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## **Original Research Article**

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Transmission studies of Leaf Crinkle Virus in Blackgram (Vigna mungo L.)

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## ABSTRACT

# Keywords

Leaf crinkle,
Blackgram, Aphis
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Blackgram (Vigna mungo) is a major pulse crop grown in Tamil Nadu and leaf crinkle virus is an important disease that infects the crops at various stages of its growth, which reduce the yield drastically. There are conflicting reports on the transmission of ULCV by insects that includes aphids, leafhopper, whitefly and beetle. Hence, a study was carried out to identify the putative vectors involved in the transmission of the disease. Found that the virus was effectively transmitted by aphid vector, Aphis craccivora in a non-persistent manner and whitefly (Bemisia tabaci). No transmission was observed by melon aphid (Aphis gossypii), cabbage aphid (Brevicoryne brassicae) and hadda beetles (Epilachna vignitioctopuntata and Epilacna dodecastigma). The per cent transmission by aphids was high compared to whiteflies to extent of 83.3 and 66.6 per cent respectively. Virus-vector relationship revealed that minimum of 5 adults of A. craccivora with an acquisition access period of 1 minute and inoculation access of 5 minutes could transmit the ULCV. However, maximum transmission (83.3%) was obtained by 10 aphids/plant with an acquisition access period of 5 to 10 minutes, inoculation feeding of 10 minutes. Minimum of five whiteflies were required to transmit the ULCV. However, sixty six per cent transmission of ULCV disease was obtained when 10 whiteflies were released per healthy plant. Acquisition access period of 36 to 48 h and inoculation period of 24h results in increased in transmission up to 66 per cent. Serial transmission up to 56 h results upto 50 per cent transmission. Beyond 72 hours no transmission of disease was observed.

### Introduction

Urdbean Leaf Crinkle Virus (ULCV) an important disease found in all the urdbean growing regions of the world and causes severe yield loss (Beniwal and Chaubey, 1979). The disease infected plants showed stunted growth, crinkling and puckering of leaves (Williams *et al.*, 1968; Nene, 1968 Bindra (1971). The disease is transmitted through mechanical inoculation (Nene, 1972;

Kadian, 1980 and Karthikeyan, 2002), seed (Ahmad *et al.*, 1997) and insect vectors (Nene, 1972; Kadian, 1980). Among the insect vector aphids (Dhingra, 1975; Nath *et al.*, 1986; Bharadwaj and Dubey, 1984 and 1986), leafhoppers (Khatri *et al.*, 1971), beetles (Beniwal and Bharathan, 1980 and Bharathan and Beniwal, 1984) and whiteflies (Narayanasamy and Jaganathan, 1973; Prasad *et al.*, 1998 and Sahay *et al.*, 1999). However, it is common that one virus known to be

transmitted by one group of insects but rather uncommon to be transmitted by several other groups of insects. Nevertheless, there are conflicting reports in literature on the transmission of ULCV by insects. The main objective of the study therefore is to identify the putative vectors involve in the transmission of the disease.

### **Materials and Methods**

## **Insect rearing**

Field collected whiteflies from mungbean host were used for transmission study after rearing on brinjal for 3 generations under controlled condition (Polston and Capobianco, 2013).A. gossipii and A. craccivora were collected from cowpea field and single matured aphid was caged on the cowpea plant and the young ones immediately after laying were transferred to healthy host daily. The hadda beetles, E. vigintioctopuntata and E. dodecastigma were tested for transmission of ULCV in blackgram. Attempts to culture the beetles on blackgram plants were not successful; therefore, fresh grubs and beetles were collected and maintained on brinjal seedlings before using for the transmission studies.

### Virus maintenance

The blackgram leaf crinkle disease was isolated through sap transmission from an early field infected blackgram cv. Pant U-19 and was maintained through seed transmission in cv. CO 6 in insect proof conditions as suggested by (Bharadwaj *et al.*, 1982).

