Association of a potyvirus with mosaic disease of gherkin (*Cucumis anguria* L.) in India

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Abstract A virus associated with severe mosaic disease of gherkin (*Cucumis anguria* L.) in south India was identified. The infected plants showed mosaic, vein banding, blistering on malformed leaves and fruits. Host range, transmission, serological and electron microscopic studies were carried out to identify the virus. The virus was readily transmitted by Sap inoculation and by aphids in a non-persistent manner. The host range of the virus was mainly limited to cucurbitaceous and chenopodium species. The virus showed positive serological relationships with members of potyvirus genus but not with cucumo, ilar and taspo viruses. Electron microscopy of leaf dip preparation of infected leaves revealed long flexuous filamentous virus particles measuring 750×12 nm. On the basis of symptomatology, host range, transmission, serology and particle morphology the virus associated with mosaic disease of gherkin might be the member of potyvirus genus.

Keywords Gherkin · Mosaic disease · Potyvirus

Introduction

Gherkin (*Cucumis anguria* L.) is an important cucurbitaceous vegetable crop grown in southern states of India like Andhra Pradesh, Karnataka and Tamil Nadu for slicing and pickling. Not only the gherkins are consumed in India but also exported to other countries. Viruses are the most common causes of diseases affecting cucurbits. These diseases result in losses through reduction in growth and yield and are responsible for distortion and mottling of fruit, making the product unmarketable. More than 25 viruses belonging to genera *Cucumo*, *Como*, *Tobamo*, *Poty* and *Ilarvirus* are known to infect cucurbits world wide [1]. The genus *Potyvirus* is the major virus group infecting cucurbits, of which *Zucchini yellow mosaic virus* (ZYMV), *Watermelon mosaic virus 2* (WMV-2) and *Papaya ringspot virus*-watermelon isolate (PRSV-W) are economically important and have world wide distribution [2]. The mosaic disease in cucurbits was reported to cause by several viruses including members of genera *Cucumo*, *Como*, *tobamo* and *potyvirus* [3].

During the survey of commercial gherkin fields around Kuppam, Andhra Pradesh in 2003, the natural infection of mosaic disease exhibiting mosaic, blistering, vein banding, malformation of leaves and fruits of gherkin has been widely observed. The disease incidence was found to be 30 to 60% with considerable loss in fruit yield. In the present communication we report the results of biological, serological and electron microscopic studies of a virus causing severe mosaic disease of gherkin in India and its identification as a member of potyvirus genus.

The infected gherkin leaf samples showing typical mosaic symptoms were collected from commercial fields and maintained both gherkin and pumpkin by mechanical

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sap inoculation for further characterization of virus. For sap inoculation, young symptomatic leaves collected from diseased gherkin plants were triturated in a pre-chilled mortar using cold 0.01 M potassium phosphate buffer pH 7.0 containing 0.2% 2-mercaptoethanol (1g leaf in 9 ml buffer). The 600 mesh carborundum dusted young leaves of healthy seedling of gherkin and pumpkin were rubbed gently with freshly prepared inoculum using muslin cloth pad.

Aphid transmission was conducted with two aphid species *Myzus persicae* and *Aphis gossypii* as described by Singh *et al* (2003) [4]. The infection on test plants were confirmed by inoculation to local lesion assay host *Chenopodium amaranticolor*.

For host range studies, plant species belonging to four dicotyledonous families (Table 1) were mechanically inoculated with crud sap as described above. Ten plants of each species were inoculated and kept under observation for the development of symptoms. The infection on plants inoculated was confirmed by back inoculation to gherkin and also to local lesion assay host *Chenopodium amaranticolor*.

Direct antigen coating ELISA was performed according to the method of Hobbs *et al* (1987) [1] to determine the serological relationships of the virus with polyclonal antiserum of *Zucchini yellow mosaic virus* (ZYMV), *Papaya*

ringspot virus-watermelon isolate (PRSV-W), *Tobacco etch virus* (TEV), *Potato virus Y* (PVY), *Cucumber mosaic virus* (CMV), *Tobacco streak virus* (TSV) and *Peanut bud necrosis virus* (PBNV). The virus infected and healthy gherkin leaf antigens, extracted in carbonate buffer, pH 9.6 at 1:10 dilution, were used to coat the plates. The crude antisera at 1:500, alkaline phosphatase labeled goat antirabbit antibodies at 1:1000 and para-nitrophenyl phosphate substrate at 5 mg/10 ml concentration were used at subsequent steps. After one hour of adding the substrate, the absorbances were measured at A_{405} in Bio-Rad Model-680 microplate reader.

Electron microscopy of infected leaves was carried out at the Regional Plant Quarantine station, Chennai. A small drop of fresh leaf dip preparation was placed on a piece of parafilm. The copper film coated grid was placed on the virus preparation (carbon film facing the leaf dip preparation). After 10 min the grid was negatively stained with 2% uranyl acetate (pH 4.5) and examined under JEOL-100-CXII electron microscope.

