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Aphis craccivora (Koch): A Potent Transmitter of Urdbean Leaf Crinkle Virus in Blackgram [*Vigna mungo* (L.) Hepper]

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ABSTRACT: Urdbean Leaf Crinkle Virus (ULCV-unclassified virus), a virus with uncharacterized etiology has turned out to be a threat to blackgram (*Vigna mungo* L.). There are conflicting reports on the transmission of ULCV through pulse aphid (*Aphis craccivora*). This study evinced that *A. craccivora* is a potent insect vector in transmitting ULCV in blackgram. The optimum number of aphids capable of transmitting Urdbean Leaf Crinkle Disease (ULCD) was 15 or more leading to 77.78 to 88.89 per cent of disease transmission. The disease transmission was 88.89 per cent when given the acquisition feeding time of 10 minutes and 55.56 per cent when the inoculation feeding period offered was 10 minutes, however, after 15 minutes, there was a declining phase in transmission. Pre-acquisition fasting of 60 minutes and post-acquisition period of 20 minutes resulted in 77.78 percent disease transmission. The data on the transmission of ULCV and its inflicted symptoms on host plants can be utilized effectively to manage ULCD before resulting to yield losses.

Keywords: Blackgram, Leaf crinkle virus, Vector Transmission, Pulse Aphid.

INTRODUCTION

Blackgram (*Vigna mungo* Linn. Hepper), the highly prized pulse crop, valued for its easily digestible protein in seeds (Tagger *et al.* 2012), is a native to India. An important short-duration Leguminosae crop grown in 23 countries, is cultivated in an area of 36.44 lakh ha with a production of 19.64 lakh tonnes. In India, a greater emphasis is given to enhance pulse production to cater to the protein needs of the vast vegetarian population. One such crop that received yield improvement attention is blackgram, however, whose yield is at stake due to innumerable biotic stresses perceived and within which the Urdbean leaf crinkle viral disease (ULCD) tops the list with a reported yield loss of 70 to 100 depending on the genotype, environmental conditions (Ashfaq *et al.*, 2008) and plant age (Beniwal and Chaubey 1979). The ULCV has been a global threat in blackgram cultivable regions (Beniwal and Chaubey 1979) especially, in the tropics (Sravika *et al.*, 2018). The ULCV inflicts loss by lamina rugosity, curled, puckered, and crinkled leaves, stunted internodal growth, and bud and pod malformation (Nene, 1968; Kolte and Nene 1972; Reddy *et al.*, 2005; Brar and Rataul 1987) and deter pollen fertility. In addition, the crop is subjected to several infestations by insects during each successive stage of crop growth (Tagger *et al.*, 2012). The black legume or cowpea aphid, *A. craccivora* is one of the important pests of blackgram and is normally seen in myriads covering leaves, inflorescence stalk, and young pods with honeydew secretion and black ant movements. Cowpea aphid infestation between the 10-leaf stage and tasseling caused 28.14 per cent yield losses (average aphid density 818 aphids/plant); while infestation through ripening stages caused 16.28 per cent yield losses (average aphid density 1038 aphids/plant) (Jain *et al.*, 2013).

Potential means of Transmission of ULCV are through mechanical sap transmission (Biswas *et al.*, 2012), also as seed-borne (Kanimozhi *et al.*, 2009) and by insect vectors such as aphids (Sravika *et al.*, 2018), whitefly (Narayanasamy and Jaganathan 1973), beetles (Beniwal and Bharathan 1980). ULCV in aphids appeared to be non-persistent according to Bhardwaj and Dubey (1986). Sravika *et al.* (2018) reported 83.3 per cent

transmission of ULCD in blackgram by *A. craccivora.* It is intriguing to note that literature has revealed conflicting reports on the transmission of ULCV by insects and especially by the cowpea aphid (*A. craccivora*). Thus, the current study has aimed to evaluate the transmission efficacy of ULCV by *A. craccivora* in blackgram.

