

**TRANSMISSION OF BEAN YELLOW MOSAIC VIRUS IN RELATION
TO APHID SPECIES (HOMOPTERA: APHIDIDAE)**

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Abstract

Experiments were carried out to determine the periods of acquisition, latent period, inoculation and retention of bean yellow mosaic virus (BYMV) when transmitted by the green peach aphid. Trials were set up with broad bean plants, and the method of indirect-ELISA was used to determine the latent carriers of the virus. Even a single viruliferous aphid was capable of transmitting the virus. However, the maximum transmission was obtained when 10 aphids per plant were employed. It was established that after acquisition of BYMV, Myzus persicae remains short time. The optimal period for acquisition of BYMV by the green peach aphid is determined to be 48 hours, and the minimal is 45 minutes. The optimal latent period of the virus in the vector for inoculation of BYMV by the green peach aphid is 48 hours and the minimal is 20 minutes.

Key words: Bean yellow mosaic virus (BYMV)- *Myzus persicae*

INTRODUCTION

Faba bean (*Vicia faba* L.) is among the most important edible crops that supply Egyptian people with protein, consumed as dry seeds, green vegetable and as processed food. Moreover, it is essential for feeding the live stock on dry seeds, as well as green and dry straw (Hassan, 1996). Assiut governorate is considered as one of the famous area cultivating with faba bean in Egypt. Insect pests, specially aphids, cause severe crop losses to faba bean, principally by direct feeding damage or as a vector and disseminator to virus disease (Manaa *et al*, 1999). Virus diseases, considered as a serious worldwide problem (Jones, 1997). The infection with certain viruses causes significant economic losses and yield reduction (estimated by 30% on susceptible cultivars of faba bean cultivars) (Khalil & Erskine, 2001). Bean yellow mosaic virus (BYMV) consider as one of the most devastating viruses affecting faba bean plants in Egypt (El-Beshehy, 1999 and Radwan *et al*, 2007). BYMV (family Potyviridae, genus Potyvirus) is an aphid transmitted virus in non-persistent mode and has a wide host range (Jones and Smith, 2004 & 2005). It causes a considerable destructive in seed yield, particularly, when plants were infected at the beginning stage of faba bean at the growing regions of Egypt. Systemic symptoms caused by BYMV infection do not kill faba bean plants, but have the potential to spread faster and further into the crop, causing greater overall yield reduction, despite inducing milder symptoms (Sidaros *et al*, 2006). BYMV are transmissible by mechanical inoculation and by aphid vectors in a non-persistent manner. More than 40 aphid species can transmit BYMV, including *Acyrtosiphon pisum*, *Aphis craccivora*, *Aphis fabae*, *Macrosiphum euphorbiae* and *Myzus persicae* (Bos, 1970).

The main propose of this work is to examine the ability of some aphid species to transmit and disseminate BYMV on faba bean, as well as certain relationship among them.

MATERIALS AND METHODS

The studies were conducted during the 2011 / 2012 and 2012/ 2013 seasons and were located at the green house of Faculties of Science, El-Azhar and Assiut University.

1-PLANTS

All the tested plants used to maintain the virus isolates and virus free aphid were grown in an artificial soil mix. It consisted of 1:1, peat moss: sand. Normal Hoaglands solution (Hewitt 1966) was watered into the mix once or twice weekly as necessary. The plants were grown in insect screened glasshouses and were treated weekly with insecticide. Giza cv461 faba bean was used. *Chenopodium quinoa* plants were used as indicator host for test inoculum of the BYMV. Seeds were sown in a mixture of sand and clay (1: 2 v/ v) in plastic pots (10 cm in diameter).

2-VIRAL ISOLATION

Twenty four samples of faba bean plants exhibiting virus-like symptoms were collected from eight different fields at Assiut Governorates. These samples were checked serologically against BYMV, alfalfa mosaic virus (AMV) and broad bean mosaic virus (BBMV) according to Hobbs *et al.*, 1987. These antibodies were obtained from the Agricultural Genetic Engineering Research Institute (AGERI), Egypt.

