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A REVIEW

Identification of the viruses causing mosaic diseases in chilli (*Capsicum annum* L.) in Tarai region of eastern U.P.

■ **Vipin Kumar, Drinkal Yadav, Ajay Kumar Pandey and Ravindra Kumar**

SUMMARY

Viruses are the most common disease causing agent in plants among them various forms of mosaic causing viruses were discovered in chilli (*Capsicum annum* L.) plant species in the Uttar Pradesh. Based on the results of serological tests and responses to various host symptomatologies, four different forms of mosaic causing viruses have been identified. Tobacco mosaic virus (TMV), potato virus X (PVX), cucumber mosaic virus (CMV) and potato virus Y (PVY) have all been found either alone or in combination with other mosaic viruses. Among them most commonly infecting one is CMV.

Key Words : Capsicum, Mosaic disease, CMV, PVX and PVY, TMV

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Several viruses can infect chilli (*Capsicum annum* L.) plant. (Ramakrishnan, 1961). Some of them, including the tobacco mosaic virus (TMV) on chilli, the potato virus Y (PVY), the cucumber mosaic virus (CMV), and the potato virus X (PVX), have been reported from India (Anjaneyulu and Apparao,1967), (Joshi and Bhargava, 1962) (Mathur *et al.,* 1966) (Mishra, 1963) (Rao *et al.*, 1970). Different kinds of mosaic symptoms were seen on chilli in U.P. during the survey and fieldwork of Tarai region. The severity of the disease increased when leaf curl, stunting, yellowing, etc. were present. Therefore, it was decided to determine which viruses are responsible for causing the various kinds of mosaic symptoms in chillies.

MEMBERS OF THE RESEARCH FORUM

Author to be contacted :
Vipin Kumar, Department of Botany, Plant Pathology Lab, M.L.K. (P.G.) College, **Balrampur (U.P.) India**
Email : vipintiwari110396@gmail.com

Address of the Co-authors:
Drinkal Yadav, Ajay Kumar Pandey and Ravindra Kumar, Department of Botany, Plant Pathology Lab, M.L.K. (P.G.) College, **Balrampur (U.P.) India**
Email : yadavdrinkal@gmail.com; ravindrabalrampur@gmail.com

MATERIAL AND METHODS

In the Tarai region of Uttar Pradesh, samples of chillies with various mosaic symptoms were collected from Balrampur, Shrawasti, Gonda and a small portion of the Bahraich district. In the insect proof chamber, a few leaves from each sample with the most pronounced disease symptoms were used to make the inoculum. Sap from the diseased leaves were extracted using phosphate

buffer (0.01M, pH 7.0). During inoculation, carborundum powder 400 mash were used as an abrasive on the hosts e.g. tobacco, *Nicotiana glutinosa, C. frutescens var. NP 46* and *N. clevelandii.* Nurseries of *Chenopodium amaranticolor, C. quinoa, Amaranthus caudatus, Datura stramomium, D. metel, Gomphrena globosa, Nicotiana glutinosa, N. rustica, N. tabacum cv. xanthi, Petunia hybrida, Phaseolus vulgaris, Cucumis sativus, Solanum melongena, Vigna unguiculata, Physalis floridana* and *Zinnia elegans* were raised individually in eight inches earthen pots. For suspected viruses, these hosts served as differentiating hosts. The transmission of insects was done using the nymphs of *Myzus persicae, Aphis gossypii* and *A. craccivora*. The Nymphs were given a one-hour starvation period before being given access for 10 minutes for acquisition and two hours for infection. Each virus isolate was tested for transmission using five nymphs per plant.

The usual techniques were used to determine the thermal inactivation point (TIP) and dilution end point (DEP). By inoculating sap that had been preserved at room temperature (20–25°C), longevity *in-vitro* (LIV) was ascertained. The inoculation were administered every two hours for the first 24 hours, and then every two days after that.

Using the double immunodiffusion assay for detecting specific antibodies (Ouchterlony) Peter Hornbeck, (2017) test in a plate coated with 0.7% noble agar in 0.01 M phosphate buffer (pH 7.5) containing 0.8% sodium azide and 0.2% sodium chloride, serological identification was carried out. As antigens, unpurified virus preparations and crude sap extracts were both employed.