MATERIALS AND METHODS

Unless otherwise specified the study was conducted in the Insectary of Department of Agricultural Entomology, Agricultural College and Research Institute, Tamil Nadu Agricultural University (TNAU), Madurai, India located at 9°58′31″N and 78°12′28″ E during the month of May 2019.

Insect rearing. Pulse aphids (*A. craccivora*) were field collected from healthy cowpea and blackgram crops from Theni and Madurai districts of Tamil Nadu, India. The stalks blooming with apterous adults of aphids were excised from the plant and carefully transferred to the research arena in small plastic boxes provided with ventilation to avoid desiccation of the sample. Each apterous matriarchal aphid was isolated using a camel brush and reared in separate insect-proof cages (wooden frames with dimensions of 150 cm x 150 cm x 75 cm designed with nylon mesh of 100-micron mesh size covered in three sides, a wooden platform, and a glass top and door) containing a healthy potted local variety of cowpea (*Vigna unguiculata*) plant aged 7 days after sowing (DAS) (Jaba *et al.*, 2010) and the emerged young ones were weekly transferred to a healthy host (Imam, 2015) in insect-proof cages.

Virus maintenance. The blackgram plants possessing the ULCD symptoms were tagged in the field at National Pulses Research Centre (NPRC), TNAU, Vamban, Tamil Nadu, and used for the collection of ULCV-infected fresh trifoliate leaves. Field collected fresh ULCV diseased leaves of blackgram were preserved in the deep freezer under -20°C until processing. The leaves weighing 1 g were then blended in a pestle and mortar using 5 ml of 0.05 M potassium phosphate buffer in cold conditions. The desired buffer was prepared by adding 8.9 ml of Solution A and 61.1 ml of Solution B. [Preparation of Solution A – 0.05 M 2.335 g of dipotassium hydrogen phosphate in 500 ml of distilled water. Preparation of Solution B- 1.575 g of potassium dihydrogen orthophosphate in 500 ml of distilled water]. In the process of blending, 0.1 per cent of mercaptoethanol was added to the buffer. The extracted sap was rubbed on healthy potted plants of 7 days old blackgram (variety T 9; susceptible check purchased from local market) (Sharma *et al.*, 2014) with a pinch of carborundum to cause abrasion on leaf lamina. After 5 to 10 minutes, the treated leaves were washed with distilled water using a hand sprayer. The symptoms appeared in the successive trifoliate leaves after inoculation in 15 to 20 days. These plants were maintained as virus inoculum for further studies in the insect-proof cages.

Determination of Transmission efficiency of *A. craccivora.* For assessing the ULCD transmission through *A. craccivora,* healthy T9 blackgram plants were grown and maintained in screen houses. The virus-free healthy seeds were sown in earthen pots (20 cm diameter) soiled with coco pith, vermiculite, properly fertilized, and watered regularly. Around three to five seeds were sown in each pot and after 14 days inferior seedlings were thinned leaving a healthy seedling behind per pot. Each grown plant was secured with an insect-proof nylon mesh cover of 100-micron mesh size to maintain an insect and virus-free plant culture.

In the forthcoming delineated controlled laboratory experiments, unless mentioned, the apterous matured aphids were given the acquisition access period of 10 minutes by exposing the aphids to the abaxial surface of the ULCV diseased leaf placed over a moist blotting paper on a Petri plate, after subjecting aphids to 60 minutes pre starvation. The aphids which were seen probing were used for the trials in 2-leaf staged blackgram T9 (susceptible variety to ULCD) plants. Six plants were tested for each replication. The experiment was replicated thrice. After each trial, the aphids were killed using insecticide and the tested plants were covered with insect-proof nylon mesh (100-micron mesh size). The results were visualised after 15 days as the plants expressed crinkling symptoms thenceforth. Control for each experiment was maintained with non-viruliferous aphids. The percentage of infection was calculated by the formula (Sravika *et al.*, 2018):

$$Per\ cent\ infection = \frac{No.of\ plants\ infected}{Total\ no.of\ plants\ observed} \times 100$$

