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# Transmission Studies on an Indian Isolate of Cowpea Mosaic Virus

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**A B S T R A C T**

An Indian isolate of Cowpea mosaic virus (CpMV) was found easily transmitted under glass house conditions by mechanical sap inoculation, using 0.1% potassium phosphate buffer (pH 7.4). Symptoms of mechanical transmission were characterized by mosaic, veinal necrosis, vein clearing, vein banding, blistering, stunting, leaf deformations, chlorosis and cupping of leaves. Three Aphid species *viz. Myzus persaicae*, *Aphis Gossypii*, and *Aphis craccivora* were found successfully transmitted the Cpmv from plant to plant. Acquisition feeding period of 40-60 seconds was found enough to acquire virus with maximum transmission of 73.3% by *Myzus persicae*. The virus was also found to be transmitted by seed and noticed high disease incidence (37.1%) with seeds collected from mechanically inoculated plants, than seeds collected from healthy seed lot (18.5%). Thus the virus under study was seed borne and sap transmissible, and it was also transmitted by Aphid vector in a non-persistent manner.

## Introduction

Cowpea (*Vigna unguiculata* L. Walp) is one of the World's important dicotyledonous leguminous food crops of millions of people in the developing countries (Summerfield *et aI.*, 1974).

Worldwide cowpeas are cultivated in approximate 8 million hectares. The total world production was estimated to be about 3.3 million tonnes of dry grain. Area under cowpea in India was 3.9 million hectares with a production of 2.21 million tonnes with the national productivity of 683 kg $ha^{-1}$ (Singh *et al.*, 2012). Major states growing cowpea are Maharashtra, Karnataka, Tamilnadu, Madhya Pradesh, Rajasthan and Andhra Pradesh. In Maharashtra, cowpea occupies an area of 11, 800 ha with an average productivity of 400 kg $ha^{-1}$ (Anonymous, 2012). Many factors contribute to very low yields and diseases are the ones responsible for reduction in cowpea yield. Among these viral diseases are major constraints to production and yield (Bashir and Hampton, 1996). More than 20 viruses affect cowpea production Worldwide (Thottappilly and Rossel, 1985).

Yield losses of almost 90% or even total crop failure have been reported (Kaiser and Mossahebi, 1975; Raheja and Leleji, 1974). Considering the economic losses caused by the virus the present investigations were carried out in the glass house during 2015 at college of agriculture, Latur.

## Materials and Methods

### Source of inoculum

Virus infected vegetable cowpea (*Vigna unguiculata spp unguiculata*) cv. Pusa Phalguni plants through the seed provided initial inoculum. Subsequently it was maintained and multiplied on cv. Pusa Phalguni through aphid and mechanical transmission and was used for different studies.

Healthy test plants of cowpea cv. Pusa Phalguni were raised in earthen pots containing steam-sterilized soil, compost and sand (2:1:1) mixture. All the transmission tests were carried out at primary leaf stage. All the inoculated plants were maintained in an insect free glass house and observations on the aspects like incubation period, percentage infectivity and symptoms were recorded.

### Transmission studies

### Mechanical transmission

For mechanical transmission, sap was extracted by crushing symptomatic leaves of cv. Pusa Phalguni cultivar of vegetable cowpea with a mortar and pestle in a chilled 0.05M potassium phosphate buffer ($P^H$ 7.4) containing 0.02M 2-mercapto ethanol. Test plants were inoculated by conventional leaf rub method with a cotton swab. Carborandum (800 mesh) was used as an abrasive. Immediately after virus inoculation, the leaves of test plants were rinsed with tap water. Test plants used for mechanical inoculation were raised from virus free seeds in earthen pots containing steam sterilized soil, sand and compost (2:1:1) mixture. Test plants were maintained in an insect-free glass house for 4-6 weeks and observations were recorded with respect of symptom development and incubation period.

