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Virus- vector relationship of yellow vein disease of calendula and the whitefly

Bushra Afreen, Geetesh Baghel, [1]Akil A. Khan and Q.A. Naqvi.
Plant Virology Lab, Department of Botany, Aligarh Muslim University, Aligarh-202 002 India.
[1]Gandhi Faiz Amm Degree College, Shahjahanpur, India, E-mail:fau2buslry@gmail.com

A B S T R A C T

Virus causing yellow vein disease on *Calendula officinalis* L. was transmitted from naturally infected *C. officinalis* to healthy seedlings of *C. officinalis* through whiteflies not through sap inoculation. A single whitefly could transmit the virus and showed 20.55% infection and fifteen whiteflies were required for 100 percent transmission. The minimum acquisition access feeding period and minimum inoculation access feeding period for the present virus were 10min and 30min, respectively. Acquisition access feeding period and inoculation access feeding period was 100 percent at 6hr and 3 hr respectively. A pre-acquisition access feeding period of 3hr or more gave 100 percent transmission. Post acquisition access feeding period of whiteflies did not have any effect in increasing transmission of CYVV. As post acquisition access feeding period increased, the transmission of CYVV was gradually decreased.

Keywords: Whitefly, Calendula, Yellow vein disease, Transmission

Introduction

Most plant viruses are transmitted by vectors from one host to another. Virus transmission by a vector is often characterized by some degree of specificity. Numerous studies suggest the involvement of a virus-ligand interaction in transmission specificity. Plant viruses can cause severe yield losses to the cereal, vegetable, fruit, and floral industries, and substantially lessen the quality of crop products. *Calendula officinalis* L. belongs to family compositae/Asteraceae. There are 25 species of calendula but only 2 species i.e. *C. officinalis* and *C. arvense* are found in India. It is an important annual ornamental plant grown in gardens during winter season for beautiful flowers. It also has importance for pharmaceutical industries as used in making antiseptic cream for sunburn, lips cracks and skin protection. Like other pathogens, plant viruses also affect the aesthetic value of *Calendula* and other ornamental plants, and have considerable economic importance in floriculture industry due to severe losses, reduction in growth, number and size of flowers caused by them.

There are several viruses reported on Calendula. In nature *Calendula* plants have been found to be infected by various plant viruses. The mixed infection of two viruses: *Cucumber mosaic virus* and *Turnip mosaic virus* was first reported on *Calendula officinalis* by Lisa *et al*. 1979 in Italy. A rosette disease transmitted by whiteflies and grafting (but not by sap and aphid transmission test) was recorded by Gupta & Verma (1983). *Calendula yellow net virus* was reported by Naqvi *et al.* (1985) from India and the virus was identified as strain of *Cucumber mosaic virus*. *Tobacco mosaic virus* from *C. officinalis* was isolated by Hristova *et al.* (1994) in Bulgaria. Among these viral diseases, rosette and yellow vein disease caused by geminiviruses. Khan *et al*. (2005) reported natural infection of begomovirus on *C. officinalis* L. plant showing yellowing of the vein and stunted growth of whole plant and named as yellow vein disease. Several studies have been reported on virus vector relationship with different viruses. (Subramanian, 1979; Salarajan *et al*.1988; Raghupati *et al*.1989; Cohen *et al*. 1983; Jayashree *et al*. 1999;

Rajnimala *et al*. 2005; Rashid *et al*. 2008). The virus vector relationship of yellow vein disease of calendula has not been studied. The present investigation was carried out to study the yellow vein disease of calendula and its relationship with its vector whitefly (*Bemisia tabaci* Genn.).

Materials and Methods

The culture of Calendula yellow vein virus was collected from naturally infected plants of calendula from botany department, Aligarh Muslim University, Aligarh and then tested for confirmation of the virus by the method of southern hybridization. The virus was maintained on *N. tabacum* cv. White Burley in insect proof cages by frequent transfers from diseased to healthy calendula plants through whitefly, *Bemisia tabaci* Genn. The colonies of whiteflies were maintained on cotton plants in insect proof cages and used for transmission studies. A wooden micro-case of 60 cm x 60 cm was made, where the top was made by glass and side covered by 60 mesh nylon net which helped to protect whitefly inside the case. This micro-case was used for whitefly transmission.

Number of whiteflies required for transmission of CYVV

In order to determine the number of whiteflies required for the transmission of CYVV, the whiteflies were given an acquisition access feeding period of 24 hr on diseased plants after which they were transferred onto 25 days old healthy plants at the rate of 1,3,5,7,9,11,15 and 20 insect per seedlings separately. After inoculation access period of 48 hrs they were killed by spraying with 0.1% insecticide (Dimecron). 10 plants were used for each treatment and the experiment was repeated twice. The inoculated plants were maintained for two months in an insect proof glass house and regularly watched for symptom expression and percentage of transmission was recorded.

