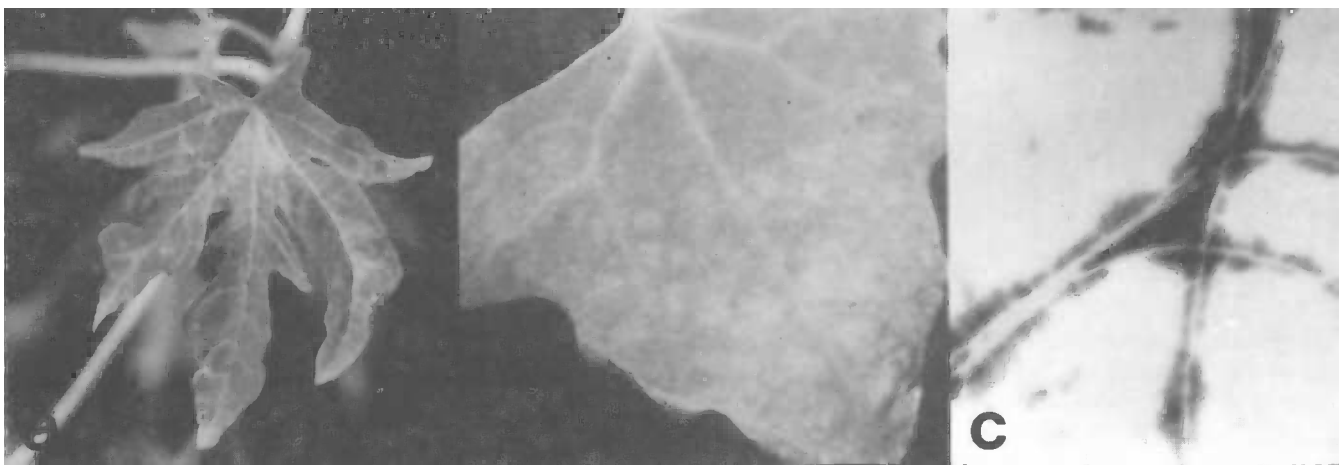

Fig. 2. Evidence proving seed transmissibility of PRV: a. Papaya seedling inoculated with sap of infected seedlings from the growing-on test (Note: Symptoms typical of PRS); b. *C. quinoa* (indictor host) inoculated with sap of infected seedling from the growing-on test (Note: Chlorotic lesions); c. Electron micrograph of flexuous or filamentous virus particles (25, oooX).

All these tests prove that the two seedlings with virus-like symptoms in the growing-on test were indeed infected with PRV, suggesting that the virus can be transmitted through the seed. This finding contradicts previous reports that PRV is not seed transmitted. Although Opina (1986) observed virus-like symptoms such as severe stunting and malformation of leaves in some seedlings, he was not able to transmit the disease to healthy papaya seedlings using sap inoculation. Espino et al (1989), on the other hand, claimed having detected the presence of PRV in papaya seeds through ELISA only, using both the polyclonal and monoclonal antibodies.

There are many factors that influence seed transmissibility of plant viruses (Matthews, 1970; Gibbs and Harrison, 1976). The conflicting observations on the seed transmissibility of PRV could be attributed to differences in weather factors, papaya varieties used in the assay, number of seeds tested, and the procedure used in the assay.

It must be pointed out that 0.15% seed transmission observed in this study may be seemingly low but it has an important epidemiological implication because the disease is capable of causing an explosive epidemic. Magdalita (1988), for instance, found that one infected plant in the field was able to trigger an epidemic in the experimental field within 24 weeks. This study was able to establish the seed transmissibility of PRV using 'Cavite'-papaya based on growing-on test, mechanical and aphid inoculation techniques, indicator host, and serology. The presence of PRV in infected seedlings was confirmed by electron microscopy.

Since PRV is seed transmissible, movement of plant materials from affected areas to geographically isolated areas should be discouraged to prevent the introduction of the disease in these potential papaya growing areas. If such movement can not be totally prevented, seedlings must be closely monitored in insect-proof enclosures for at least two months and those showing PRS symptoms must be properly disposed. Otherwise, sensitive serological tests can be done to detect the presence of the virus. It must be remembered that even a single infected plant can start an epidemic which may seriously damage the papaya industry of an area such as Mindanao and other geographically isolated areas.

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