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Transmission studies of Cucumber mosaic virus in Cucumber

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ABSTRACT

Cucumber mosaic virus (CMV) is one of the viruses affecting cucumbers (Cucumis sativus). It is transmitted by aphids (Aphis gossypii) in a non-persistent manner. A minimum number of one aphid was required to transmit the virus. However, the Maximum percent transmission of CMV disease was obtained when 30 aphids were released per plant. Twenty minutes of Acquisition Access Feeding period and an Inoculation Access Feeding Period of 24 hrs were required by aphids for higher percent transmission of CMV. The percentage of transmission decreased with an increase in the acquisition access feeding period and inoculation access feeding period of more than 20 minutes and 24 hrs respectively. A pre-acquisition starvation period of 2 hrs ensured the higher percent transmission of CMV.

Keywords: *Cucumber, Cucumber mosaic virus, Aphis gossypii, Pre-acquisition starvation period, AAFP, IAFP, Vector density, seed transmission, TAS-ELISA*

INTRODUCTION

Cucumber is an important exportable vegetable crop grown in tropical and temperate regions of the world. India ranks 27 by producing 215,797.28 tonnes of cucumber in the world [1]. In India, cucumber is cultivated in an area of 109 ha with a production of 1,869.48 tonnes [2]. In India, cucurbitaceous crops contribute about 5.6 % of the total vegetable production [4]. In Tamil Nadu, cucumber is cultivated in an area of 57 ha with a production of 188.49 tonnes [5]. Among Cucurbits, cucumber is the second most cultivated crop after the watermelon, which was prone to mosaic, anthracnose, wilt, root rot, leaf spot, seedling blight, downy and powdery mildew diseases in which Cucumber mosaic virus causes economic yield losses up to 40-60 per cent [6]. This crop is prone to more viral diseases, among which the *Cucumber mosaic virus* (CMV) is one of the devastating diseases affecting the yield. [7] reported that 80 species of aphids belonging to 33 genera were able to transmit CMV in a non-persistent manner. Some of the viruses particularly those belonging to the genera *Potyvirus, Cucumovirus, Tobamovirus, Tymovirus*, and *Nepovirus* were transmitted by seed. [16,17,18]. [3] reported that CMV was transmitted by seed and the rate of transmission was cultivar dependent.

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MATERIALS AND METHODS

To maintain the culture of CMV in a glass house, cucumber seeds of the Vallanadu local variety were sown in small pots containing a mixture of soil, sand and FYM in the ratio of 2:1:1. The typical mosaic symptom collected from the cucumber field was sap inoculated on cucumber plant and maintained in insect proof cage for symptom development.

Maintenance of aphids

Aphis gossypii from the CMV- infected cucumber field were collected and observed under the LEICA SAPO image analyzer for morphological confirmation. The *Aphids gossypii* were transferred to the healthy cucumber plants raised in insect - proof cages using camel hair brush and allowed for multiplication [8]. The pure culture of aphids was made viruliferous by allowing them for an acquisition access feeding period (AAFP) for 20 minutes on CMV infected cucumber plants and an inoculation access feeding period of 16 hrs in the healthy cucumber plants for CMV transmission. Subsequently, the aphids were killed by spraying 0.2% Dimethoate. The inoculated plants were maintained in insect- proof cages for symptom development.

VIRUS VECTOR RELATIONSHIP

Preliminary studies on viral vector relationships were conducted under insect-proof glass house, to standardize the pre-acquisition starvation period, Acquisition Access Feeding Period (AAFP), Inoculation Access Feeding Period (IAFP) and a number of aphids required for maximum percent transmission of CMV.

Effect of the pre-acquisition starvation period on CMV transmission

To determine the effect of different pre-acquisition starvation periods on the rate of transmission of CMV, the adults of *Aphis gossypii* were allowed to starve for different periods viz., 0.5 hr, 1 hr, 2 hr, 4 hr, 6 hr and 8 hr in test tubes placed in dark space. Then the aphids were given an acquisition access feeding period of 20 minutes and transferred to one- week- old healthy cucumber plants. After the inoculation access feeding period of 24 hr, the aphids were killed by spraying 0.2% Dimethoate. For each treatment, ten cucumber plants were inoculated. The plants were observed for symptom expression and the percent transmission was calculated by recording the number of infected plants [9].