## **Transmission efficiency**

The apterous adults were given pre-virus acquisition fasting of one hour, acquisition access of 10 min and inoculation access of 24 h after 20 min of post-acquisition fasting. Acquisition access for a definite period was given by liberating starved aphids on the

underside of a freshly detached diseased urdbean leaf, kept reversed in a petri plate over a moist blotting paper. The aphids seen probing by a hand lens, were lifted by sterilized camel hair brush and fasted before transferring 5 aphids/test plant of 10 day old seedling grown in an insect free net-house. After test feeding the insects were killed with 0.1 % malathion. Unless mentioned, 10 plants were inoculated with each treatment. The inoculated plants were kept under insect free nylon mesh cage. In all inoculation trails only fully grown apterous aphids and young healthy plants of CO 6 at 3-4 leaf stage were used as a test plant.

# Determination of optimum number of viruliferous aphids required for transmission

A large number of aphids were starved for 60 minutes and then divided in 10 groups consisting of 1, 2, 5, 10, 15, 20, of each group were given acquisition feeding for 10 minutes on blackgram leaves severely infected with ULCV. Viruliferous aphids from each group were transferred to healthy blackgram plants separated for test feeding.

# Determination of the acquisition threshold of *A.craccivora*

In order to determine the time required by the aphid vectors to acquire the virus from infected plants, large number of aphid colonies were collected and given a pre-acquisition starvation of 60 minutes since this period was found to be the efficient in the transmission. Each batch of 10 aphids were given an acquisition feeding period of 1, 2, 5, 10, 15, 30, 60, 120 minutes respectively on virus diseased plant before transferring them to test plants for inoculation feeding. Then aphids were transferred to healthy plants under insect free condition and allowed to feed for 10 minutes.

# Determination of the inoculation threshold of *A.craccivora*

In order to determine the time required by the viruliferous aphids to transmit the virus to healthy test plants, a large number of virus-free colonies of aphids were starved for 60 minutes.

Aphids were distributed in batches consisting of 10 aphids each and then, transferred to test plants for inoculation feeding for, 1, 5, 10, 15, 30 minutes and 1, 2, 4, 6, 12, 24 hour respectively, an inoculation access period of 10 minutes were given for the transmission of the virus.

# Serial transmission Persistence/nonpersistence of ULCV in the insect vectors in successive transfers

The experiments were conducted to determine how long the viruliferous aphids would remain infective in successive transfers to healthy plants without access to a fresh infection source. For this purpose aphids were given pre-acquisition fasting and acquisition feeding as mentioned earlier. Then the individual aphids were transferred in succession to a series of five healthy test plants. Different feeding intervals were given to different series such as 2, 5, 10, 15, 30 minutes and 1, 2 and 4 hours respectively.

# **Determination of optimum number of whiteflies required for transmission**

Whiteflies were collected from rearing cages using aspirator and were transferred to the clip cages at rate of 1, 3, 5, 10, 15, 20 numbers. Non-viruliferous whiteflies were allowed to acquire the virus from infected plant with an acquisition access period (AAP) of 24 hours. Then viruliferous whiteflies were allowed to feed on seedlings of 2 leaf stage. The experiment was replicated three times.

# Determination of the acquisition threshold and inoculation threshold of *B.tabaci*

Ten one day old whiteflies were collected from whitefly rearing cage using aspirator and transferred to clip cages. Similarly healthy whiteflies were separately allowed for different acquisition and inoculation feeding period's viz., 1, 4, 8, 12, 24, 36 and 48 h (inoculation feeding period of 48 hours) and 1, 4, 8, 12, 24, 36 and 48 h (Inoculation access period of 24 h) on ULCV infected blackgram plants. The viruliferous whiteflies were released on healthy blackgram seedlings of 2 leaf stage at the rate of 10 to 15 whiteflies per plant.

## Serial transmission persistence/nonpersistence of ULCV

The experiments were conducted to determine how long the viruliferous whiteflies would remain infective in successive transfers to healthy plants without access to a fresh infection source. For this purpose whiteflies given pre-acquisition fasting and acquisition feeding as mentioned earlier. The individual whiteflies were then transferred in succession to a series of five healthy test plants. Different feeding intervals were given to different series such as 6, 12, 24, 48, 56 and 68 hours respectively. The vectors were later killed by spraying 0.1 per cent malathion. The plants were kept under observation for 45 days. The percentage of infection is calculated by the formula

Per cent infection= (Number of plants infected/ Total number of plants observed) × 100