### Aphid transmission

For aphid transmission, *Aphis craccivora* Koch. *Aphis gossypii* Glov, and *Myzus persicae* Sluz, raised from single aphid colony were used. For raising an aphid colony, the healthy leaves of cotton (*Gossypium hirsutum* L.) and groundnut (*Arachis hypogea* L.) were placed in a petridishes on slightly wet filter paper and an apterous form of aphids were transferred separately with small camel hair brush to the leaves. Petridishes were closed for 8 hours and the newly born aphids were used for transmission studies. The apterous forms of aphids were transferred to clean petridishes for 2 hours fasting. This was followed by an acquisition feeding of 40 to 60 seconds on virus infected detached leaves of source plant.

Aphids were allowed to make only brief probes of 40 to 60 seconds duration. Aphids still in probing position at 40 seconds were picked up with camel hair brush and transferred in batches of 25 to healthy test plants for inoculation feeding of four hours. The test plants were kept in muslin cages. Later, aphids were killed by spraying with 0.02 percent imidacloprid (17.8 EC) insecticide and plants were maintained in an insect free glass house for three to four weeks. Observations were recorded for the symptoms on test plants. The healthy seedlings receiving non viruliferous aphids served as control.

### Seed transmission

For seed transmission, the seeds were collected from the virus infected cowpea test cultivars. Seeds were sown in earthen pots containing steam sterilized soil, sand and compost (2:1:1) mixture and maintained in an insect free glass house. Observations were recorded for percent transmission and the plants with perceptible symptoms were counted in total population for determining the level of transmission.

## Results and Discussion

### Mechanical transmission

The results on sap inoculation indicated that, the virus was readily transmitted by mechanical means from infected cv. Pusa Phalguni to uninfected cv. Pusa Phalguni. The systemic symptoms produced by mechanical inoculation on cv. Pusa Phalguni of cowpea were fine vein clearing (Fig. 1) and veinal necrosis which were evident on first trifoliate leaves after 8 to 10 days of virus inoculation. This was followed by interveinal chlorosis and green vein banding on second and third trifoliate leaves apparently evident after 18 days of virus inoculation. Irregular mosaic (Fig. 2), cupping (Fig. 3) and puckering along with the main central vein (Fig. 4) were evident on subsequent trifoliate leaves after 21 to 25 days of virus inoculation. The systemic symptoms produced by the virus upon mechanical inoculation on cv. Pusa Phalguni of cowpea were similar to those produced by seed transmission on the same cultivar.

Similar result regarding sap transmission was reported earlier by Gahukar and Kalore, (1984); Sekar and Sulochana, (1986); Damiri *et al.,* (2013).

### Aphid transmission

The results on aphid transmission of cv. Pusa Phalguni of cowpea virus is shown in Table 1 and graphically in Figure 5. It is revealed from the table, that the virus was transmitted by three aphid species in the non-persistent manner from infected cv. Pusa Phalguni to uninfected cv. Pusa Phalguni. These aphids included *Aphis craccivora, A. gossypii* and *Myzus persicae.* Of these aphids, *Myzus persicae* was found to be the most efficient vector. The *Aphis craccivora* and *A. gossypii* were next in order for their efficiency in transmitting the seed borne virus of cv. Pusa Phalguni. Aphids picked up the virus from seed borne plants showing vein clearing and chlorotic vein banding symptoms and transmitted it to the healthy test plants of cv. Pusa Phalguni of cowpea.

Similar results of aphid transmission was reported earlier by Bock, (1973); Bashir *et al.*, (2002); Anitha and Nandihalli, 2008); Kitajima *et al.,* (2008); Damiri *et al.,* (2013) and Santoshi *et al.,* (2015).

From Table 2, it is revealed that transmission efficiency of aphid transmissible virus infecting cowpea by *Myzus persicae* and *Aphis craccivora* was found to be dependent on the number of aphids used/test plant. When 1, 5, 10, 15 and 20 Aphids/test plants were used the per cent transmission obtained was 0%, 30%, 40%, 60% and 75%, respectively by *Myzus persicae* and 0%, 20%, 30%, 50% and 70% per cent respectively by *Aphis craccivora*. 80% transmission was obtained in both the cases by using 25 aphids, respectively. The systemic symptoms produced on cv. Pusa Phalguni of cowpea by aphid inoculation were similar to those produced on same cultivar by sap inoculation and plants infected through seeds.