Samples giving only BYMV positive results were mechanically inoculated on *C. quinoa* and then isolated by single local lesion according to El-Afify *et al.*, 2007. The virus isolate was maintained on broad bean plants under greenhouse conditions for subsequent experiments.

3- REARING INSECTS:

Two sets of healthy Faba bean plants were sorted in two cages (36 x 40 x 60 cm), to maintain the virus- free aphid colonies. Two species of *Aphis craccivora* and *Myzus persicae* were collected from broad bean plants to obtain stocks of virus- free aphids. The plants were renewed weekly in each cage.

4-INSECT TRANSMISSION:

Two aphid species were used to compare the vector specificities to BYMV isolate. Insect from each aphid culture were starved for 24 hours at 4°C. Subsequently, the aphids given acquisition access three to five minutes to *V. faba* leaves infected with the virus isolate in Petri dishes and then transferred to healthy broad bean plants for 24 hours. They were then sprayed with an insecticide before their return to the glasshouse. The experiments were repeated four times. The aphid-isolate combination involved ten aphids per plant and ten plants per treatment. Probability of transfer (p) was calculated using the function of Burrows (1987):

$$p = 1 - [(2kR + k - 1) / (2kN + k - 1)]^{1/k}$$

where: N = number of test plants, R = number of healthy plants, and k = number of aphids transferred to a single host plant.

COMPARISON OF APHID SPECIES

Aphids from each colony were tested for their ability to transmit BYMV. *V. faba* plants were used as virus sources and as test plants. Each test was done in duplicate using ten aphids per plant. Acquisition and inoculation access periods were three days of each. Effect of number of each aphid on virus

transmission were allowed to feed on infected plants for 72 hours and then placed singly or in groups of five or ten on healthy broad bean seedlings. Subsequently, the aphids were killed with insecticide.

PERSISTENCE OF BYMV ON APHIDS:

Virus-free colony of *A. craccivora* and *M. persicae* given acquisition access to infected *V. faba* plants for 72 hours. Twenty aphids were then transferred singly onto new sets of healthy test plants every 24 hours until all the aphids had died. The stage of development of each aphid was recorded daily after they were transferred.

ACQUISITION THRESHOLD PERIOD:

A free- virus individuals of *A. craccivora* were given acquisition access for 10, 20, 30, 45 minutes or 1, 2, 4, 6, 12, 24, 48 or 72 hours. At the end of the respective acquisition access periods, the aphids were placed in groups of 10 aphids per plant of the healthy test plants until six days after commencement of the experiment.

INOCULATION THRESHOLD PERIOD:

Non-viruliferous individuals of *A. craccivora* were fed for 72 hours on infected plants and then transferred in groups of 10 to each test plant (ten test plants were used). The aphids were given access to the test plants for either 10, 15, 20, 25, 30 or 45 minutes or 1, 2, 4, 6, 12, 24, 48 or 72 hours. The aphids were killed as mentioned above.

RESULTS AND DISCUSSION

VIRUS ISOLATION

One collected samples which gave positive reaction against BYMV antiserum was only used as virus source to inoculate the plants of *C. quinoa* as a differential host. Inoculated *C. quinoa* leaves showed chlorotic local lesions. The virus was isolated via single chlorotic local lesion technique on faba bean three times.

Variations of symptoms were appeared after aphid transmission. The terminal bud twisted slightly after 6-7 days of inoculation, followed by initiation of very narrow leaves from new buds and was more evident after 15 days of inoculation (Fig. 1). As the time advanced, proliferation of both terminal and axillaries branches occurred resulting into long and thin branches consisting of very narrow leaves. The leaves below the proliferated branches exhibited yellowing or inter venial chlorosis or mosaic. The overall height of the plant was reduced. Such plants produced very few distorted flowers that converted to very small pods.

