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Bitter gourd Mosaic: A Complex Viral Disease in Kerala

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***Abstract* – Bitter gourd (*Momordica charantia* L.) is one of the important vegetable crops that occupy a pivotal position among fruit vegetables, particularly in south India. The three different viruses causing mosaic in bitter gourd are cucumber mosaic virus (CMV), poty virus and bitter gourd distortion mosaic virus (BDMV). As these viruses causes mixed infection in field, the separation of individual viruses was carried out using systemic indicator host plants. Systemic indicator host plants were used for separation of CMV and poty virus, where as white fly transmission was adopted for separation of BDMV. The pure cultures of these viruses were maintained on susceptible bitter gourd variety Preethi and symptoms were studied.**

***Keywords* – Bitter gourd mosaic, Bitter gourd distortion mosaic virus, Cucumber mosaic virus, Poty virus.**

I. Introduction

Bitter gourd (*Momordica charantia* L.) a native of Indo-Burma is one of the important cucurbit vegetable crops that occupies pivotal position among fruit vegetables in South India. It is also known as bitter melon, bitter squash or balsam-pear. The fruits of this crop which have a high commercial value are being used as supplementary food as they contain large quantities of minerals, vitamins and essential amino acids and also in various medicinal preparations as it is having anti diabetic property. One major limiting factor in its cultivation is its low productivity due to various diseases of which mosaic is the most important one.

The bitter gourd mosaic is caused by different viruses. Cucumber mosaic virus (CMV) infection of bitter gourd reported for the first time from Coimbatore, India [7]. The virus was transmitted by five different species of aphid vectors and was tentatively named as bitter gourd mosaic virus. The infection of papaya ring spot virus (PRSV) a poty virus was described from cucurbitaceous plants with variable symptoms like vein clearing, mottling, malformed leaves and filimorphism [3]. The association of Bitter gourd distortion mosaic virus (BDMV) with bitter gourd was first reported in India from Kerala [5]. The symptoms of BDMV infection in bitter gourd consists of upward curling, shortening of internodes, distortion of leaves, stunting of plants and deformation of fruits [4]. The simultaneous occurrence of different viruses in bitter gourd plants results in the mosaic complex. Cucumber mosaic, watermelon mosaic and bitter gourd distortion mosaic are the major viral diseases of bitter gourd [6]. Symptom of various mosaics of bitter gourd in Kerala and artificial inoculation of bitter gourd distortion mosaic virus (BDMV) was reported [11]. The association of Indian cassava mosaic virus (ICMV) with yellow mosaic disease of bitter gourd has been reported from Tamil Nadu, South India [9]. The congenial climatic conditions for multiplication of insect vectors of the viruses and susceptibility of cultivated bitter gourd varieties contribute to the high incidence of bitter gourd mosaic in Kerala. At this juncture, it becomes necessary to elucidate the complex symptoms of bitter gourd mosaic in Kerala by separating individual viruses and evaluating symptoms of individual viruses. Hence this study was carried out to separate viruses of bitter gourd mosaic complex and to study symptomatology of individual viruses.

II. Materials and Methods

i) *Separation of virus cultures*

Separation of CMV and poty virus was carried out using sap transmission on systemic indicator plants and that of BDMV was carried out using vector transmission on healthy bitter gourd seedlings. As per [8], the ornamental crop *Cosmos sulphureus* is a systemic host of CMV. As the crop is not infected by any other viruses, it was selected as an indicator host for separation of CMV from bitter gourd samples infected by different viruses.

Young leaves of bitter gourd plants having mosaic symptom were collected from field, washed with tap water, dried with blotting paper and weighed for the preparation of standard extract. Ten ml of 0.1 M potassium phosphate buffer and ten gm of the infected leaves were added into a chilled mortar and ground with pestle. After thorough grinding, the homogenized leaf sap was filtered through double layered muslin cloth to get filtered extract. A pinch of carborundam powder (600 mesh) was added to the extracted sap and it was swabbed using a cotton pad soaked in the standard extract, on the leaves of seven days old *C. sulphureus*. Swabbing was done only in one direction that was from petiole to apex of the leaf by supporting it from below using a cardboard. After five minutes of inoculation, the leaves were washed with sterile distilled water to remove the excess inoculum and extraneous particles. The plants inoculated with the buffer without the infected sap was served as control. The inoculated plants were kept in the insect proof net house and observed daily for the development of symptoms.

Papaya ring spot virus (PRSV), a poty virus infecting papaya reported to induce variable symptoms like vein clearing, mottling, filimorphism and malformed leaves in different cucurbitaceous plants including bitter gourd [3] and [2]. As *C. papaya* is not infected by any other sap transmissible viruses, it was selected as an indicator host for separation of poty virus from bitter gourd samples infected by different viruses. Young leaves of bitter gourd plants having mosaic symptom were collected from field and the standard extract was prepared and inoculated on the leaves of seven days old papaya seedlings as mentioned in the case of CMV on *C. sulphureus*.

White fly (*Bemisia tabaci*) transmission to healthy bitter gourd seedlings was used for separation of BDMV, a

begomo virus. The healthy whiteflies from the culture of whiteflies maintained on healthy brinjal plants were given an acquisition access period of 24 h on infected bitter gourd plants. These viruliferous whiteflies were then given an inoculation access period of 24 h on healthy bitter gourd seedlings at the rate of 10 whiteflies per plant which were then killed by spraying quinalphos.

ii) *Maintenance of virus culture*

The pure cultures of CMV and poty virus obtained in *C. sulphureus* and *C. papaya* respectively were subsequently transferred to 10 days old healthy bitter gourd seedlings of the susceptible variety Preethi by mechanical inoculation. The pure culture of CMV and poty virus obtained initially in bitter gourd was maintained periodically by sap transmission of healthy bitter gourd seedlings. The pure culture of BDMV obtained initially in bitter gourd was maintained periodically by whitefly transmission of healthy bitter gourd seedlings.