Similar results regarding transmission efficiency of *Aphis craccivora* and *Myzus persicae* was earlier reported by Syed, (1988).

### Seed transmission

The studies revealed that, seed transmission of virus in commercial seeds of cv. Pusa Phalguni was found to vary from 17.6 to 19.2 per cent in two tests conducted separately. On an average, seed transmission of commercial seed was found to the extent of 18.5%. However, no significant reduction in percentage seed germination was found in commercial seed of cv. Pusa Phalguni of cowpea.

**Table.1** Aphid transmission of a virus infecting cowpea cv. Pusa Phalguni

| Sr. No. | Test aphid | No. of aphids/plant | Virus source* | Test plants** | Per cent Transmission | Reaction*** | |
|---|---|---|---|---|---|---|---|
| | | | | | | cv. Pusa Phalguni | |
| | | | | | | Local | Systemic |
| 1. | *Aphis craccivora* | 20 | cv. Pusa Phalguniof cowpea with vein clearing symptoms on primary leaves | 21/30 | 70.0% | _ | Vc, Vb, M |
| 2. | *A. gossypii* | 20 | -do- | 18/30 | 60.0% | _ | Vc, Vb, M |
| 3. | *Myzus persicae* | 20 | -do- | 22/30 | 73.3% | _ | Vc, Vb, M |

* = cv. Pusa Phalguni of cowpea with seed transmitted symptoms on primary leaves.
** = cv. Pusa Phalguni of cowpea: no. of plants with disease symptoms/total no. of plants inoculated with the virus.
*** = Vc = Vein clearing; Vb = Vein banding; M = Mosaic; − = Test plants not infected.

**Table.2** Efficiency of *Myzus persicae* and *Aphis craccivora* vectors in transmitting the virus infecting cowpea (cv. Pusa Phalguni)

| Sr. No. | No. of aphids/ plant | *Aphis craccivora* | | | *Myzus persicae* | | |
|---|---|---|---|---|---|---|---|
| | | No. of plants inoculated | No. of plants with symptoms | Transmission (%) | No of plants inoculated | No. of plants with symptoms | Transmission (%) |
| 1. | 1 | 20 | 0 | 0 | 20 | 0 | 0 |
| 2. | 5 | 20 | 4 | 20 | 20 | 6 | 30 |
| 3. | 10 | 20 | 6 | 30 | 20 | 8 | 40 |
| 4. | 15 | 20 | 10 | 50 | 20 | 12 | 60 |
| 5. | 20 | 20 | 14 | 70 | 20 | 15 | 75 |
| 6. | 25 | 20 | 16 | 80 | 20 | 16 | 80 |

Cv = Cultivar

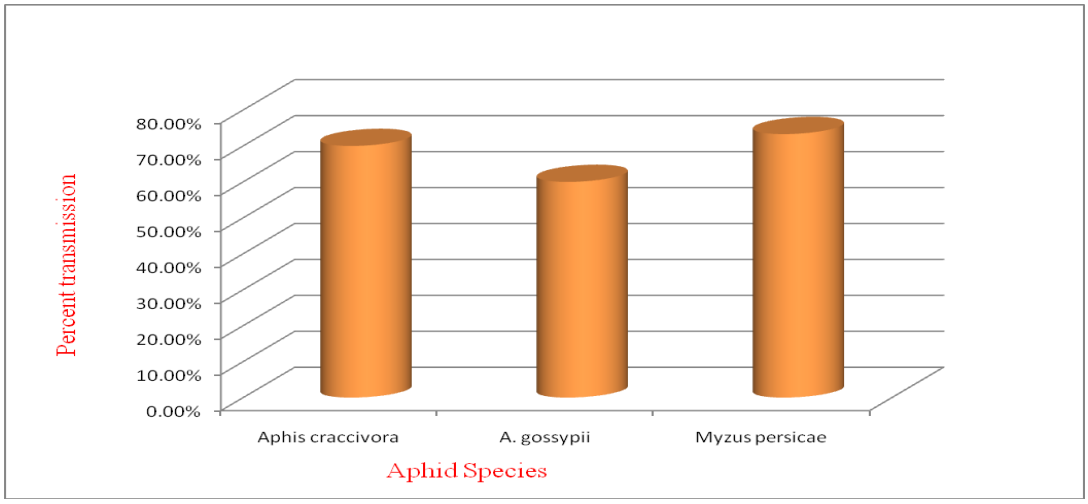
**Fig.1** Cowpea leaves showing vein clearing