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Virus isolates :

After conducting the following analysis on the samples, four distinct virus isolates were observed :

Chilli virus isolate 1:

Vein clearing and mosaic were both visible on the leaves of infected chilli plants. Later, the tips of these leaves developed into filiform structures with inward rolling (Fig. 1). Fruits started to develop chlorotic spots. *Myzus persicae*, *Aphis gossypii* and mechanical transmission are all potential means of virus transmission. While *C. sativus*, *N. tabacum* and *N. glutinosa* displayed mosaic symptoms, *A. caudatus* plants became stunted and displayed narrowing of the leaves and mosaic. Localized necrotic lesions were caused by *Chenopodium amaranticolor* and *Vigna sinensis*. TIP was between 60 and 65°C, DEP was between 1: 200 and 1: 500 and LIV was between 10 and 12 hours. With CMV antiserum, crude and partially purified sap reacted favourably.

Chilli virus isolate 2:

On the leaves of chilli plants, dark green vein-banding and mottling were clearly visible symptoms. Some of the younger leaves displayed crinkling (Fig. 2). Less flowers were produced and the size of the fruits that did form was greatly diminished. Mechanical inoculation, as well as *M. persicae* and *A. gossypii*, were effective methods of virus transmission. The onset of symptoms took 12–15 days. TIP was between 60 and 65°C, DEP was between 1: 10000 and 1: 100000, and LIV was between 4 and 6 days. However, the virus caused local lesions on *C. amaranticolor* and mosaic symptoms on *V. sinensis*, while failing to infect *D. stramonium*. The virus tested positive with antiserum against PVY in crude sap and in partially purified form, but negative with antibodies against PVX, CMV and TMV.

Chilli virus isolate 3:

Infected plants' leaves displayed mosaic symptoms and a few noticeable yellow spots. Younger leaves had leaf laminae with wavy margins that rolled inward (Fig. 3). Aphids were a simple means of spreading the virus. It causes localised lesions on *C. amaranticolor* and *G. globosa*. On *D. stramonium*, inoculated leaves developed concentric local lesions, but subsequent leaves displayed mosaic mottling. Between 10^{-4} and 10^{-5} was the dilution endpoint, between 65 and 70 degrees were the TIP and between 18 and 20 days were the LIV. With PVX antiserum, the virus in crude sap and partially purified form responded favourably.

Chilli virus isolate 4:

Infected plants displayed the typical light and dark green spots on their leaves, a reduction in fruit wall thickness and growth as well as displayed leaf malformation, excessive dwarfing and dark green vein

banding (Fig. 4). It was easily transmitted by sap, but not by aphids. On *N. tabacum* cv. *Xanthi*, *N. glutinosa*, and *C. amaranticolor*, it caused localised necrotic lesions. It did not, however, infect *G. globosa*. It had a LIV of more than 30 days, a DEP between 10^{-9} and 10^{-10}, and a TIP between 90 and 95°C. With TMV antiserum, the virus in crude sap produced a noticeable reaction.

Infection with several viruses :

In the same plant, infections with CMV and PVY were found in 18 samples. Such plants displayed leaf malformation, excessive dwarfing and dark green vein banding. The terminal leaves had a crown-like shape. Fruit production was almost non-existent and flowering was extremely poor. By inoculating *D. stramonium*, which was infected by CMV but not by PVY, CMV was isolated from PVY. By using a serological test, (Wetter, 1965 and Peter Hornbeck, 2017) it was confirmed that the same plant had PVY and CMV mixed infections.

The majority of the current studies of virus identification relies on serological tests and responses from various hosts. Additionally, consideration has been given to signs, transmission potential, physical characteristics and host range. On the basis of symptoms, transmissibility, response on various hosts, including *D. stramonium, C. amaranticolor, C. sativus* and *N. glutinosa* and serological tests, the chilli virus isolate-1 has been identified as being similar to CMV. *A. craccivora* did not transmit the CMV; *M. persicae* and *A. gossypii* transmit. Similar CMV symptoms were reported by Anjaneyulu and Apparao (1967) when using chilli plant. They reported host reactions and physical characteristics that were fairly similar to our findings. They did not, however, perform a serological test. In Madras, CMV was found on chillies, according to Kandaswamy *et al*. (1963). Host reaction and serological tests were used in Mauritius to record and identify CMV Dossa and Mungur (1982). As in the current instance, their isolate produced mosaic on *N. glutinosa* and *N. tabacum*. In addition, Tobias *et al*. (1982) identified CMV from sweet chilli using serology and host plant reactions. The majority of the samples used in the current study were CMV-infected. This may be because CMV has a very diverse range of hosts, including a number of weeds. In addition, nature contains a number of effective aphid vectors. According to Conti and Masenga (1977), *Capsicum annum* was the plant where CMV and TMV were most prevalent in Italy.

