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Virus- Vector Relationship Of Sunflower Leaf Curl Virus (SuLCV) In Relation To Disease Spread

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Abstract: ***A Single viruliferous whitefly is able to transmit the disease. However, transmission efficiency will increased with increasing the number of whiteflies. Five viruliferous whiteflies were required for 100 per cent transmission of SuLCV from sunflower to sunflower, when acquisition and inoculation access periods were 24 h each. A minimum period of 30 min is necessary for B. tabaci to acquire SuLCV and an AAP of at least 6 hr is required for 100 per cent transmission by whiteflies. A minimum IAP of 30 min is recorded to achieve 20 per cent transmission efficiency and IAP of 6 h resulted in 100 per cent transmission. The test carried out for persistence of SuLCV in the vector whitefly B. tabaci. It's found that SuLCV could persist in B. tabaci for eight days.***

Keywords: Sunflower, Whitefly, Acquisition Access Period, Inoculation Access Period.

I. INTRODUCTION

Sunflower as an oilseed is a newly introduced crop in the country. This crop has gained importance due to its short duration of maturity, excellent quality oil, photo-insensitivity, wide adaptability in different agroclimatic regions and drought tolerance. Sunflower is grown as inter crop with crops such as groundnut, pigeon pea, castor, soybean and urd bean. Since it is a photo-insensitive crop, it can be grown throughout the year. The crop has been found suffering from many diseases like *Alternaria* leaf spot, downy mildew, powdery mildew, charcoal rot, scleorotium rot or wilt, rhizopus head rot, sunflower necrosis virus, and cucumber mosaic virus (Saharan *et al.*, 2005). Among viral disease affecting sunflower, very recently leaf curl disease caused by begomovirus of the geminiviridae family was reported for the first time from Main Agricultural Research Station (MARS), University of Agricultural Sciences (UAS) campus, Raichur, Karnataka, India. Causal agent of the disease was confirmed as ss DNA begomovirus which is clustered next to Tomato leaf curl Karnataka virus isolate Lucknow (ToLCKV-[Luc] (Accession no. EU604297.2) and Tomato leaf curl virus - Bangalore II (ToLCBV-[Ban2]) (Accession no. EU604297.2) and shared 97.5 per cent nucleotide identities (Govindappa *et al.*, 2011). Further, viral full genome was sequence and the analysis of the study revealed that, leaf curl virus having DNA-A and the associated satellite beta DNA components of 2761 and 1375 (Nucleotides) in length respectively. The DNA-A molecule shared maximum identity with tomato leaf curl Karnataka clone IKH12 (ToLCKV- IKH12) (Vanitha, 2012).Leaf curl disease being the first and foremost kind of disease appeared on sunflower, Research efforts were very meager in relevance to its distribution, symptomatology and virus-vector relationships in relation to its nature of transmission sunflower genotypes were very much lacking. Hence present investigations were conducted.

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II. MATERIALS AND METHODS

A. MAINTENANCE OF SULCV CULTURE

Sunflower plants showing characteristic leaf curl virus symptoms of vein thickening, upward leaf curling, enation and stunted growth was brought to the laboratory from sunflower fields of Main Agricultural Research Station, University of Agricultural Sciences, Raichur, and virus culture was maintained by inoculating 8-10 days old healthy sunflower seedlings using whiteflies (*B. tabaci*). All process was carried out under nylon net (40 mesh) protected greenhouse (Plate 1).

Plate 1: SuLCV culture maintained on sunflower hybrid KBSH-44 at MARS, Raichur

B. MAINTENANCE OF WHITEFLY (B. TABACI) CULTURE

Initially, whiteflies (*B. tabaci*) were collected from sunflower plants at MARS, Raichur and the colony was established on freshly grown cotton, *Gossypium hirsutum* plants kept in insect proof net house. There after a generation, freshly emerged whiteflies were collected using an aspirator and were transferred onto freshly grown cotton plants kept in an insect proof net house. The colony so developed was referred to be pure (a-viruliferous) and further periodically maintained by frequently introducing healthy cotton plants grown in pots (6 x 10 cm) into the insect proof net house which was maintained at temperature of 28 to 30°C in an insect proof polyhouse (Plate 2).