Standardization of AAFPs on CMV transmission

To determine the effect of different acquisition access feeding periods on the rate of transmission of CMV, aphids were allowed for different acquisition feeding periods (AAFPs) *viz.,* 1, 5, 10, 15, 20, 30 and 60 minutes. The viruliferous aphids were transferred on one- week- old healthy cucumber plants and they were allowed for an inoculation access feeding period of 24 hr. After that the aphids were killed by spraying 0.2% Dimethoate. For each treatment, ten cucumber plants were inoculated. The plants were observed for symptom expression and the percent transmission was calculated by recording the number of infected plants [10].

Standardization of IAFPs on CMV transmission

To determine the effect of different inoculation access feeding period on transmission of CMV, aphids were given an acquisition access feeding period of 20 minutes. The viruliferous aphids were transferred on a week healthy cucumber plant and allowed different IAFP viz.,1 hr, 3 hr, 5 hr, 7 hr, 9 hr, 12 hr, 16 hr, 24 hr and 30 hr. After that the aphids were killed by spraying 0.2 % Dimethoate. The inoculated plants were kept in insect-proof cages for symptom development. For each treatment, ten cucumber plants were inoculated. The plants were observed for symptom expression and the percent transmission was calculated by recording the number of infected plants [11].

Determination of the number of aphids required for CMV transmission

To determine the number of aphids required for the successful transmission of the CMV, the aphids were given an AAFP of 20 minutes on infected cucumber plants, after which they were transferred to one- week- old healthy cucumber plants at the rate of 1, 3, 7, 10, 16, 19, 24 and 30 aphids per plant separately. After an IAFP of 24 hr the aphids were destroyed by spraying 0.2% Dimethoate. For each treatment, ten cucumber plants were inoculated. The plants were observed for symptom expression, and the percent transmission was calculated by recording the number of infected plants [8].

Seed transmission studies

Cucumber fruit was collected from the CMV-infected cucumber plants maintained under a glass house. From the fruit, seeds were extracted and dried. For transmission studies 25 seeds were randomly taken and sown individually in each pot containing a mixture of soil, sand and FYM in the ratio of 2:1:1. Similarly, seeds were extracted from the fruit of a healthy cucumber plant maintained in an insect-proof cage. From this, 25 seeds were randomly selected and sown individually in pots for healthy control.

The plants observed periodically and symptoms were recorded [3]. Triple antibody Sandwich Enzyme-Linked Immune Sorbent Assay (TAS-ELISA) was done to confirm the presence of CMV in seeds from CMV-infected plants maintained in the seed transmission study.

RESULTS

Effect of the pre-acquisition starvation period on CMV transmission

To determine the effect of pre-acquisition starvation periods of aphids on CMV transmission, the aphids were starved for different periods viz.,0.5 hr,1 hr, 2 hr, 4 hr, 6 hr and 8 hr and were allowed for 20 minutes of AAFP and released on one-week old healthy cucumber plants for 24 hr of IAFP. The results revealed that the pre-acquisition starvation period of 2hr resulted in a maximum transmission efficiency of 91 percent whereas the lowest transmission efficiency of 11 percent was recorded at a pre-acquisition starvation period of 8 hr (Table 1).

Standardization of AAFPs on CMV transmission

To determine the acquisition threshold for the vector, the non-viruliferous aphids were allowed for different AAFPs viz., 1 min, 5 min, 10min, 15 min, 20 min, 30 min and 60 min on diseased plants followed by IAFP of 24 h on one-week old healthy cucumber plants. The results revealed that the aphids required a minimum of one min AAFP to become viruliferous with three per cent transmission. The maximum transmission of 89 per cent was achieved with an acquisition access feeding period of 20 min. The AAFP of 5 min, 10 min, 15 min, 30 min and 60 min resulted in 17,37,67,51 and 33 per cent transmission of CMV respectively. The aphids lose their virulence when the acquisition feeding period increased by more than 20 minutes (Table 2)