The chilli virus isolate-2 was identified as PVY based on similar observations to the earlier case. As differentials, *D. stramonium, N. glutinosa* and *C. amaranticolor* were employed. The infected chilli plants had mosaic, crinkling and dark green vein banding. As well as *M. persicae, A. craccivora,* and *A. gossypii,* the virus was mechanically transmitted by these organisms. On the basis of symptomatology, PVY's current observations of *C. annuum* concur with those of Nagaraju and Reddy (1981). Filiform leaves and the same symptoms as those seen in the aforementioned isolate were observed by Joshi and Bhargava in 1962. In contrast to the 16–24 hour LIV of the PVY virus isolate described by Nagaraju and Reddy in 1981, the PVY isolate used in the current studies was infectious for 4-6 days. Despite the fact that isolate-2 did not exhibit filiformy, it was present in all other studies, and the current isolate resembled that reported by Joshi and Bhargava (1962). The physical characteristics of the PVY isolates examined by Jeyarajan and Ramakrishnan (1969) and Dossa and Mungur (1982) differed from those of the current isolate.According to Jeyarajan and Ramakrishnan (1969),PVY could mechanically and orally be transmitted by *A. gossypii* but not by *M. persicae or A. craccivora. But M. persicae, A. craccivora* and *A. gossypii* could mechanically transmit the current isolate. Both Conti and Masenga (1977) and Dossa and Mungur (1982) performed serological tests on the virus and noted a positive response to PVY antiserum. In isolate-2, similar observations were made and recorded.

PVX was recognised as isolate 3 of the chilli virus. On leaves with rolled and waved margins, this virus on chilli caused mosaic and yellow spots. Similar symptoms on *Capsicum annuum* and *C. frutescens* have also been reported by Putnam (1937), Dijkstra (1939), Ramakrishnan (1961) and Rao *et al.* (1970). It could spread mechanically, but not through aphids. PVX on chilli as reported by Rao *et al*. (1970) and the ribgrass strain of PVX as reported by Sharma and Raychaudhuri were similar in terms of physical characteristics to virus isolate-3 (1962). On *D. stramonium*, concentric rings were seen on leaves that had been inoculated, and mosaic was seen on leaves that followed. Similar responses were noted when the ring spot strain of PVX was inoculated on *D. stramonium* by Matthews, (1949) and Ladeburg *et al.* (1950).

TMV was recognised in isolate 4 of the chilli virus. The symptoms displayed by this virus isolate on capsicum

Fig.1

Fig.2

Fig.3

Fig.4

were distinct from those reported by Wetter *et al.* (1984) but resembled those of TMV reported by Conti and Masenga, (1977). Fruits with defects and blemishes were observed by Conti and Masenga in 1977. On leaves, there were some depressed yellow whitish spots or stripes, as well as large discolored areas that appeared more frequently near the top. On some apical leaves, transient mottle and necrosis later appeared. An unusual strain of TMV on chilli that caused mosaic on *C. annuum* and local lesions on *C. frutescense* was described by Feldman and Oremianer, (1972). Isolate-4's physical characteristics revealed high TIP, DEP, and LIV values. The serological response to TMV antiserum was favorable. Contrary to Conti and Masenga's (1977) report, isolate-4 failed to infect *G. globosa*. A strain of TMV that affected chillies and caused wilting, stunting, necrosis on stems, defoliation, and mosaic with deformed leaves and fruits was described by Erkan and Yorgani (1983). Similar result were observed by Ansari *et al*. (2004); Pandey *et al.* (2004).

The chilli plants in the current study that had concurrent CMV and PVY infections displayed consistent symptoms. According to Conti and Masenga (1977), PVY and/or TMV have been implicated in multiple CMV infections. Four instances of each type of symptom and the relevant virus have been noted. Dossa and Mungur, (1982) discovered viruses from the same pepper plant, but they were unable to establish a reliable correlation between PVX and PVY co-existing in a single plant or CMV with PVY. Only one such chilli plant was discovered.

Out of 120 samples analysed, 40 had CMV infection, 27 PVY infection, 13 TMV infection and 9 PVX infection. 18 samples were unable to be identified. All of these viruses have a very broad range of hosts, which includes numerous weeds. However, while CMV and PVY are effectively transmitted by a number of efficient insect vectors, namely *M. persicae, A. gossypii* and *A. craccivora*, TMV and PVX are not known insect vectors. One of the primary causes of the widespread prevalence of CMV and PVY on chilli in the tarai region of Uttar Pradesh could be attributed to their presence in nature.

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