To determine the optimum number of viruliferous aphids required for transmission. Pre-starved animate aphids were given an acquisition feeding for 10 minutes on ULCD infected leaves placed in Petri dishes on moist blotting paper. The aphids seen probing were considered viruliferous aphids. The test insects were then transferred to healthy blackgram plants varying the number of aphids as 1, 2, 5, 10, 20, and 25 for each trial (Sravika *et al.,* 2018). After providing the inoculation period of 10 minutes, the test insects were killed using insecticide. The treatments were replicated thrice. For each replication, six test plants of T9 blackgram were used. Non-viruliferous aphids were used in the mock test (control). The results were visualised after 15 days as the plants expressed crinkling symptoms.

To determine the required pre- acquisition fasting of viruliferous aphids for transmission. Non-viruliferous aphids were subjected to varied pre-acquisition fasting of 0, 10, 15, 30, 60, 90, 120, and 240 minutes and divided into batches. Each batch consisted of 10 aphids. The latter were given 10 minutes of acquisition feeding period and transferred to the healthy

test plants. The test insects were then subjected to 10 minute inoculation period (Sravika *et al.,* 2018). Then the test insects were killed after the feeding on the host plants. The treatments were replicated thrice. For each replication six plants of T9 blackgram were used. Control trials were conducted as mentioned above. The results were visualised after 15 days as the plants expressed crinkling symptoms.

To determine the acquisition threshold of *A. craccivora.* Pre-starved animate aphids were given an acquisition feeding for 1, 2, 5, 10, 15, 30, 60, 120, and 240 minutes on ULCD infected leaves. Then each batch of 10 numbers of viruliferous aphids was given the acquisition feeding period as mentioned above and transferred to healthy blackgram plants for each trial and given the inoculation period of 10 minutes (Sravika *et al.,* 2018). The test insects in every trial plant were killed using the insecticide. The experiments were replicated thrice. For each replication six plants of T9 blackgrams were used. Control trials were conducted as mentioned above. The test insects in every trial plant were killed using the insecticide. The results were visualised after 15 days as the plants express crinkling symptoms.

To determine the post-acquisition fasting of viruliferous aphids required for transmission. Aphids given the pre-starvation of 60 minutes and acquisition feeding for 10 minutes were divided into groups consisting of 10 matured aphids. Each batch was subjected to varied post- acquisition fasting of 0, 10, 20, 40, 80, 120, 160, and 240 minutes and then transferred to healthy test plants. They were provided with an inoculation period of 10 minutes in the test plants (Sravika *et al.,* 2018). Each treatment was replicated thrice (Six test plants were used in each replication). Control trials were conducted as mentioned above. Then the test insects were killed using insecticide after feeding in the host plants. The results were visualised after 15 days as the plants express crinkling symptoms.

To determine the inoculation threshold of *A. craccivora*

Each batch consisting 10 pre-starved animate viruliferous aphids was given an inoculation feeding for 1, 5, 10, 15, 30, 60, 120, and 240 minutes on ULCD infected leaves (Sravika *et al.,* 2018) and an inoculation access period of 10 minutes on healthy test plants (T9 blackgram). Then, the viruliferous aphids used in treatments were killed and the control was maintained as mentioned above. The experiments were replicated thrice (six test plants for each replication). The results were observed after 15 days of inoculation as the symptom occurred thenceforth.

Statistical Data Analysis. All the experiments were conducted under Completely Randomized Block Design (CRBD). Data were statistically analyzed using SPSS for Windows (version 22) (IBM Corp 2013) software to carry out ANOVA. Grouping of data was done using Tukey's HSD (Honestly Significant Difference) test (Tukey, 1977).

