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Viru-vector relationships of yellow mosaic virus and whitefly (*Bemisia tabaci*) in horsegram

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Abstract
The present investigations were undertaken with different aspects on virus-vector relationship for yellow mosaic disease of horsegram which includes minimum number of whiteflies required for transmission, acquisition accesses period, inoculation accesses period, persistence period of yellow mosaic virus in the vector and starvation period. *Horsegram yellow mosaic virus* was transmitted to healthy plants only by the whitefly, *Bemisia tabaci* and not through sap inoculation. Single viruliferous whitefly can able to transmit the HYMV. However, transmission efficiency was increased with increased number of whiteflies. More than ten whiteflies were required for 100 per cent transmission of *Horse gram yellow mosaic virus* with common acquisition and inoculation access periods of 12 h. Similarly minimum period of 1 h was necessary for whitefly to acquire the virus and transmit the disease to an extent of 3.33 per cent and an AAP of at least 12 h was required for 100 per cent transmission by whiteflies. A minimum IAP of 1 h was recorded to achieve 6.66 per cent transmission efficiency and IAP of 12 h resulted in 96.6 per cent transmission. When healthy whiteflies provided 1 h pre acquisition starvation period resulted in 20 per cent transmission. As the pre acquisition starvation period increases, the extent of virus transmission was also increased and obtained cent per cent transmission at 12 h. As per the results of persistence of HYMV in the vector whitefly *B. tabaci*, it was found that, the virus could persist in *B. tabaci* for nine days and gradually decreased over a period of time after acquisition. This showed that, the virus is whitefly borne and transmit in persistent manner.

Keywords: HYMV, AAP, IAP, persistence, starvation, virus, vector

Introduction
Horsegram (*Macrotyloma uniflorum* (Lam.) Verdc.) popularly known as "Kulthi" which is known for its easily digestible quality protein and originated from South West India. It belongs to family Leguminosae and sub-family Papilionaceae. Pods are short and hairy. It is an excellent source of dietary fiber with positive effects on intestine and colon, besides other homeostatic and therapeutic functions in human nutrition (Yadahalli *et al.,* 2012) [18]. It has good feed and fodder value with valuable protein (23-30%) and vitamins. It is an under exploited grain legume with great potential in sustainable agriculture, as it enriches soil considerably by fixing atmospheric nitrogen and increasing the organic matter of soil.

Horsegram is one of the important pulse crop being cultivated over a larger areas in many dry land regions of Karnataka. Crop is known to suffer from several diseases including fungal, bacterial and viral diseases. Important diseases which causes crop production losses such as powdery mildew (*Erysiphe polygoni*), Dry root rot (*Macrophomina phaseolina*), Anthracnose (*Colletotrichum lindemuthianum*), Rust (*Uromyces phaseoli typica*), Leaf spot (*Cercospora cruenta*) and cottony stem rot (*Sclerotinia sclerotiorum*) and Yellow Mosaic Disease (YMD).

Among the diseases, Yellow mosaic disease (YMD) caused by begomovirus belongs to family geminiviridae is one of the major constraints for crop cultivation in peninsular India and in many parts of Karnataka. Yellow mosaic disease transmitted by whitefly *B. tabaci* (Gennadius) more prevalent in most parts of South India (Muniyappa and Reddy, 1976) [7]. The emergence of the whitefly transmitted geminivirus complex around the world depends on various factors, such as exchange of genetic information by recombination plays a role in the evolution of viruses, evolution of variants of the viruses, changes in the biology of vectors, movement of infected planting materials, introduction of new crops and host susceptibility genes through the exchange of germplasm, changes in cropping systems, and climatic factors (Ramappa *et al*., 1998; Varma and Malathi, 2003; Shivalingama *et al.,* 2007 and Kaur *et al.,* 2015) [14, 16, 15, 5].

Horsegram YMD transmitted by whitefly *B. tabaci* known to cause yellow discoloration on the leaves that leads to irregular, small, greenish yellow mosaic symptoms. Severe infection leads to stunted growth of the plant and reduction in the leaf size (Prema, 2013) [10]. Isolation, purification, electron microscopic and serological studies of *Horse gram Yellow Mosaic Virus* (HYMV) has been studied (Muniyappa *et al*., 1987) [8].