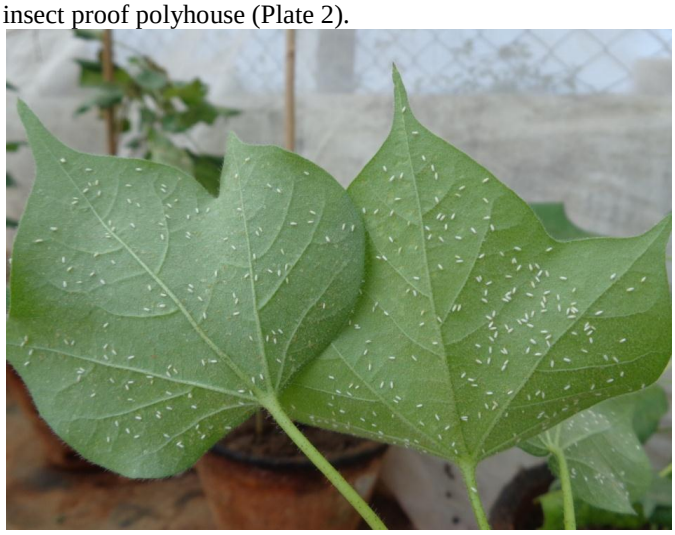

Plate 2: Maintenance of Whiteflies (Bemisia tabaci) colony on cotton (Gossypium hirsutum)

C. RAISING OF HEALTHY SEEDLINGS

Healthy seedlings of sunflower hybrid KBSH-44 required for virus-vector relationship studies were raised from seeds collected from healthy sunflower plants. The seedlings were raised in 4"x 6" polyethylene bags filled with soil and compost mixture in 2:1 proportion. These plants were kept in insect proof cages and used throughout the period of investigations.

D. VIRUS- VECTOR RELATIONSHIP STUDIES

Healthy seedlings of sunflower plants were raised in polythene bags containing soil mixture in insect proof glasshouse for virus-vector relationship studies. Plastic or polyvinyl chloride (PVC) bottles (20 x 7.5 cm) were used for acquisition access feeding of *B. tabaci* and for inoculation studies. Whiteflies were collected by using aspirator. To determine the virus- vector relationship, the SuLCV local isolate maintained on Sunflower hybrid KBSH-44 and the whitefly *B. tabaci* colony was maintained on cotton hosts were used for transmission studies. Both the cultures were used to determine the various parameters such as Acquisition Access Period (AAP), Inoculation Access Period (IAP) of whitefly and to find out type of persistence.

E. DETERMINATION OF NUMBER OF WHITEFLIES (*B. TABACI*) REQUIRED FOR TRANSMISSION OF SULCV

To determine the minimum number of whitefly required for achieving the transmission of virus onto a susceptible sunflower hybrid KBSH-44 (Economic threshold level ETL), Whiteflies were tested at different number varied from 1, 3, 5, 10, 15 and 20/plant and method of virus acquisition and inoculation was followed. After 24 h of inoculation access period, seedlings were sprayed with hostathion 40EC at 1.5 ml/L and kept for symptoms expression. Observation was made on number of seedlings exhibits leaf curl symptoms among the total plants inoculated per each test number of *B. tabaci*.

F. DETERMINATION OF AAP OF SULCV

The healthy whiteflies maintained on cotton plants were collected using aspirator and allowed to feed on SuLCV infected sunflower leaf for different acquisition access periods *viz.*, 30 min, 1 hr, 2 hr, 3 hr, 6 hr, 12 hr and 24 hr (AAP). The viruliferous vectors of the respective AAP were again transferred to healthy sunflower seedling to inoculate the virus for a specific period of 24 hr. After, 24 hrs of inoculation access periods, whiteflies were killed using systemic insecticide Hostathion 40 EC @ 1.5 ml/L. The inoculated seedlings were maintained in insect proof cages till the expression of symptoms. Observation was recorded on time period of AAP required to achieve minimum and maximum per cent transmission based on number of seedlings exhibits diseases symptoms upon inoculation of virus.

G. DETERMINATION OF IAP OF SULCV

The healthy colonies of whiteflies were allowed to feed on SuLCV infected sunflower leaf sample for a specific period of 24 hrs. After 24 hrs of AAP, such viruliferous whiteflies were allowed to feed on healthy sunflower seedlings for different inoculation access periods *viz.*, 30 min, 1 hr, 2 hr, 3 hr, 6 hr, 12 hr and 24 hr (IAP) to inoculate the virus. After, respective periods of inoculation access periods, whiteflies were killed using systemic insecticide Hostathion 40 EC @ 1.5 ml/L. Later inoculated seedlings were maintained in insect proof cages till the expression of symptoms. Observation was recorded on time period of IAP required to achieve minimum and maximum per cent transmission based on number of seedlings exhibits diseases symptoms upon inoculation of virus.

H. DETERMINATION OF PERSISTENCE OF SULCV DISEASE IN WHITEFLY *B. TABACI*

To determine the type of persistence of SuLCV in whitefly *B. tabaci*, whiteflies were given 24 h of acquisition access on sunflower leaf curl infected leaves. Later, single viruliferous whitefly were inoculated to healthy sunflower seedling (KBSH-44) and allowed to feed for 24 hr of inoculation access period. After every 24h of inoculation access period, same whitefly was inoculated onto healthy seedling till the adult life span (7 days). Seedlings were sprayed with hostathion 40 EC at 1.5 ml/l and kept for symptoms expression. Observation was made on seedlings which exhibits leaf curl symptoms upon inoculation till the survival of adult.