DAC ELISA was performed to confirm the presence of CMV and poty virus in the systemic indicator plants (*C. sulphureus* and *C. papaya*) and back inoculated bitter gourd seedlings using polyclonal antiserum of the virus collected from NRCB, Trichy.

III. Results and Discussion

i) *Separation of virus cultures*

The ornamental crop summer cosmos (*Cosmos sulphureus*), was used as a systemic indicator host for separation of CMV from the mixed infected bitter gourd field samples. Initial symptoms were developed on newly emerged leaves after 21 days of inoculation. Mosaic accompanied with yellowing was the initial symptoms and this was followed by vein clearing and mild curling. Leaf distortion and shoestring appearance were the prominent symptoms observed in later stages of infection. Necrosis of the infected leaves was also observed in severely infected plants. Leaf size of the infected plants was drastically reduced as compared to the healthy plants (Plate 1a). Similar symptoms were reported in *C. sulphureus* [8], on mechanical inoculation of CMV.

Seedlings of *Carica papaya* were used as a systemic indicator host for separation of poty virus from field infected bitter gourd samples. Initial symptoms were observed after 15 days of inoculation on newly emerged leaves. Mosaic, down ward puckering of leaves and stunted growth of plants were the symptoms observed (Plate1b). PRSV infection in bitter gourd was reported as vein clearing, blistering and filimorphism of leaves [3] and [2]. But symptoms in *C.papaya* upon inoculation of bitter gourd poty virus were not reported earlier.

Whitefly transmission was carried out with 24 h acquisition access period and 24 h of inoculation access period for separation of BDMV from the infected field samples to healthy bitter gourd seedlings. Cent per cent transmission was recorded in the inoculated bitter gourd seedlings. Initial symptom was observed after 10 days of inoculation. The symptoms produced in the inoculated plants were mosaic, puckering of leaves, reduced leaf size, profuse hairy growth on twigs and stunted growth of plant. Similar symptoms were also reported by earlier workers [5], [4] ,[1]and [10].

ii) *Maintenance of virus culture*

The pure culture of CMV and poty virus obtained from *C. sulphureus* and *C. papaya* were mechanically inoculated to healthy seedlings of susceptible bitter gourd variety Preethi. The symptom was developed in CMV inoculated bitter gourd seedlings after 10 days of inoculation and the symptoms observed were vein clearing, puckering, deformation and reduction in leaf size. Leathery leaf and marginal leaf rolling were also observed in some plants. Even though symptoms of artificial inoculation of CMV in bitter gourd were similar to those reported earlier, leatheriness and marginal rolling of leaves were not reported earlier and hence it is the first report (Plate2).

The symptom was developed in poty virus inoculated bitter gourd seedlings after 13 days of inoculation and the symptoms observed were vein clearing, puckering, deformation and reduction in leaf size. The symptoms of PRSV inoculation in bitter gourd was reported as vein clearing, blistering and filimorphism of leaves [3] and [2]. The symptoms recorded in back inoculated bitter gourd plants of the study was also in accordance with the earlier reports.

The pure cultures of CMV and poty virus obtained in bitter gourd plants were maintained subsequently by periodical sap transmission and that of BDMV by periodical whitefly transmission to healthy bitter gourd seedlings of susceptible variety Preethi.

DAC ELISA was performed to confirm the presence of CMV and poty virus in the indicator plants and the back inoculated bitter gourd seedlings using polyclonal antiserum. The absorbance value of infected *C. sulphureus* and bitter gourd samples were 0.381 and 0.374 where as that of healthy samples of these plants were 0.163 and 0.153 respectively. Similarly absorbance value of infected *C. papaya* and bitter gourd samples were 0.608 and 0.328 where as that of healthy samples of these plants were 0.272 and 0.162 respectively. Based on the results of DAC ELISA the presence of CMV and poty virus infection was confirmed in indicator plants and back inoculated bitter gourd plants.

IV. Conclusion

The present study developed a method for separation of individual viruses from mixed infected bitter gourd field samples using systemic indicator hosts.

V. Acknowledgement

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Vein clearing

Shoestring

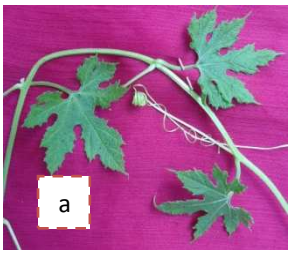
a. Symptoms of CMV on *Cosmos sulphureus*

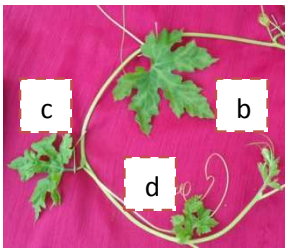
Puckering

Mosaic

b. Symptoms of poty virus on *Carica papaya*

Plate 1. Symptom of CMV on *Cosmos sulphureus and* poty virus on *Carica papaya*





a. Leathery leaf b. Vein clearing
c. Puckering d. Reduced leaf size

Plate 2. Symptoms of CMV under artificial inoculation