Currently, there is megar information on virus-vector relationship of *Yellow Mosaic Virus* (YMV) infecting horsegram from southern India. With this backdrop, the present study on the virus-vector relationship was taken up.

Material and Methods

The present investigations on yellow mosaic disease of horsegram were carried out at Department of Plant Pathology, UAS, Raichur situated at north eastern dry zone (zone-2) of Karnataka located at 16° 12' N latitude and 77° 20' E longitude with an altitude of 389 meters above the mean sea level during 2018-19.

Raising of healthy seedlings

Healthy seedlings of horsegram required for various transmission studies were raised from seeds collected from healthy 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.

Maintenance of yellow mosaic virus (YMV) culture

Horsegram plant showing characteristic symptoms of yellow mosaic disease was collected in the surveyed field. Using whiteflies (*B. tabaci*), the virus cultures was transmitted to the healthy horsegram plants. Whiteflies were given for 12 h acquisition and later transferred to 10-12 days old healthy horsegram seedlings in insect proof glasshouse. The inoculation access period of 12 h was given on healthy seedlings. The virus culture was maintained in insect proof cages by periodically inoculation to healthy seedlings by *B. tabaci* and further used for various studies.

Maintenance of whitefly culture and handling of whiteflies

The most preferred hosts for whiteflies are brinjal and cotton. Therefore the brinjal and cotton plants were raised in insect proof cages. An aspirator made with tube was used for the collection of whiteflies from cotton fields around MARS, UAS, Raichur. The collected whiteflies were transferred to the 40 days old brinjal and cotton plants grown in insect proof glasshouse. After one generation the whiteflies were continuously maintained on cotton and brinjal plants in insect proof nylon mesh. Later the flies were used for further transmission studies.

Preparation of acquisition cages

Plastic or Poly vinyl chloride (PVC) bottles (20 cm x 7.5 cm) tapering towards the narrow mouth was taken, the bottom portion of the bottles was removed with the help of a soldering rod and they were covered with black muslin cloth. The narrow mouth of the bottle was cut up to neck below the screw cap and plugged with black muslin cloth rapped with non-absorbent cotton ball to prevent flies escaping from the bottle during usage.

Preparation of inoculation cages

Plastic tubes (7.5 cm x 2.5 cm) were taken and the bottom of the tubes was removed using soldering rod. The bottom ends was sealed with a black muslin cloth to avoid accumulation of excess moisture inside the cage and also to provide aeration. A small hole of 0.5 cm diameter was made in the middle portion of the tube to facilitate release of whiteflies. The open end of the tube was plugged with cotton after inserting young leaflets into the tube.

Preparation of aspirators and collection of whiteflies

An aspirator made of a glass tube (30 cm x 0.5 cm x 40 cm) and a rubber tube of 40 cm length was used for the collection of whiteflies. The leaves colonized with healthy whiteflies were turned slightly upward and the flies were sucked into the glass tube. Later they were gently blown in to the plastic tubes. Such collected whiteflies were cultured on cotton and brinjal further used for investigation.

YMV transmission *by* whitefly (*B. tabaci*)

Transmission of YMV by whitefly (*B. tabaci*) was carried out in protected cage under glass house condition. For the study, adult whiteflies were collected from the culture house in PVC acquisition bottle with the help of an aspirator and the YMV diseased plant was inserted inside the bottle. Whiteflies were allowed to feed for 12 hr and then such whiteflies were released on caged healthy test seedlings at the rate of 10 per seedling. After 12 hr Innoculation access period (IAP), whiteflies were removed from individual plants. The plants were sprayed with 0.03 per cent imidachloprid and kept in insect proof cages for symptom development.

Virus vector relationships

Virus-vector relationship was determined only for *Yellow Mosaic Virus* (YMV) infecting horse gram using *B. tabaci* maintained on cotton.