Standardization of IAFPs on CMV transmission

To determine the impact of IAFP on CMV transmission, the aphids were allowed for an AAFP of 20 min on diseased plants and then the viruliferous aphids were released on one- week-old healthy cucumber plants for different IAFPs viz.,1hr, 3hr, 5hr, 7hr, 9hr, 12hr, 16hr, 24 hr and 30 hr. The results revealed that the aphids required a minimum of 1hr IAFP to inoculate the virus in the plant with a transmission efficiency of 4 per cent. The maximum transmission efficiency of 98 per cent was recorded with the IAFP of 24 hr. IAFPs of 3 hr, 5 hr, 7 hr, 9 hr, 12 hr, 16 hr and 30 hr resulted in 9, 30, 47, 69, 89, 92, and 37 per cent transmission of CMV respectively. The transmission efficiency of CMV was reduced gradually when the IAFP increased by more than 24 hr (Table 3).

Determination of aphids required for CMV transmission

To find out the optimum number of aphids required for maximum transmission efficiency of CMV, different numbers of inoculative insects were used to transmit CMV on one-week-old cucumber plants. The aphids were allowed for 2hr of starvation period, 20 minutes AAFP and released on one-week-old healthy cucumber plants for 24 hr of IAFP. The results indicated that one number of aphids could transmit the virus to the extent of 6.0 per cent. However, the maximum transmission of 99 per cent was recorded when thirty numbers of Viruliferous aphids were released per plant (Table 4).

Seed Transmission

About twenty-five seeds randomly selected from CMV-infected cucumbers were grown individually in pot under insect proof glass house condition to test the possibility of virus transmission by seeds. Twenty-five seeds were randomly selected from healthy cucumber plants and were also grown individually for healthy control. Among the 25 seeds taken from infected and healthy cucumber plants, 18 and 20 seeds were germinated respectively. Among the 18 plants grown 15 plants were infected with CMV and expressed the mosaic symptom whereas, no symptoms were observed from the plants grown from healthy seeds. This study revealed that CMV is transmitted through seed at 83.33 percent (Table 5).

Detection of CMV in seeds

For this study, seeds were extracted from the CMV-infected and healthy cucumber plants maintained in seed transmission studies and TAS-ELISA was done. The results revealed that seeds from infected plants showed a positive reaction to CMV polyclonal and monoclonal antiserum. The A405 value of infected seeds ranged from 0.416 to 0.562 whereas the A405 value in the range of 0.199 to 0.293 was recorded in healthy seeds (Table 6).

DISCUSSION

Insects were the most prevalent vectors for plant viruses. Among these, 50 per cent of plant viruses were transmitted by aphids [12,13]. [6] reported that among the different species of aphid tested Aphis gossypii showed 100 per cent transmission of *Cucumber mosaic virus* in cucumber. *A. gossypii* consistently had a better transmission efficiency than *M. persicae* [14]. [15] reported that the transmission efficiency of *A. gossypii* was significantly more than *M. persicae* and *A. fabae.* It is essential to determine the preacquisition starvation period, Acquisition Access Feeding Period, Inoculation Access Feeding Period and number of aphids required for CMV transmission. This present study revealed that the maximum transmission efficiency (91 percent) of CMV was recorded in the cucumber plants released with aphids starved for a period of 2 hr. This result is similar to the findings [8] who reported that the transmission efficiency of CMV was more in bottle gourd released with the aphids starved for a period of 2 hr. The maximum transmission efficiency of 89 percent was recorded with the AAFP of 20 minutes. This finding is in line with [8], where they reported that the maximum transmission of CMV on gherkin was achieved with the aphids allowed for an AAFP of 10 min. The IAFP of 24 hr achieved the maximum transmission of 98 percent. Similarly, [11] reported that maximum transmission of CMV on musk melon was achieved with the IAFP of 24 hr. A minimum transmission of 3 percent and 4 percent was recorded with the AAFP of one minute and IAFP of one hour respectively. A maximum of 30 viruliferous aphids were required for maximum transmission of 99 percent of CMV. This result is in accordance with [8]. They reported five numbers of aphids were required to achieve 100 percent transmission of CMV on gherkin. However minimum of one aphid also transmits CMV with a transmission efficiency of 6 percent.