### **Results and Discussion**

The infectivity of ULCV was confirmed by using the vectors aphids, whiteflies, beetles and leafhoppers. The results revealed that both

aphid (A.craccivora) and whitefly (B.tabaci), efficiently transmitted the ULCV infected plants to healthy plants to an extent of 83.3 and 66.6 per cent respectively. The other vectors tested viz., A. gossipii, beetles (E. vigintioctopuntata and E. dodecastigma) and leafhopper (E. kerri) failed to transmit the virus in the preliminary studies. Preliminary studies carried out with A. craccivora and B. tabaci gave positive results in transmission of the disease. Typical symptoms of the disease were observed between 35 to 45 days after inoculation. Hence, a detail study of virusvector relationship was taken up and the results presented in the tables from 1 to 8. As the number of aphids increased from one to five, five to ten, and ten to fifteen, the success in transmission has also increased from 0 to 33.3, 33.3 to 66.6 and 66.6 to 83.3 per cent respectively. Nevertheless, when the number of aphids was further increased to 20 and 25, the per cent transmission remained the same. The results pertaining determination of the acquisition threshold of A. craccivora (Table 1). Significant difference in the transmission was observed with respect to different acquisition access periods used in the experiment. Maximum transmission (83.3 per cent) was recorded, when 5 and 10 minutes acquisition access was given. As acquisition access increased to 15, 30, 60 and 120 minutes. success in transmission decreased to 66.6, 50, and 33.3 per cent respectively (Table 2). Significant difference in the transmission of the disease was observed with respect the different inoculation Maximum access period. transmission (83.3%) was recorded when 10 minutes of inoculation feeding was given. transmission was observed when 120, 240 and 360 minutes, inoculation access were given and as the inoculation access was increased to 50 and 83.3, per cent respectively, when the inoculation access was increased to 5 to 10 minutes. The per cent transmission decreased from 66.6 to 50 and 50 to 16.6, when the

inoculation feeding increased from 15 to 30 and 30 to 60 minutes (Table 3). Disease symptoms were observed between 35 and 45 days after inoculation irrespective of the inoculation access periods. When viruliferous aphids were transferred different intervals 2, 10, 30, 60, 180, 360, 720 minutes, only 2, 10 and 30 minutes showed higher transmission rates. Further increase in feeding of 60 to 180 minutes, decrease in transmission of 66.6 to 16.6 per cent (Table 4). The results indicated that a single whitefly couldn't transmit the virus, however at least 5 whiteflies are required for 33.3 per cent greater transmission and transmission observed with 10 to 20 number of whiteflies to the tune of 66.6 per cent. When the number of whiteflies was further increased to 20 and 25, the per cent transmission remained the same (Table 5). There existed a positive correlation between the number of whiteflies and ULCV transmission. Whiteflies required a minimum AAP (Acquisition Access Period) of 12h and maximum of 36h to become viruliferous, which resulted in 66.6 per cent transmission. Acquisition feeding period of 72h or more resulted in decreased per cent transmission to 33.3 (Table 6). With increase in AAP, the percentage of insects becoming viruliferous increased, as observed from the higher percentage of infected plants, and the days required for symptom expression became less. The results from inoculation threshold study revealed that an IFP (Inoculation Feeding Period) of 8h by the viruliferous vectors caused 33.3 per cent transmission (Table 7). With increase in IFP, there was a gradual increase in the percentage of infected plants up to 66.6 per cent. The present study revealed that as acquisition period increased, the per cent transmission remains same. Beyond the 56h decrease in transmission rates was observed. Further increase in feeding of 72 to 144, decrease in transmission was up to 33.3 when whiteflies were transferred after 72 h.

Table.1 Optimum number of Aphis craccivora required for transmission of ULCV

Number of aphids per plant	Number of plants tested	Number of plants infected	Per cent transmission*
1	6	0	0(11.59)
2	6	0	0(11.60)
5	6	2	33.3(36.36)
10	6	4	66.6(47.71)
15	6	5	83.3(54.81)
20	6	4	66.6(47.75)
25	6	4	66.6(47.75)
			SEd = 2.1085
			CD(.05) = 4.5228

Table2 Acquisition access period of Aphis craccivora required for transmission of ULCV