Determination of number of whitefly vector for transmission of HYMV

To standardize the number of whiteflies required for transmission, the whiteflies were allowed at the rate of 1, 2, 3, 5, 8 and 10 per plant. For the study, healthy colonies of whiteflies were allowed to feed on infected plants for 12 h to acquire the virus. The viruliferous whiteflies were then inoculated at the rate of 1, 2, 3, 5, 8 and 10 per plants separately to one week healthy seedlings and given 12 hr as inoculation feeding period. For each test number of whiteflies, ten plants were inoculated. After 12 h of inoculation access period, whiteflies were killed by spraying 0.03 per cent imidacloprid 17.8 SL and seedlings were kept in insect proof cages for symptom expression. Observations were taken on per cent transmission based on number of plants showing mosaic symptoms among the total number of plants inoculated and the incubation period per test number of *B. tabaci.*

Determination of Acquisition access period (AAP)

The effect of AAP on the rate of transmission of YMV was tested by allowing *B. tabaci* to feed for varying AAP of 1 h, 2 h, 4 h, 6 h and12 h on YMV infected plants separately. After the prescribed AAP, the whiteflies were transferred on to 10-12 days-old healthy seedlings at the rate of 10 whiteflies per plant. For each treatment (AAP period), 10 plants were inoculated. After 12 h of IAP from all the test AAP period, insects of the respective treatment were killed by spraying 0.03 per cent imidacloprid. Plants were kept in the glasshouse for symptom development. Observations were taken on per

cent transmission based on number of plants showing mosaic symptoms among the total number of plants inoculated and the incubation period per test acqusition access period.

Determination of inoculation access period (IAP)

To determine the effect of varied IAP on transmission of HYMV, large group of *B. tabaci* were collected in acquisition bottle and allowed for 12 h AAP on YMV-infected plants separately. Such viruliferous whiteflies were then transferred to 10-12 days old horsegram seedlings (at the rate of 10 per seedling) and allowed to varied IAP of 1 h, 2 h, 4 h, 6 h and 12 h separately. For each IAP (Treatment), ten plants were inoculated. Whiteflies were then killed by spraying 0.03 per cent imidachloprid 17.5 SL and plants were kept in an insect-proof glasshouse for symptom development and determine the minimum and maximum IAP required for transmission of virus from each test period.

Determination of pre-acquisition starvation period (PASP)

A group of (500-800) healthy colonies of whiteflies were collected with the help of acquisition bottles from the whitefly culture (Maintained on cotton). Those non viruliferous whiteflies were given a different starvation periods *viz*., 1 h, 2 h, 4 h, 6 h and 12 h in a test tube covered with muslin cloth for aeration.

After particular starvation period the whiteflies were transferred to symptomatic horse gram plants to acquire the virus. The whiteflies were given 12 h of acquisition access period and viruliferous whiteflies were inoculated to one week old healthy seedlings for 12 h as inoculation feeding period. For each test number of whiteflies, ten plants were inoculated. After 12 h of inoculation access period, whiteflies were killed by spraying 0.03 per cent imidacloprid 17.8 SL. The inoculated seedlings were kept in insect proof cages for symptom expression. Observations were taken on number of seedlings showing mosaic symptoms among the total plants inoculated per test period.

Determination of persistence of virus in vector

To determine the persistence of YMV in adult *B. tabaci* a group of 500 whiteflies were allowed for 12 h AAP on YMV infected horsegram plant. Then such viruliferous whiteflies were transferred to healthy non host cotton seedlings. Later 5 viruliferous whiteflies from the above group were transferred onto healthy horsegram seedlings and allowed to feed for 12 h as inoculation access period. After 12 h IAP, seedling was allowed for symptom expression to know the persistence of virus on first day. Likewise the viruliferous whiteflies maintained on non-host cotton plant were serially transferred to healthy horsegram seedling each day with 12 h IAP until the insects were alive.

