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# SOME PROPERTIES OF THE VIRUS CAUSING PAPAYA MOSAIC FROM PAKISTAN

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**Abstract.** Papaya mosaic virus was transmitted by mechanical inoculation from *Carica papaya* L. to young papaya seedlings where it caused systemic mosaic and leaf curl symptoms, and to *Glycine max* (L.) where it caused few chlorotic lesion on inoculated cotyledons and systemic mosaic symptoms developed later. Local lesions were produced on *Chenopodium quinoa* Willd. Which was used as an assay plant. In crude sap, virus was inactivated at 55°C for 10 min and stood for dilutions of 1:10,000. The virus had a wide range of pH and inactivated after 3 days at 25°C.

Mosaic disease of papaya (*Carica papaya* L.) has been recognized earlier in other counteries and recently in Pakistan. Wallace and Wallace[15] were the first who attributed this to papaya mosaic virus, but offered no proof. Kulkarni and Shefield[6] continuously isolated virus from affected trees and concluded that virus infection was implicated. More recently, several authors[7,10,11,12] have described the transmitter of the disease by sap inoculation and have studied some of their properties. From Pakistan Moshin *et al.*[8] described the disease symptoms, which were similar to the disease concerned, but virus has not been identified.

The aim of the present work was to isolate the virus, to identify the virus on the basis of reaction on test plants and to study the properties *in vitro*, of papaya mosaic virus (PaMV) which appear very identical to those of Moshin, *et al.*[8]

## Materials and Methods

### *Virus Source*

Isolations were made from infected papaya leaves, inoculum was prepared by homogenizing the infected leaves tissue in a pestle and mortar with an equal amount (weight/volume) of 0.01*M* phosphate buffer, pH, 7.5. The extract was mechanically inoculated to carborandum-dusted leaves of young papaya seedlings. Isolates were maintained in young papaya seedlings and soyabean. Inoculum for the host range and properties determination was usually taken either from papaya or soyabean.

### *Inoculation Method*

All seedlings raised in 4-in pots under ordinary green-house conditions were inoculated at the cotyledon stage or 3-4 leaves stage. Inoculated plants that showed no symptoms after 4 weeks were back indexed on *C. quinoa*. *C. quinoa* was used as a virus assay plant. Control plants of each species were kept and inoculated with water.

### *Properties Determination*

The crude sap was expressed through cheese cloth and 1:10 dilution was made with 0.01M phosphate buffer, pH, 7.5. The diluted sap was used for the study of the following properties.

(a) *Thermal Inactivation Point* (*TIP*). A sample of 5 ml. crude sap was placed in a thin-walled glass tube and each test sample was heated for 10 min to the following temperatures (30,35,40,45,50,55 and 60°C) The heated sap was cooled and inoculated immediately on *C. quinoa* seedlings.

(b) *Dilution End Point* (*DEP*). Five dilutions (1:10,1:100,1:1,000,1:10,000 and 1:100,000) were made. The inoculation procedure was the same as previously described.

(c) *Longivity in Vitro* (*LIV*). The 1:10 diluted sap was kept at 25°C. Each day the sample was taken out for inoculation and this procedure continued for 8 days.

(d) *Tolerance to pH.* A sample of 5 ml were placed in a glass tube and adjusted to the following pH (4.0, 5.0, 6.0, 7.0, 8.0 and 9.0) on a pH-meter by adding IN solution of HCl or NaOH. The inoculation procedure was the same as described before.

## Results

### *Field Symptoms*

The disease begins earliest when the tree is 6-8 months old, affected plants are stunted with a reduction of leaf number and size. Young leaves show vein clearing, in severe cases the youngest leaves are distorted, crinkled, back rolled and more rarely blistered (Fig. 1-A). Frequently mottle or discrete yellow spotting is seen.

### *Virus Isolates*

Young papaya leaves showing prominent disease symptoms were ground in a pestle and mortar with a few drops of phosphate buffer, pH 7.5. The extract was mechanically inoculated to carborandum-dusted leaves of young papaya seedlings which developed mosaic and leaf curl symptoms after 15 days.