RESULT

The test insect, *A. craccivora* has been proven to transmit ULCD in blackgram efficiently in a non-persistent manner with considerable variation in the optimum number of viruliferous aphids needed to transmit the disease/plant, pre-starvation period, inoculation feeding period, and acquisition feeding period. The confirmation of the transmission of ULCD through *A. craccivora* was determined from the observation of crinkling symptoms in the successive trifoliate leaves of test plants after the inoculation of virus isolates by probing of viruliferous *A. craccivora* adults.

In regards to the optimum number of aphids efficient in transmitting the ULCV disease, it was proven that about five viruliferous aphids were capable of transmitting the disease up to 33.33 per cent (Table 1). The transmission of disease increased with the increase in the number of viruliferous aphids. There was a significant increase in disease transmission up to 88.89 per cent for 25 viruliferous aphids (Table 1).

Disease transmission of 33.33 per cent was witnessed from one minute of the acquisition feeding period. The data revealed that the maximum disease transmission of 88.89 per cent was observed when the acquisition feeding time was 10 minutes, which eventually dwindled to 11.11 per cent, and zero per cent transmission when given the acquisition feeding period of 120 and 240 minutes, respectively (Table 2).

The inoculation feeding of five minutes resulted in 11.11 per cent of disease transmission in the host plant which hiked up to 55.56 per cent of disease transmission for 10 and 15 minutes of the inoculation feeding period. Later, a decline in disease transmission was witnessed with the increase in the inoculation feeding period. There was zero transmission of disease when given the inoculation time exceeding 60 minutes (Table 3).

According to the pre-acquisition fasting trial data, zero per cent of disease transmission was noted when the aphids were given zero pre-acquisition fasting period. This transmission of disease had escalated gradually from 22.22, 55.56, 66.67, and 77.78 per cent given the pre-acquisition fasting for 10, 15, 30, and 60 minutes, respectively (Table 4). The disease transmission was found to diminish as the pre-acquisition fasting exceeded 60 minutes *i.e.* with pre-acquisition fasting of 90, 120, and 240 minutes, the transmission dropped from 11.11 to 0.00 per cent (Table 4).

Distinguishable transmission of disease of about 33.33 per cent was observed when given the post-acquisition period of zero minutes. The vector transmission of the disease was notably increased by 44.44 and 77.78 per cent with an increase in the post-acquisition period of 20 and 40 minutes, respectively. There was a significant decline in disease transmission of 66.67, 33.33, 11.11, and 0.00 per cent with 40, 80, 120, and 160 minutes of post-acquisition period respectively (Table 5).

Table 1: Determination of optimum number of viruliferous *A. craccivora* (apterous matured) aphids required to transmit Urdbean leaf crinkle viral disease (ULCD) in susceptible blackgram (accession: variety T9).

No. of aphids/plant	Pre-acquisition fasting (min)	Acquisition access period (min)	Inoculation feeding period (min)	% disease transmission#
1	60	10	10	0.00 ± 0.00 (4.05)^{d}
2	60	10	10	0.00 ± 0.00 (4.05)^{d}
5	60	10	10	33.33 ± 0.33 (5.16)^{c}
10	60	10	10	66.67 ± 0.35 (6.15)^{ab}
15	60	10	10	77.78 ± 0.38 (6.44)^{a}
20	60	10	10	77.78 ± 0.19 (6.48)^{a}
25	60	10	10	88.89 ± 0.18 (6.76)^{a}
SEd				**0.57**
P value				**0.001****

#Mean values of three replications are represented as mean± standard deviation
Figures in the parentheses are arc sine transformed values
Means followed by the same letter are not significantly different from each other, Tukey's test ($p \leq 0.05$)
SEd: Standard Error of the difference; ** Highly Significant

Table 2: Determination of acquisition threshold time for feeding of viruliferous *A. craccivora* (apterous matured) aphids to transmit Urdbean leaf crinkle viral disease (ULCD) in susceptible blackgram (accession: variety T9).