**Fig.2** Cowpea leaves showing irregular mosaic

**Fig.3** Cupping of leaf

**Fig.4** Puckering of cowpea leaves along the central vein

**Fig.5** Per cent transmission of aphid transmissible virus infecting cowpea by three aphid species



On the other hand, the seed transmission was found to be at higher level when seed was collected from mechanically inoculated plants, on first trifoliate leaves than from commercial seed. In case of mechanically inoculated plants the seed transmission ranged from 36.3 to 41.9 per cent in three tests conducted independently. The average seed transmission was up to the extent of 37.1 per cent. Similarly, the reduction in seed germination was found to be varying from 17.3 to 8.3 per cent with an average of 12.5%. Results also indicated that, disease incidence was high (37.1%) in seeds collected from mechanically inoculated plants than commercial seed (18.5%).

Similar results on seed transmission were reported earlier by Syed, (1988); Tsuchizaki *et al.,* (1970); Bock and Conti, (1974); Neya *et al.,* (2007); Damiri *et al.,* (2013) and Neya *et al.,* (2013). Neya *et al.,* (2013) also assessed disease incidence percentage in virus free seeds and seeds contaminated with virus and reported that, propagation of cowpea mosaic caused by CABMV was low with varieties having low ability of virus transmission by seed in particular with virus free seeds or seeds slightly contaminated.

Symptoms on experimental field and mechanical transmission were characterized by mosaic, veinal necrosis, vein clearing, vein banding, blistering, stunting, leaf deformations, chlorosis and cupping of leaves. Similar result regarding sap transmission was reported earlier by Gahukar and Kalore (1984), Sekar and Sulochana (1986) and Damiri *et al.*, (2013).

The results on aphid transmission revealed that, cowpea aphid transmissible virus was transmitted by three aphid species in the non-persistent manner from infected Pusa Phalguni cultivar to uninfected Pusa Phalguni cultivar. These aphids included *Aphis craccivora, A. gossypii* and *Myzus persicae.* Of these aphids *Myzus persicae* was found to be the most efficient vector. The *Aphis craccivora* and *A. gossypii* were next in order for their efficiency in transmitting the seed borne virus of cv. Pusa Phalguni. Similar results of aphid transmission was reported earlier by Bock (1973), Bashir *et al.,* (2002), Anitha and Nandihalli (2008), Kitajima *et al.,* (2008), Damiri *et al.,* (2013) and Santoshi *et al.*, (2015).

The results of seed transmission revealed that, the average per cent transmission of aphid transmissible virus infecting cowpea in commercial seed and in seeds collected from mechanically inoculated plants was 18.5% and 37.1%, respectively. Results also indicated that, disease incidence was high (37.1%) in seeds collected from mechanically inoculated plants than commercial seed (18.5%). Similar results on seed transmission were reported earlier by Syed, (1988); Tsuchizaki *et al.,* (1970); Bock and Conti, (1974); Neya *et al.,* (2007); Damiri *et al.,* (2013) and Neya *et al.,* (2013). Neya *et al.,* (2013) also assessed disease incidence percentage in virus free seeds and seeds contaminated with virus and reported that, propagation of cowpea mosaic caused by CABMV was low with varieties having low ability of virus transmission by seed in particular with virus free seeds or seeds slightly contaminated.

Based on symptamatology and transmission studies present virus under study was similar to cowpea aphid borne mosaic virus or black eye cowpea mosaic virus. Some workers regarded BICMV and CABMV are closely related or synonymous with each other (Bock and Conti 1974; Taiwo *et al.,* 1982).

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