In this present study it is concluded that pre-acquisition starvation period of 2 hr, AAFP of 20 minutes, IAFP of 24 hr and 30 viruliferous aphids were required for maximum transmission of CMV in cucumber. In this study, 25 seeds each from the fruits of CMV- infected cucumber plants and healthy cucumber plants were randomly selected, sown individually in pots and maintained under glass-house conditions. Among the 25 seeds taken from infected and healthy cucumber plants, 18 and 20 seeds were germinated respectively. Among the 18 plants grown from the seeds of infected fruits, 15 were infected with CMV and produced mosaic symptoms. However, no symptom was observed in the plants grown from the healthy seeds. The result of this study revealed that CMV was transmitted through seed at 83.33 percent. Similar findings were also reported by [9] in bottle gourd. He reported that CMV was transmitted through seed at 77.77 per cent.

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CONFLICT OF INTEREST: The authors declare that they have no conflict of interest.

Table 1. Effect of pre-acquisition starvation period on CMV Transmission

S. No.	Pre - acquisition starvation period (hrs)	Percent Transmission*
1.	0.5	46 (42.49)
2.	1	65 (53.95)
3.	2	91 (75.46)
4.	4	49 (44.4)
5.	6	31(33.60)
6.	8	11 (14.97)
SEd		4.3036
CD (P=0.05)		8.6281

*Mean of ten replications
Figures in the parentheses are arc-sine transformed values

Table 2. Influence of Acquisition Access Feeding Period on CMV Transmission

S. No.	Acquisition Access feeding Period (min)	Percent Transmission *
1.	1	3(5.53)
2.	5	17(23.97)
3.	10	37(37.31)
4.	15	67(55.13)
5.	20	89(72.81)
6.	30	51(45.60)
7.	60	33(34.86)
SEd		3.2610
CD (P= 0.05)		6.5379

*Mean of ten replications
Figures in the parentheses are arc-sine transformed values

Table 3. Influence of Inoculation Access Feeding Period on CMV Transmission

S. No.	Inoculation Access Feeding Period (hrs)	Percent Transmission *
1.	1	4(7.37)
2.	3	9(14.53)
3.	5	30(33.02)
4.	7	47(43.24)
5.	9	69(56.39)
6.	12	89(72.81)
7.	16	92(77.31)
8.	24	98(86.31)
9.	30	37(35.42)
SEd		4.2423
CD(P=0.05)		8.4569

*Mean of ten replications
Figures in the parentheses are arc-sine transformed values

Table 4. Influence of vector density of A. gossypii on CMV Transmission

S. No.	No. of aphids/plant	Percent Transmission *
1.	1	6 (11.06)
2.	3	10(15.34)
3.	7	27(30.94)
4.	10	36 (36.56)
5.	16	53(46.78)
6.	19	68(55.79)
7.	24	91(75.46)
8.	30	99(88.15)
SEd		3.7839
CD (P=0.05)		7.5432

*Mean of ten replication
Figures in the parentheses are arc-sine transformed values

Table 6. Detection of CMV in cucumber seeds through TAS-ELISA

Seed No.	Absorbance value at 405 nm	
	Infected	Healthy
1.	0.463	0.203
2.	0.491	0.211
3.	0.571	0.290
4.	0.251	0.291
5.	0.434	0.209
6.	0.562	0.215
7.	0.445	0.199
8.	0.495	0.268
9.	0.544	0.277
10.	0.236	0.255
11.	0.443	0.293
12.	0.482	0.244
13.	0.441	0.257
14.	0.224	0.284
15.	0.489	0.239
16.	0.542	0.246
17.	0.432	0.211
18.	0.416	0.251

Table 5. Transmission of CMV from seeds of cucumber plants

S. No.	Crop	No. of seeds		No. of seeds germinated		No. of plants infected		Transmission percentage	
		Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected
1.	*Cucumis sativus*	25	25	20	18	0	15	0	83.33

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