Acquisition feeding (min)	No. of aphids/plant	Pre-acquisition fasting (min)	Inoculation feeding period (min)	% disease transmission#
1	10	60	10	33.33 ± 0.33 (5.16)^{c}
2	10	60	10	33.33 ± 0.29 (5.16)^{c}
5	10	60	10	66.67 ± 0.57^{b} (6.15)
10	10	60	10	88.89 ± 0.20 (6.75)^{a}
15	10	60	10	55.59 ± 0.24 (5.87)^{bc}
30	10	60	10	44.44 ± 0.18 (5.55)^{bc}
60	10	60	10	11.11 ± 0.11 (4.44)^{d}
120	10	60	10	11.11 ± 0.15 (4.44)^{d}
240	10	60	10	0.00 ± 0.00 (4.05)^{e}
SEd				**0.60**
P value				**0.00****

#Mean values of three replications are represented as mean± standard deviation
Figures in the parentheses are arc sine transformed values
Means followed by the same letter are not significantly different from each other, Tukey's test ($p \leq 0.05$)
SEd: Standard Error of the difference; ** Highly Significant

Table 3: Determination of inoculation period required for viruliferous *A. craccivora* (apterous matured) aphids to transmit Urdbean leaf crinkle viral disease (ULCD) in susceptible blackgram (accession: variety T9).

Inoculation feeding (min)	No. of aphids/plant	Pre-acquisition fasting (min)	Acquisition access period (min)	% disease transmission#
1	10	60	5	0.00 ± 0.00^{c} (4.05)
5	10	60	5	11.11 ± 0.19 (4.44)^{c}
10	10	60	5	55.56 ± 0.25 (5.87)^{a}
15	10	60	5	55.56 ± 0.50 (5.76)^{a}
30	10	60	5	33.33 ± 0.33 (5.16)^{b}
60	10	60	5	11.11 ± 0.17 (4.44)^{ab}
120	10	60	5	0.00 ± 0.00^{c} (4.05)
240	10	60	5	0.00 ± 0.00^{c} (4.05)
SEd				**0.62**
P value				**0.03***

#Mean values of three replications are represented as mean± standard deviation
Figures in the parentheses are arc sine transformed values
Means followed by the same letter are not significantly different from each other, Tukey's test ($p \leq 0.05$)
SEd: Standard Error of the difference; *Significant

Table 4: Determination of pre-acquisition threshold time required for viruliferous *A. craccivora* (apterous matured) aphids to transmit Urdbean leaf crinkle viral disease (ULCD) in susceptible blackgram (accession: variety T9).

Pre acquisition fasting (min)	No. of aphids/plant	Acquisition access period (min)	Inoculation feeding period (min)	% transmission#
0	10	5	10	0 ± 0 (4.05)^{b}
10	10	5	10	22.22 ± 0.17 (4.84)^{ab}
15	10	5	10	55.56 ± 0.47 (5.76)^{ab}
30	10	5	10	66.67 ± 0.27 (6.16)^{ab}
60	10	5	10	77.78 ± 0.38 (6.44)^{a}
90	10	5	10	11.11 ± 0.16 (4.45)^{ab}
120	10	5	10	0 ± 0 (4.05)^{b}
240	10	5	10	0 ± 0 (4.05)^{b}
SEd				**0.66**
P value				**0.005****

#Mean values of three replications are represented as mean± standard deviation
Figures in the parentheses are arc sine transformed values
Means followed by the same letter are not significantly different from each other, Tukey's test ($p \leq 0.05$)
SEd: Standard Error of the difference; ** Highly Significant

Table 5: Determination of post acquisition threshold time required for viruliferous *A. craccivora* (apterous matured) aphids to transmit Urdbean leaf crinkle viral disease (ULCD) in susceptible blackgram (accession: variety T9).