III. RESULTS

A. MINIMUM NUMBER OF WHITEFLIES (*B. TABACI*) REQUIRED FOR TRANSMISSION

To ascertain the minimum number of *B. tabaci* required for efficient transmission, different number of whiteflies (Ex., 1, 3, 5, 10, 15, and 20) per plant were used for virus inoculation. Viruliferous whiteflies were enclosed on test plants with AAP and IAP of 24 h each. Single whitefly was able to transmit SuLCV with 20 per cent efficiency (Table 1). The transmission efficiency increased to more than 50 percent when 3 whiteflies caged on healthy sunflower seedlings. Transmission efficiency of 100 per cent with 5 or more whiteflies per plant was achieved. This indicates that the single whitefly enough to transmit the disease further number of insects and the transmission efficiency was positively correlated (Fig 1).

B. ACQUISITION ACCESS PERIOD (AAP)

A group of five non–viruliferous adult whiteflies were allowed to feed on SuLCV infected leaves for 30 min to 24 hr. The whiteflies were then enclosed on healthy plants for 24 hr IAP to estimate the comparative efficiency of AAP of *B. tabaci*. A minimum AAP of 30 min was necessary for *B. tabaci* to acquire SuLCV, which resulted in 30 per cent transmission (Table 2). An AAP of at least six hr was required for 100 per cent transmission from whiteflies. Results of this experiment also revealed that the per cent transmission increased with the increase in AAP (Fig 2).

C. INOCULATION ACCESS PERIOD (IAP)

A group of five non–viruliferous adult whiteflies were allowed for inoculation of SuLCV. The inoculation period ranged from 30 min to 24 hr (Table 2). Viruliferous whiteflies required a minimum IAP of 30 min to achieve 20 per cent, transmission efficiency. An IAP of 6 h resulted in 100 per cent transmission. The results also indicated that per cent transmission increased with the increases of IAP (Fig. 2).

D. PERSISTENCE OF SULCV IN VECTOR WHITEFLY *B. TABACI*

To determine the type of persistence of SuLCV in whitefly *B. tabaci*, a single whitefly was serially transferred to healthy sunflower seedlings separately at 24 h interval upto eight days. It was found that SuLCV could persist in *B. tabaci* upto 8 days and can transmit the virus serially (Table 3).

Number of whiteflies	Number of plants infected /inoculated plants	Transmission (%)
1	2/10	20
3	6/10	60
5	10/10	100
10	10/10	100
15	10/10	100
20	10/10	100

AAP: 24 h, IAP: 24 h, Culture: SuLCV; Sunflower hybrid: KBSH-44

Table 1: Determination of number of whiteflies (B. tabaci) required for Transmission of leaf curl virus to sunflower in 24 hours

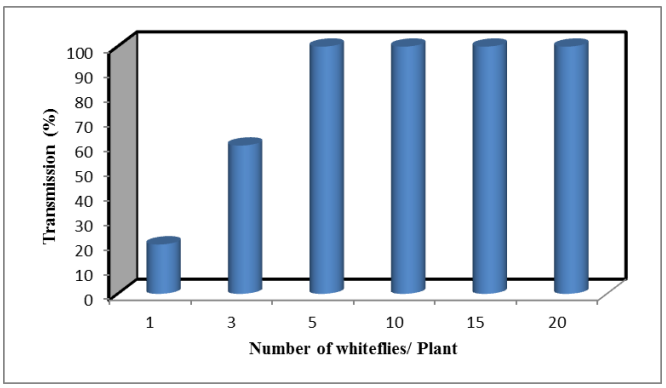

Figure 1: Determination of number of whiteflies (B. tabaci) required for transmission of leaf curl virus to sunflower in 24 hours

AAP	Number of plants infected/ inoculated plants	Transmission (%)	IAP	Number of plants infected/ inoculated plants	Transmission (%)	Number of plants infected/ inoculated plants
30 min	3/10	30	30 min	2/10	20	3/10

1 hr	5/10	50	1 hr	3/10	30	5/10
2 hr	7/10	70	2 hr	5/10	50	7/10
3 hr	9/10	90	3 hr	7/10	70	9/10
6 hr	10/10	100	6 hr	10/10	100	10/10
12 hr	10/10	100	12 hr	10/10	100	10/10
24 hr	10/10	100	24 hr	10/10	100	10/10