Acquisition access feeding periods

To determine the minimum acquisition feeding period, the non-viruliferous whiteflies were allowed to feed the diseased calendula plants by giving an acquisition feeding period of 10 min, 15 min, 30 min, 1 hr, 3 hrs, 4 hrs, 6 hrs, 8 hrs and 12 hrs after which they were transferred accordingly to healthy calendula plants for inoculation feeding period of 12 hrs. Ten viruliferous whiteflies per plants were allowed for feeding the test plants for each test and then the plants were sprayed with 0.1% insecticide (Dimecron) for killing the whitefly. The seedlings were then allowed to grow.

Inoculation access feeding periods

To determine the influence of inoculation access feeding periods (IAFP) on transmission of CYVV, insects were allowed for an AAFP of 12 hr and were transferred onto 25 days old healthy plants. They were allowed different IAFP, viz., 30 min, 1hr, 2hr, 3hr, 5hr, 7hr, 9hr, 12hr and 16 hr. Ten whiteflies per plants were allowed for feeding the test plants for each test and then the plants were sprayed with 0.1% insecticide (Dimecron) for killing the whitefly. The seedlings were then allowed to grow.

Pre- and post-acquisition starvation periods

In order to determine the effect of pre-acquisition starvation periods on the rate of transmission of CYVV, the whiteflies were collected and then allowed to starve for different periods, viz., 30 min, 1hr, 2hr, 3hr, 4hr and 6hr. They were then given 12 hrs acquisition and 12 hrs inoculation feeding periods. To determine the effect of post- acquisition starvation periods on the transmission rate of CYVV, the non-viruliferous *Bemisia tabaci* were allowed to

acquire the virus for 12 hrs and then subjected them to different starvation period viz. 0.0 minute, 30 minutes, 1 hr, 2 hrs, 3 hrs and 4 hrs. Thereafter, an inoculation feeding period of 24 hrs was given for these whiteflies to feed on the test plants. Then the plants were sprayed with Dimecron (0.1%) and finally allowed to grow.

Results

Number of whiteflies required for transmission of CYVV

This experiment was conducted for the determination of number of whiteflies required for transmission of calendula yellow vein virus. It was found that even single whitefly could transmit the virus to an extent of 20.55 percent. The transmission was 25.66, 56.82, 62.18, 82.37 and 91.25 percent when numbers of whiteflies per plant were 3, 5, 7, 9 and 11. The rate of transmission was 100 percent when 15 whiteflies were transferred onto healthy plants. It was found that a maximum of 15 whiteflies required for transmission of CYVV.

Acquisition access feeding periods

The results from table 2 indicated that the percentage of infection increased with the increase in acquisition feeding period. The vector of calendula yellow vein virus, *Bemisia tabaci* required a minimum AAFP of 10 min to become viruliferous which resulted in 15.77 percent transmission. The vector required 6hr or more for successful transmission of CYVV which resulted 100% transmission into calendula plants and showed disease symptoms.

Inoculation access feeding periods

The results from inoculation access feeding periods study revealed that at least 30 minutes of inoculation feeding period were required to transmit the virus CYVV and the per cent of transmission was 49.95. With increase in IAFP, there was a gradual increase in the percentage of infected plants. 100.00 per cent transmission was recorded when 3 hrs of inoculation feeding period was given to whitefly.

Pre- and post-acquisition starvation periods

The results from table 4 revealed that a pre-acquisition starvation period of 3hr or more resulted in 100% transmission. Pre-acquisition starvation period required for the vector for successful transmission of the virus was 15 minutes though the percentage of infection was increased with the increase in pre-acquisition starvation period.

It is clear from table 5 that the hundred percentage transmission of CYVV could be obtained when vectors were not starved after acquisition. The study reveal that post-acquisition starvation period of whiteflies did not have any effect in increasing CYVV transmission. As post- acquisition starvation period increased, the transmission of CYVV was gradually decreased.