The disease could be easily transmitted by mechanical sap inoculation from naturally diseased gherkin to healthy seedlings of gherkin and pumpkin. After 12–15 days of inoculation the plants showed typical symptoms of the disease such as mosaic, blistering (Fig. 1b), vein banding (Fig. 1c&d) malformation of leaf margins and dark green irregular shaped blisters on malformed fruits (Fig. 1a). The virus was transmitted in a non-persistent manner by both the aphid species tested, but the efficiency differed with aphid species. *Aphis gossypii* (80–90%) transmitted more efficiently than *Myzus persicae* (60–80%). Symptoms of plants infected by aphid transmission were similar to those observed in the field.

Host range of the virus isolate was mainly confined to the members of the family *Cucurbitaceae*. They showed mainly systemic mosaic and dark green vein banding symptoms after 12–15 days of inoculation. *Chenopodium* species produced chlorotic local lesions. No symptoms were observed on other non-cucurbitaceous plant species even after 30 days of inoculation (Table 1). Similar findings with respect to transmission and host range have been reported in case of yellow mosaic disease on zucchini caused by a potyvirus [7] and WMV-1 causing mosaic syndrome in cucurbits in Punjab state [6].

In DAC-ELISA the virus isolate was positively reacted with polyclonal antisera of PRSV-W, ZYMV, and TEV but not with CMV, PVY, TSV and PBNV. Long flexuous filamentous particles measuring 750×12 nm could be observed in electron microscopy of leaf dip preparation of diseased leaves (Fig. 2).

On the basis of symptomatology, transmission, host range, serological relationships and particle morphology, the authors conclude that the virus causing mosaic disease

Table 1 Reaction of different host plants to sap inoculation with the extract of infected gherkin leaves.

Host plant	Type of reaction
Cucurbitaceae	
<i>Cucumis sativus</i>	VC, VB
<i>Cucumis melo</i>	M
<i>Cucurbita pepo</i>	M, VB, LD
<i>Cucurbita maxima</i>	M,VB
<i>Luffa acutangula</i>	NI
<i>Lagenaria vulgaris</i>	M
<i>Momordica charantia</i>	M
Chenopodiaceae <i>Chenopodium</i>	
<i>amaranticolor</i>	CLL
<i>Chenopodium quinoa</i>	CLL
Fabaceae	
<i>Cajanus cajan</i>	NI
<i>Dolichos lablab</i>	NI
<i>Phaseolus vulgaris</i>	NI
<i>Vigna unguiculata</i>	NI
<i>Vigna radiata</i>	NI
<i>Vigna mungo</i>	NI
Solanaceae	
<i>Lycopersicon esculentum</i>	NI
<i>Petunia hybrida</i>	NI
<i>Datura metal</i>	NI
<i>Capsicum annum</i>	NI
<i>Nicotiana rustica</i>	NI
<i>N. glutinosa</i>	NI
<i>N. tabacum</i> var. White burley	NI
<i>N. benthamiana</i>	NI

Fig. 1 (a) Infected gherkin fruit showing malformation and dark green blisters (b) Infected gherkin leaf showing blisters (c) Infected gherkin leaf showing malformation and vein banding (d) Infected pumpkin leaf showing vein banding

Fig. 2 Electron micrograph of leaf dip preparation of infected gherkin leaf showing flexuous rod shaped particles. (Mag. 80,000 X, Bar represents 100 nm).

of gherkin might be the member of genus *potyvirus* of the family *potyviridae*. However, the exact taxonomic identity of the virus requires its molecular characterization. In India, potyviruses have been reported on several cucurbits [7] and an outbreak of tobacco streak ilarvirus causing necrosis disease in gherkin has been reported [8]. But, no other viral infections in gherkin were identified so far and this is probably the first report on natural occurrence of potyvirus on gherkin.

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References

1. Lovisolo O (1980) Virus and viroid diseases of cucurbits. *Acta Horti* 88:33–82
2. Lecoq H, Wisler G and Pitrat M (1998) Cucurbit viruses: the classics and the “emerging.” In: *Cucurbitaceae 1998: Evaluation and Enhancement of Cucurbits Germplasm*. (Ed. McCreight JD). ASHS press, Alexandria, VA; 30/11-04/12/1998, Pacific Grove, CA., pp. 126–142
3. Mukhopadhyay S (1985) In: *Prospectives in Virology*. (Eds. Gupta IBM, Singh BP, Verma HN and Srivastava KM), Print House (India), Lucknow, pp 75–104
4. Singh SJ, Raj Verma, Ahlawat YS, Singh RK, Satya Prakash and Pant RP (2003) Natural occurrence of a yellow mosaic disease on zucchini in India caused by a Potyvirus *Indian phytopathol.* 56(2):174–179
5. Hoobs HA, Reddy DVR, Rajeswari R and Reddy AS (1987) Use of direct antigen coating methods and protein-A coating ELISA procedures for detection of three peanut viruses. *Plant Dis* 71:747–749
6. Sandhu PS and Kang SS (2007) Variability in virus isolates causing mosaic syndrome of cucurbits in Punjab. *Indian J Viro* 18(2):75–78
7. Singh SJ (1983) Viral and phytopathological diseases of vegetable crops. *IIHR Technical Bulletin* No. 9
8. Krishnareddy M, Devaraj, Lakshmi Raman, Salil Jalali and Samuel DK (2003) Outbreak of tobacco streak virus causing necrosis of cucumber (*Cucumis sativus*) and gherkin (*Cucumis anguria*) in India. *Plant Dis* 87:1264