### *Host Range*

Several species were used as test plants for the

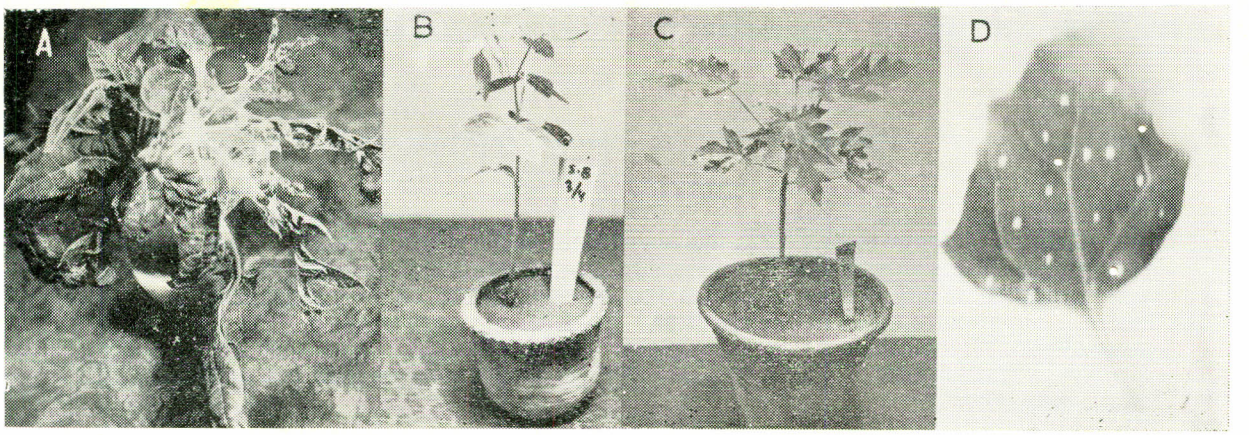


Fig. 1. (A) Field symptoms of papaya leaf showing mosaic, deformation and blistering; (B) Systemic mosaic symptoms in soyabean, inoculated with PaMV, and recovery of virus from young leaves; (C) Papaya seedlings, inoculated with papaya mosaic virus showing systemic mosaic, deformation and blistering symptoms; (D) Leaf of *C. quinoa* L., showing local lesions due to PaMV.

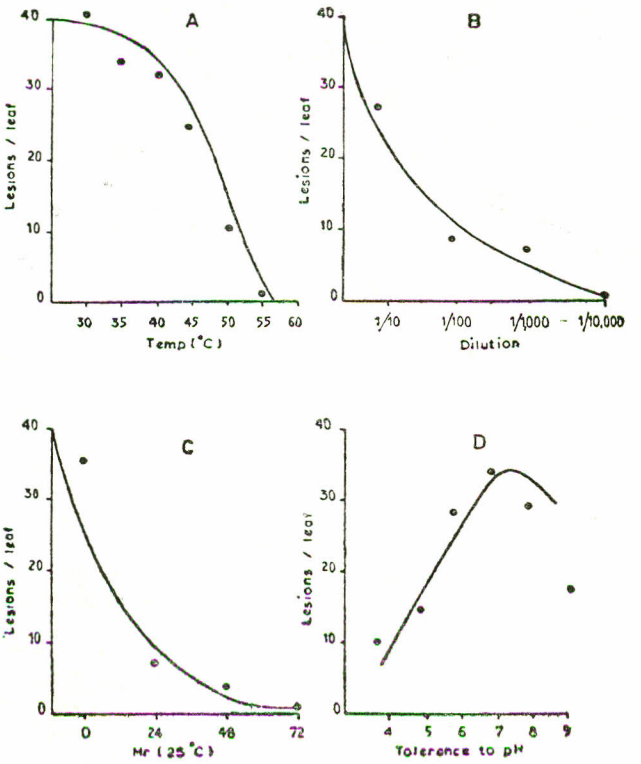


Fig. 2. Physical properties of PaMV determined in crude sap. (A) thermal inactivation point (TIP); (B) Dilution end-point (DEP); (C) Longivity *in vitro* (LIV); (D) Tolerance to pH. Each point represents average lesions per leaf of six C. *quinoa*.

susceptibility of the virus and for the search of a good local lesion host for assay work. Papaya mosaic virus (PaMV) has a narrow host range and *C. quinoa* was selected as a good local lesion host for further work.

A list of test plants used in this work are described in Table 1 and the symptoms produced on susceptible plants are as follows.

(a) *Carica Papaya* L. It developed systemic mosaic and leaf curl symptoms after 15 days of inoculation (Fig. 1C). The symptoms in green house were similar to those observed in the field.