No. of whiteflies/seedlings: 5, Culture: SuLCV
Sunflower hybrid: KBSH-44, AAP: 24 h, IAP: 24 h

Table 2: Determination of AAP and IAP on transmission of Leaf curl virus by B. tabaci to sunflower

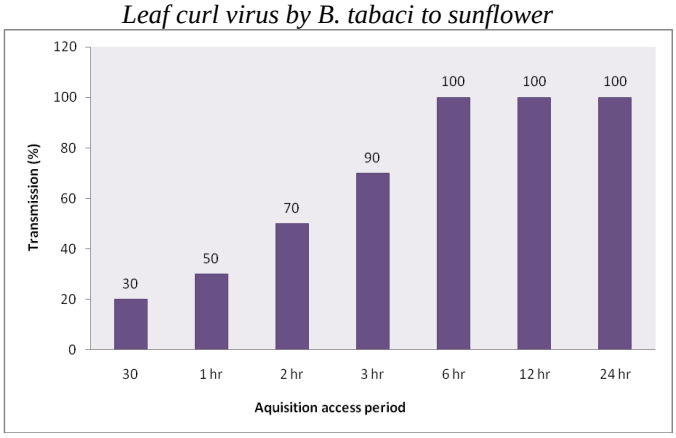

Figure 2: Determination of Acquisition Access Period (AAP) on transmission of SuLCV by B. tabaci to sunflower

No. of Expt.	Days old whiteflies 1	2	3	4	5	6	7	8	9	10	11
Expt-1	+	+	+	+	+	+	+	+	-	-	-
Expt-2	+	+	+	+	+	+	+	+	-	-	-

Culture: SuLCV; Sunflower hybrid: KBSH-44
Single WF/seedling, same WF transferred to next seedling for different days; IAP: 24h

Table 3: Determination of persistence of SuLCV in viruliferous whitefly (B. tabaci)

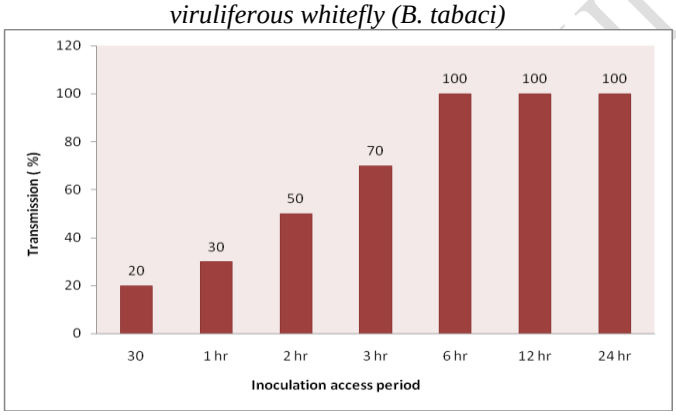

Figure 3: Determination of Inoculation Access Period (IAP) on transmission of SuLCV by B. tabaci to sunflower

IV. DISCUSSION

The present studies on virus-vector relationship revealed that, single whitefly transmitted SuLCV to an extent of 20 per cent. However, five or more whiteflies were required for 100 per cent transmission. There is a positive correlation between the number of whiteflies and SuLCV transmission. This finding is in confirming with other whitefly transmitted geminiviruses in different crops.

The whiteflies required a minimum AAP of 30 min to become viruliferous, which resulted in 30 per cent SuLCV transmission when group of 5 adults were used with 24 hr IAP. AAP of 6 hr or more resulted in 100 per cent transmission. Viruliferous natures of whiteflies and per cent transmission have positive correlation with increase in AAP. The results from IAP study revealed that a minimum of 30 min IAP by the viruliferous vectors caused 20 per cent transmission. With increase in IAP, there was a gradual increase in the percentage of infected plants. An IAP of 6 hr or more resulted in 100 per cent transmission. Persistence of sunflower leaf curl virus in viruliferous whiteflies (*B. tabaci*) study revealed that the virus may persist upto 8 days with continuous transmission. Similar work done on Indian cassava mosaic virus (ICMV) (Nair, 1975; Mathew, 1991) and ICMV (Tri) (Maruthi *et al*., 2002) and tomato leaf curl virus (Saikia and Muniyappa, 1989; Ramappa, 1993). and similarly intermittent transmission like other most related begomoviruses such as Zinnia leaf curl virus (Shivakumar, 2010) Croton leaf curl virus (Mahesh *et al*., 2010).

V. CONCLUSION

A Single viruliferous whitefly can transmit the SuLCV. However five viruliferous whiteflies were required for 100 per cent transmission. A minimum period of 30 min is necessary for *B. tabaci* to acquire and to inoculation of the SuLCV and Whitefly remained viruliferous upto 8 days for transmission of virus.

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