Results and Discussion

Determination of number of whiteflies (*B. tabaci*) for transmission of *Horsegram yellow mosaic virus*

Among different number of whiteflies (1, 2, 3, 5, 8 and 10) per plants was used for inoculation, single whitefly was able to transmit HYMV with 20 per cent at incubation period of 20-22 days. The transmission efficiency was increased by increase in whitefly population, where as 2, 3, 5 and 8 whiteflies shown transmission efficiency of 23.33, 43.33, 56.66 and 90.0 per cent respectively at different incubation period. Transmission efficiency reached 100 per cent when 10 whiteflies per plant used for inoculation. As the number of whiteflies increased, the transmission efficiency was also increased and incubation period for symptoms expression reduced from 22 days to 12-14 days (Table.1). YMV transmitted by whitefly *B. tabaci* on horsegram known to cause yellow discoloration on the leaves that leads to irregular, small, greenish yellow mosaic symptoms. Severe infection leads to stunted growth of the plant and reduction in the leaf size.

Table 1: Determination of number of whitefly vector for transmission of *Horsegram yellow mosaic virus*

No of viruliferous whiteflies used for transmission	Average per cent transmission *	Incubation period (days)
1	20.00	20-22
2	23.33	20-22
3	43.33	18-20
5	56.66	15-18
8	90.00	12-13
10	100.00	12-14

Whiteflies allowed 12h of each acquisition and inoculation access periods were used.
No. of plants inoculated: 10 in each treatment.
* values are in average of three replication.

Determination of Acquisition access period (AAP) for transmission of *Horsegram yellow mosaic virus*

The studies indicated that a minimum AAP of 1 hour was necessary for *B. tabaci* to acquire the virus, which resulted in 3.33 per cent transmission with incubation period of 17-19 days. The maximum transmission of 100 per cent was obtained at AAP of 12 hours. It has been shown that, the per cent transmission of the disease increased with the increase in AAP. At the same time incubation period required for symptoms expression was reduced from 19 to 12 days as the AAP increased from 1 to 12 hours (Table.2).

Table 2: Effect of different Acquisition access period (AAP) on transmission of *Horsegram yellow mosaic virus* through whitefly, *B. tabaci*

Acquisition access period (AAP) (hours)	Average per cent transmission *	Incubation period (days)
1	03.33	17-19
2	16.66	15-16
4	43.33	15-16
6	86.66	13-15
12	100.00	12-14

Inoculation access period: 12h
No. of whiteflies per plant: 10
No. of plants inoculated: 10
*Average values of three replication.

Determination of inoculation access period (IAP) for transmission of *Horsegram yellow mosaic virus*

A minimum IAP of 1 h was necessary for *B. tabaci* to transmit the virus, which resulted in 6.66 per cent transmission with incubation period of 15 to 17 days. The maximum transmission of 96.60 per cent was obtained at IAP of 12 hours. It has been shown that, the per cent transmission of the disease increased with the increase in IAP. At the same time incubation period required for symptoms expression was reduced from 17 to 12 days as the IAP increased from 1 to 12 hours (Table. 3).

Table 3: Effect of different Inoculation access period (IAP) on transmission of *Horsegram yellow mosaic virus* through whitefly, *B. tabaci*

Inoculation access period (IAP) (hours)	Average per cent transmission*	Incubation period (days)
1	6.66	15-17
2	10.00	15-17
4	30.00	12-15
6	56.66	12-14
12	96.60	12-14

Acquisition access period: 12h
No. of whiteflies per plant: 10
No. of plants inoculated: 10
*average values of three replication.

Determination of pre-acquisition starvation periods of Horse gram yellow mosaic virus

The investigation on determination of the effect of pre acquisition starvation period of whitefly on the transmission of HYMV revealed that, when whiteflies provided with 1 h pre acquisition starvation period resulted in 20 per cent transmission with incubation period of 15 to 17 days. The maximum transmission of 100 per cent was achieved when pre acquisition starvation period of 12 h with incubation period of 12 to 13 days. As the pre acquisition starvation period increases, the extent of virus transmission also increases and also the incubation period reduces from 17 days to12 days (Table. 4)

Table 4: Effect of pre acquisition starvation period for whiteflies on transmission of *Horsegram yellow mosaic virus*

Pre-Acquisition starvation period (hours)	Average per cent transmission	Incubation period (days)
1	20.00	15-17
2	46.66	14-15
4	60.00	14-15
6	73.33	12-13
12	100.00	12-13