Acquisition feeding (min)	Number of plants tested	Number of plants infected	Per cent transmission*
1	6	2	33.3(35.20)
2	6	2	33.3(33.55)
5	6	5	83.3(68.54)
10	6	5	83.3(66.10)
15	6	4	66.6(54.79)
30	6	3	50(45.00)
60	6	2	33.33(35.19)
120	6	0	0(0.28)
240	6	0	0(0.28)
60	6	0	0(0.28)
			SEd = 4.4686
			CD(.05) = 9.3213

Table.3 Inoculation feeding period of Aphis craccivora required for transmission of ULCV

<b>Inoculation feeding (min)</b>	Number of plants tested	Number of plants infected	Per cent transmission*
1	6	0	0(4.04)
5	6	3	50(44.99)
10	6	5	83.3(68.50)
15	6	4	66.6(54.72)
30	6	3	50(45.00)
60	6	1	16.6(24.02)
2	6	0	0(4.02)
4	6	0	0(4.03)
6	6	0	0(4.04)
12	6	0	0(4.04)
24	6	0	0(4.04)
			SEd = 3.8278 CD(.05)= 7.9384

Table.4 Serial transmission for Aphis craccivora required for transmission of ULCV

Serial transmission (min)	Number of plants tested	Number of plants infected	Per cent transmission*
2	6	3	50(44.99)
10	6	4	66.6(54.77)
30	6	4	66.6(54.97)
60	6	2	16.6(24.05)
180	6	0	0(4.05)
360	6	0	0(4.05)
720	6	0	0(4.02)
			SEd = 3.0778
			CD(.05) = 6.6020

Table.5 Optimum number of Bemisia tabaci required for transmission of ULCV

Number of whiteflies per plant	Number of plants tested	Number of plants infected	Per cent transmission*
1	3	0	0(4.04)
3	3	0	0(4.04)
5	3	1	33.3(35.26)
10	3	2	66.6(54.72)
15	3	1	33.3(35.31)
20	3	2	66.6(54.78)
30	3	2	66.6(54.96)
			SEd = 2.9247 CD(.05) = 6.2735

Table.6 Acquisition access period of Bemisia tabaci required for transmission of ULCV

Acquisition feeding (hrs)	Number of plants tested	Number of plants infected	Percent transmission*
1	3	0	0(4.04)
4	3	0	0(4.04)
8	3	0	0(4.04)
12	3	1	33.3(35.23)
24	3	1	33.3(35.11)
36	3	2	66.6(54.78)
48	3	2	66.6(54.96)
72	3	1	33.3(35.18)
			SEd = 2.8544
			CD(.05) = 6.0512

**Table.7** Inoculation feeding period of *Bemisia tabaci* required for transmission of ULCV

Inoculation feeding (hrs)	Number of plants tested	Number of plants infected	Percent transmission*
1	3	0	0(4.04)
4	3	0	0(4.04)
8	3	1	33.3(35.18)
12	3	1	33.3(35.23)
24	3	2	66.6(54.79)
36	3	1	33.3(35.22)
48	3	2	66.6(54.86)
72	3	2	66.6(64.96)
			SEd = 3.5825
			CD(.05) = 7.5947

Table.8 Serial transmission of Bemisia tabaci required for transmission of ULCV

Serial transmission (hrs)	Number of plants tested	Number of plants infected	Percent transmission*
6	3	1	50(44.9)
12	3	2	66.6(54.77)
24	3	2	66.6(54.97)
48	3	2	50(45)
56	3	2	50(45)
68	3	1	33.3(25.22)
72	3	0	0(4.02)
144	3	0	0(4.02)
			SEd = 3.1096
			CD(.05) = 6.5922

ULCV is transmitted by insect vectors viz., whitefly (B. tabaci), aphids (A. craccivora) hadda not through beetle vigintioctopuntata and H. dodecastigma), melon aphid (A. gossypii) and leafhopper (E. failed to transmit kerri) the virus. Narayanaswamy and Jaganathan (1973)reported the transmission of ULCV by whitefly, B. tabaci (Table 8). The casual virus of leaf crinkle disease under study was easily transmitted by A. craccivora in a nonpersistent manner. The vector could acquire the virus, when given an acquisition access of 5 minutes and transmitted the virus to healthy plants in inoculation feeding 10 minutes.