Table 1.
Numbers of whiteflies required for transmission of CYVV

Number of whiteflies per plant	Transmission (%)	Incubation period (d)
1	20.55	15.02
3	25.66	10.82
5	56.82	11.07
7	62.18	6.79
9	82.37	7.12
11	91.25	7.42
15	100.00	6.32
20	100.00	6.30
CD (P=0.05)	3.25	0.06

Table 2.
Influence of acquisition access feeding period for the transmission of CYVV

Acquisition access feeding period(h)	Transmission (%)	Incubation period (d)
0.16	15.77	12.58
0.25	25.67	14.11
0.50	38.78	13.28
1	60.77	11.40
3	79.66	11.98
4	90.33	10.23
6	100.00	10.01
8	100.00	9.44
12	100.00	9.40
CD (P=0.05)	3.20	0.06

Table 3.
Influence of Inoculation access feeding period for the transmission of CYVV

Inoculation access feeding period(h)	Transmission (%)	Incubation period (d)
0.5	49.95	16.18
1	69.79	14.35
2	80.71	14.49
3	100.00	11.98
5	100.00	11.21
7	100.00	11.00
9	100.00	10.61
12	100.00	10.35
16	100.00	10.21
CD (P=0.05)	2.00	0.07

Table 4.
Influence of Pre-acquisition starvation feeding period for the transmission of CYVV

Pre-acquisition starvation feeding period (h)	Transmission (%)	Incubation period (d)
0.25	12.85	13.45
0.50	29.98	11.35
1	69.68	10.98
2	79.98	9.68
3	100.00	8.95
4	100.00	8.25
6	100.00	8.00
CD (P=0.05)	2.69	0.08

Discussion

In the present investigation a single whitefly could transmit the calendula yellow vein virus. This is similar to the previous findings of Seetharama Reddy & Yaraguntaiah (1981); Monsour & Al-Musa (1992); Jiang *et al*. (2000); Varma (1952); Capoor & Verma (1975); Jayashree *et al*. (1999); Rashid *et al*.(2008).

Table 5.
Influence of Post-acquisition starvation feeding period for the transmission of CYVV

Post-acquisition starvation feeding period (h)	Transmission (%)	Incubation period (d)
0.0	100.00	12.00
0.5	95.17	11.55
1	65.79	11.41
2	55.66	11.00
3	53.31	10.79
4	51.43	10.01
6	50.01	10.00
CD (P=0.05)	3.10	0.08

For 100 percent transmission of calendula yellow vein virus 15 whiteflies were required in our study. This finding corroborates with finding of Subramanian (1979) & Rashid *et al*. (2008). They reported that 15 whiteflies were required to cause 100 percent transmission of yellow mosaic virus in *Lablab niger* and TYLCV in tomato respectively. Salalrajan (1988) and Raghupathi (1989) reported that 15-20 whiteflies were required to cause effective transmission of yellow mosaic virus diseases of urdbean and soyabean, respectively. The minimum acquisition feeding period required for the vector (*B. tabaci*) for successful transmission of CYVV was found to be 30 minutes. This has been supported by Reddy and Yaraguntaiah (1981); Jayashree *et al*. (1999); Jiang *et al*. (2000) and Rashid *et al*. (2008). In

their studies, the minimum period of 30 minutes was required as acquisition feeding period of tomato yellow leaf curl virus. In the present study, AAFP of 6h was required for 100 percent transmission. The findings of the present study are in accordance with the findings of Subramanian (1975) and Jayashree *et al*. (1999). However, Salalrajan (1988) reported that AAFP of 2h was required to transmit yellow mosaic virus to urdbean plants and Rashid *et al*. (2008) found that 8h was required as AAFP for TYLCV in tomato. The results obtained from the present investigation, proved that 30 min was sufficient as inoculation access feeding period for the successful transmission of calendula yellow vein virus. The percentage of infection increases with the increase of inoculation access feeding period. This is similar to the findings of Cohen and Nitzany (1966); Seetharama Reddy &Yaraguntaiah (1981); Jiang et al. (2000); Jayashree *et al*. (1999) Rashid *et al*. (2008). However, Salajrajan (1988) reported that 2h was required as IAFP for the transmission of yellow mosaic virus in urdbean. In the present study, the minimum pre-acquisition starvation period required for the vector for successful transmission of CYVV was 15 min. 3hr or more resulted for 100 percent transmission. The percentage of transmission was increased with the increase in pre- acquisition starvation feeding period. This finding is similar to the findings of Capoor (1949) and Jayashree *et al*. (1999).

In case of post acquisition starvation feeding period, the hundred percent transmission of CYVV could be resulted when vectors were not starved after acquisition. The vectors (*B. tabaci*) did not have any effect in increasing CYVV after post acquisition starvation feeding period. Capoor and Ahmad (1975) reported that 2h starvation of vectors before acquisition gave maximum infection while starvation after acquisition led to reduction in transmission efficiency in PWMV inoculated pumpkin plants. While Varma, (1952) reported that 4h post acquisition starvation period was effective in case of OYVMV.

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