Whiteflies allowed 12h of each acquisition and inoculation access periods were used.
Total No. of whiteflies per plant: 5
No. Of whiteflies per plant: 10

Persistence of *Horsegram yellow mosaic virus* in whitefly (vector)

The study was conducted to determine the type of persistence of HYMV in vector whitefly *B. tabaci*. It was noticed that, as the number of post acquisition access period (days) increases from 1 to 9 days the virus transmission efficiency reduces to 3.33 per cent with more incubation period of 18 to 21 days. The result indicated that the transmission efficiency decreased as the post acquisition access period increases up to 9 days. After 9 days of post acquisition access period, the whiteflies could not able to transmit the virus to healthy plants indicating that, the virus can persists in vector for 9 days (Table. 5).

The comprehensive approach on transmission efficacy of HYMV indicated that virus was successfully transmitted by an insect vector *B. tabaci* and exhibited mild to severe mosaic in 2 to 3 weeks after inoculation. Single viruliferous whitefly can transmit the YMV and more than 10 viruliferous whiteflies required for 100 per cent transmission. A minimum period of 1 hour was necessary for *B. tabaci* to acquire and to inoculation of the YMV. Whitefly remained viruliferous upto 9 days for transmission of virus. The efficacy in transmitting virus depends on different mechanisms involved in vectors. Protein–protein interactions between plant viruses and their insect vectors are an essential molecular interface that determines acquisition from infected host plants and transmission to new hosts (Ralf *et al.*, 2016) [13]. In connection to this, majority of transmission of virus involves coat proteins in virus vector relationship (Raccah *et al.*, 2009) [11]. Several studies indicated the efficacy of transmission depends on protein-protein interaction in specific Potyviruses, which encode a helper protein called Helper Component-Proteinase (HC-Pro) which is essential for virus transmission, as it facilitates virion retention in aphid stylets (Wang *et al.*, 1996) [17]. Similarly, Caulimoviruses have also adopted a helper-dependent transmission strategy, but in a rather more complex manner than potyviruses. CaMV (cauliflower mosaic viris) requires two viral-encoded nonstructural proteins, P2 and P3. A P2-P3-virion complex is formed, with P2 binding to the aphid whereas P3 binding to the virions. Plant viruses can modify insect vector behavior directly and indirectly by manipulating plant hosts, leading to enhanced transmission efficiency and spread (Blanc *et al*., 2016) [1]. The geminivirus tomato yellow leaf curl virus (TYLCV) was shown to manipulate feeding behaviors of whiteflies to increase virus transmission (Liu *et al.*, 2013) [6].

Table 5: Determination of the persistence of *Horsegram yellow mosaic virus* in whitefly, *B. tabaci*

Days after Acquisition access period	Average per cent transmission	Incubation period (days)
1	100.00	12-14
2	96.66	12-14
3	86.66	12-15
4	66.60	14-16
5	60.00	14-16
6	53.43	14-16
7	36.60	18-19
8	16.66	18-19
9	3.33	18-21
10	0.00	-

Inoculation access period: 12h
Acquisition access period: 12h
No. of plants inoculated: 10
No. of whiteflies per plant: 5

Virus-induced biochemical and physiological changes in the host-plant have been shown to influence vector insect host preference (Mauck *et al.*, 2014) [9]. Virus-infected plants can emit volatiles that make them more attractive to insect feeding (Bosque *et al.*, 2011; Rajabaskar *et al.*, 2014) [2, 12]. Virus-infected plants generally appear superior quality hosts for vectors compared to uninfected plants, thus enhancing vector life history and virus spread. However, examples of reduced host plant quality leading to rapid vector dispersal have also been reported. Luteovirid acquisition by aphids appears to alter host selection behavior to prefer uninfected plants while non-viruliferous aphids tend to prefer virus-infected plants, thereby promoting both virus acquisition and transmission (Ingwell *et al.*, 2012) [4]. Similar virus effects on host preferences of the vector were observed for reovirus-infected and virus-free planthoppers. The nuclear inclusion a (NIa) protease protein of turnip mosaic potyvirus has been implicated in manipulating host plant physiology to attract aphid vectors and to promote their reproduction (Casteel *et al.*, 2014) [3].

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