Increase in number of viruliferous vector increased the efficiency of transmission. Similar results were obtained for ULCV which is vectored by *A. craccivora* was demonstrated by several workers (Bindra, 1971; Dhingra and Chenulu, 1981; Dubeyet al., 1983; Nath et al., 1986; Vijay Kumar, 1993 and Suneela, 1996). In the present study increased transmission with increase in number of aphids per plant was observed. Maximum transmission (83.3%) was recorded when 15 aphids/plant were used although a five viruliferous aphids could transmit the disease to an extent of 33.3 per cent. Dhingra and Chenulu (1981); Vijay Kumar (1993) and

Suneela (1996) also reported similar observations with ULCV on blackgram with Serial transmission A.craccivora. viruliferous aphids showed increase in per cent transmission up to 30 minutes (66.6 per cent), beyond 60 minutes no transmission was observed. The fall in per cent transmission of the virus may be due to the formation of salivary sheath which will be wipe-off the virus from the stylet during its reensheathment or moulting. With the increase in acquisition access period beyond ten minutes, there was decrease in 83.3 to 66.6 per cent transmission by the vector. The fall in per cent transmission of the virus due to prolonged acquisition access periods beyond 10 minutes by the vector, A.craccivora may be due to the formation of salivary sheath which will be wipe-off the virus from the stylet during its re-ensheathment. This was supported by the findings of Bhardwaj and Dubey (1986) in case of non-persistently transmitted by viruses by aphid vectors. In the present study ULCV could not be transmitted another aphid species, A.gossypii and Brevicoryne brassicae. Present isolate of the virus could not be transmitted by beetle (E.vignioctopuntata and E.dodecastigma. In contrast transmission of ULCV by Epilachna beetle was reported by Beniwal and Barathan (1980) and Barathan and Beniwal (1984). When the transmission efficacies of different vectored are compared, ten or more whiteflies successfully transmitted. A similar feeding reported by Narayanaswamy was Jaganathan They successfully (1973).transmitted the ULCV. A similar finding was reported by using 15 to 20 whiteflies per plant. Generally viruses transmitted by sap are not transmitted by whiteflies but ULCV seems to an exception. The possible reason might be the involvement of more than one strain of ULCV in producing leaf crinkle disease on blackgram. In the present study, ten or more aphids are required for the successful transmission of ULCV but Dhingra

and Chenulu (1981) could attain the maximum transmission of ULCV by using ten aphids per plants. Barathan and Beniwal (1984) reported that a single adult beetle could transmit the virus though groups of five or more were required for 100 per cent transmission. In the present findings whiteflies and aphids were found to transmit the virus, maximum percentage was affected only through aphids and whiteflies. Epilachna beetles couldn't transmit the disease, may be due to failure of feeding to regurgitate the virus. Hence, aphids (A.craccivora) and whiteflies (B. tabaci) seems to the potential vector under field conditions. Similar results were reported in Tamil Nadu, ULCV was transmitted by whitefly, B.tabacibut the reports are in contrast in persistency of non-persistent transmission, a transmission was repoted by Narayanasamy Jaganathan (1973); from Meghalaya (Prasad et al., 1998 and Sahay et al., 1999). Bharathan and Beniwal (1984) reported positive transmission of ULCV with beetle vector, E.dodecastigma. In the present study, the virus causing leaf crinkle disease was successfully transmitted by Aphis craccivora in a non-persistent, manner which confirms the findings Vijay Kumar (1993). The results are in agreement with those of Dhingra and Chenulu (1981) who reported that a short acquisition access period of 30 seconds to 2 necessary for successful minutes was transmission of the virus. Vijay Kumar (1993) reported that 10 aphids with an acquisition access period of 2 minutes. A short inoculation feeding period of 10 to minutes are efficient in the present study which in contrast with the inoculation access periods of 24 hours could bring maximum success in transmission of the virus reported by Vijay Kumar (1993) and Vijay Kumar and SubbaRao (1994). The present findings with respect to vector transmission confirms the work of Dhingra and Chenulu (1981); Dubey et al., (1983) and Bhardwai and Dubey (1984

and 1986); Vijay Kumar (1993); Vijay Kumar and SubbaRao (1994) and Suneela (1996).

These studies indicated the vector specificity in transmission of various isolates of the virus causing ULCV from different parts of the country. Further work on the work on the transmission of leaf crinkle virus needs to be carried to know the virus isolate vector specificity pertaining to the leaf crinkle isolated reported from different parts of the country.

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