(b) *Chenopodium quinoa* L. Mechanically inoculated leaves developed small chlorotic yellow lesion within 20 days (Fig. 1D). These lesions occasionally developed into chlorotic rings at later stage and rarely developed systemic symptoms.

(c) *Glycine max* L. Two or three chlorotic spots 6–8 mm developed on inoculated leaves within 10 days. Later, systemic infection developed showing

TABLE 1. REACTIONS OF 15 PLANT SPECIES AFTER INOCULATION WITH CRUDE SAP OF PAPAYA MOSAIC VIRUS.

| Plant species | Plants infected* | Sym-ptoms | Back index |
|---|---|---|---|
| *Beta vulgaris* L. | 0/20 | — | — |
| *Brassica oleracea* L. | 0/12 | — | — |
| *Capsicum frutescens* L. | 0/36 | — | — |
| *Chenopodium quinoa* Willd. | 10/12 | LL | + |
| *Cucumis sativus* L. | 2/30 | YS | — |
| *Cyamopsis tetragonoloba* L. | 0/32 | — | — |
| *Glycine max* (L.) Merr. | 32/40 | CL,SM,VC | + |
| *Phaseolus mungo* L. | 0/10 | — | — |
| *Phaseolus vulgaris* L. | 4/30 | YS, M | — |
| *Lycopersicon esculentum* Mill. | 0/22 | — | — |
| *Nicotiana tabacum* L. | 0/8 | M | — |
| *Pisum sativum* L. | 0/10 | — | — |
| *Vigna sinesis* Savi. | 4/35 | CL, M | — |
| *Vinca rosea* L. | 0/6 | — | — |
| *Zinnia elegans* | 0/4 | — | — |

*Total number of plants infected/total number of plants inoculated. LL, local lesion; CL, chlorotic lesion; YS, yellow spots; VC, Vein clearing; SM, Systemic mosaic; M, Mottle; — no reaction or virus is not recovered on back indexing; + virus recovered on back indexing.

vein clearing, faint mottle and mosaic symptoms (Fig. IB).

*Properties in vitro.* Properties determined in crude sap for PaMV are shown in the following tabulation.

| Virus properties | Virus recovered on *C. quinoa* |
|---|---|
| Thermal inactivation point | 55°C |
| Dilution end-point | 1:10,000 |
| Longevity *in vitro* | 3 days |
| Tolerance to pH | 6–8 |

The virus had a wide range of pH in crude sap and could stand pH 6.0–8.0, but best results were obtained at pH 7.5 (Fig. 2D). The virus remained active after an exposure of 10 min at 55°C but not at 60°C (Fig. 2A). Thus the TIP point lies between 55–60°C. The virus retained the infectivity at a dilution of 1:10,000 but not at a dilution of 1:100,000 (Fig 2B). It was found that virus remained infective after 3 days, kept at 25°C but not thereafter. (Fig. 2C).

## Discussion

The first report of leaf shreading of Papaya tree from Pakistan by Moshin *et al.*[8] encrouged me to investigate this problem in some detail. The other advantage to concentrate on this was because papaya mosaic virus was reported to be sap transmissible (Puricifull and Hiebert[11]).

In the present investigations papaya mosaic virus (PaMV) has been transmitted experimentally to young papaya seedlings and to many other plant species. It was found that PaMV has a very narrow host range. The symptomatology and physical properties of PaMV in crude sap are in close agreement with other findings.[7,11,12] This suggests its close relationship to the type strain of PaMV and probably all these viruses are strains of one and the same virus. It is concluded tentaviely here, that the symptoms observed on papaya in Sind region is caused by PaMV.

Besides PaMV, other viruses have been mentioned in the literature, and very few of these were well defined. Bunchy top of papaya, a disease caused by mycoplasma,[13] papaya leaf curl[5] a disease transmitted by grafting[9] yellow crinkle[3] a disease caused by tomato spotted wilt virus and have hosts among *Solanaceae*[14] papaya ringspot virus[1] which infect *Cucurbita pepo*[2] *Citrullus vulgaris*[4], *Cucumis sativus*[16] and decline viruses of pawpaw[7], which infect *Gompherena globosa*, *Glycine max* and *C. quinoa*. Like decline viruses of Pawpaw[7] *Glycine max* and *C. quinoa* are the hosts of the virus, but none of the other hosts seem to be susceptible to the virus under study here.

Further studies comprising the purification, serological investigations and biochemical properties of PaMV will be necessary to elucidate its relationships with the virus isolates from Pakistan with the viruses prevalent in other countries.

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