Post acquisition period (min)	No. of aphids /plant	Acquisition access period (min)	Inoculation feeding period (min)	% transmission#
0	10	10	10	33.33 ± 0.33 (5.23)^{ab}
10	10	10	10	44 .44 ± 0.19 (5.55)^{ab}
20	10	10	10	77.78 ± 0.38 (6.42)^{a}
40	10	10	10	66.67 ± 0.33 (6.15)^{ab}
80	10	10	10	33.33 ± 0.58 (5.04)^{ab}
120	10	10	10	11.11 ± 0.19 (4.44)^{ab}
160	10	10	10	0 ± 0 (4.05)^{b}
240	10	10	10	0 ± 0 (4.05)^{b}
SEd				**0.68**
P value				**0.01***

#Mean values of three replications are represented as mean± standard deviation
Figures in the parentheses are arc sine transformed values
Means followed by the same letter are not significantly different from each other, Tukey's test ($p \leq 0.05$)
SEd: Standard Error of the difference; *Significant

DISCUSSION

The above study evinced that the test insect vector, pulse aphid, *A. craccivora* has the potential to transmit ULCD (Dhingra 1975; Dhingra and Chenulu 1981; Bhardwaj and Dubey 1986; Brar and Rataul 1987; Sravika *et al.* 2018). The insect vector was capable of transmitting the virus isolates when given the acquisition access period of one minute and the inoculation feeding period of five minutes in the plants that served as the virus inoculum. Though, the transmission efficiency had increased when the pre-acquisition fasting was given for 60 minutes.

The aphids, when given with the acquisition feeding period exceeding 15 minutes had shown a decline in disease transmission and this might have been a cause of insect vector stylet's having been free from pathogen due to re-ensheathment and proving the vector to be non-persistently transmitting the ULCV. The above-mentioned report was supported by Khurana *et.al.* (1973) and Bhardwaj and Dubey (1986), concerning the re-ensheathment mechanism aiding the aphids to be a non-persistent vector transmitter.

In the present study, 15 to 25 aphids were efficient in transmitting the disease (77.78-88.89 %). Corresponding results were given by Sravika *et al.* (2018), in which 10 numbers or more numbers of aphids were required for maximum transmission of disease. On contrary, Dhingra and Chenulu (1981) reported that the maximum disease transmission was achieved with 10 viruliferous aphids. The study was supported by Nageswas Rao (2002) who stated that a single viruliferous adult of pulse aphid given the acquisition period of 2 min and inoculation access period of 24 h could transmit ULCV but the maximum transmission was achieved by 10 viruliferous/plant with the pre- starvation period of 60 min, acquisition period of 2 min and inoculation access period of 24.

In the research conducted, 10 minutes of acquisition feeding period was efficient in achieving 88.89 per cent. Contradictory findings on the acquisition feeding period by Dhingra and Chenulu (1981) whose reports suggested that an acquisition access period of 30 seconds to 2 minutes was successful in transmitting ULCD.

This study proved that 10 numbers of aphids given the acquisition feeding period and inoculation feeding time of 10 minutes was efficient in ULCD transmission. Whereas, the reports of Kumar and Subha Rao (1994) proved that acquisition feeding period of 2 minutes and inoculation feeding time of 10 minutes were efficient in disease transmission by 10 numbers of aphids.

A number of 15 or more viruliferous pulse aphids are efficient in inflicting ULCD (77.78 to 88.89 %) in blackgram provided the pre-starvation period of 60 min, an acquisition and inoculation feeding period of 10 min. The post acquisition period of 20 mins was more effective resulting in 77.78 per cent disease incidence, which gradually declined with increase in post acquisition period. The research conducted in determining whether *A. craccivora* is a putative insect vector in transmitting Urdbean leaf crinkle viral disease

(ULCD) on blackgram turned out to be positive. The knowledge on studies on the transmission of ULCD on blackgram (T9 cv.) by *A. craccivora* can be implemented to practice effective methods of management of ULCD's spread through application of insecticides that targets insect vectors and also to find remedies in inhibiting the virus replication in host plants.

CONCLUSION

This research on insect vector-virus relationship will ensure in prescribing preventive measures in controlling the ULCD and further studies on the etiology of virus in future might help in developing Anti-viral principles and vector targeting insecticides.

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Conflict of Interest. None.

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