APHID TRANSMISSION

Result obtain in table (1) and Fig. (1) show that the individual of the two examined aphid species were able to transmit BYMV isolate. The *M. persicae* was able to transmit it more readily (82%) than the *A. craccivora* (70%). The efficiencies with the two aphids to transmit BYMV via individual insect were often at variance. *M. persicae* was more effective in transmitting BYMV (0.19). The work of Makkouk *et al.* (1990) reported that the virus affecting faba bean in West Asia and North Africa was identified as Broad Bean Wilt Faba virus (BBWV). The isolates from Syria and Egypt found to be positively transmitted non- persistently by 4 aphid species, most efficiency by *Myzus persicae*. The work of Su *et al.* (1989) declared that *M. persicae* was more suited to the transmission of BBWV than that of *A. craccivora*, even both of two species have the ability to disperse and transmit the virus. Manna *et al.* (1999) in their study on BBWV at Assiut locality found that two previous mention species were found on the infested faba bean plants. Therefore, the dominant density of *A. craccivora*

blamed it as responsible of virus dissemination. *A. cracivora* was the most dominant species during the two successive season of study. Several small colonies of *M. persicae* were scattered within those of *A. cracivora*. Therefore, the dominant density of *A. cracivora* blamed it as responsible of virus transmission.



Figure (1): Mosaic and vein-banding symptoms induced on broad bean leaves after *M. persicae* inoculation.

The relation among the density of aphid individuals on the host plant and the successful transmission of virus was estimated, data recorded in table 2. The rate of transmission increased when larger numbers of aphids were transferred from virus source to test plants. One hundred percent of transmission was obtained when ten *M. persicae* were allowed to feed on each test plant. These experiments indicated that *M. persicae* was efficient vectors of BYMV from infected broad bean plants to healthy ones, the percentage of transfer by a single aphid averaged 36 as compared with 12 in *A. crassefora*. The experiment showed that a single aphid is able to transmit BYMV to a healthy plant. This result confirms the data of Curpetino (1995) who reports that after a single aphid acquires the potato leaf roll virus (PLRV) from a potato plant, it is able to transmit it to a healthy *Physalis floridana* test plant.

Our results revealed that this virus disease has more chance to disseminate at high abundant level of aphid infestation. Also, the result was agreement with Lampert *et al.*, 1988, they reported significant results in tobacco etch virus (TEV) transmission by increasing aphid numbers per plant.

The acquisition access period (AAP) and the inoculation access period species were timed and illustrated in Table 3. The results suggested the suitable timing of these indicators when *M. persicae* was tested as BYMV vector. No transmission of BYMV was recorded when AAP was less than 45 minutes (Table, 3). Increasing the AAP beyond this time increased the efficiency of transmission and the optimal AAP was at least 24-48 hours. Similarly, *M. persicae* was able to transfer BYMV within 20 minutes after becoming infective. The rate of transmission increased as IAP was extended up to 6 hours. The *M. persicae* transmitted the BYMV with 100% efficiency when the IAP was 12 hours. These results revealed that *M. persicae* seems to be a successful vector and disseminator agent for BYMV. Aphid transmission of non-persistent viruses is almost completely restricted to very brief (5-10 seconds) intracellular punctures of epidermal or mesophyll cells (López *et al.*, 1969; Powell, 1991). A model in which egestion through the food canal injects virus into the plant does not take account of the anatomical fact that the food and salivary canals in the maxillary stylets fuse at 2-8 µm from the tips (Forbes, 1969).

In test to examine the persistence transmission of virus isolate by *M. persicae*, it was found that 5 of 13 aphids acquired virus and infected test plants. The aphids retained their infectivity after transfer to test plants and only one insect did so after developing into adult alatae. In the serial transfer tests, individual aphids varied considerably in their abilities to transmit the virus. Transmission usually occurred intermittently. *M. persicae* was efficient in virus transmission only during the first ten days after the commencement of the acquisition access period and then largely lost its transmitting ability at the subsequent transfer periods.

Table (1): The ability of Aphid species to transmit BYMV by aphid species.

Aphids	% transmission	Transmission efficiency*
<i>Aphis craccivora</i>	14/20 (70)**	0.12
<i>Muzus persicae</i>	19/22 (82)	0.19

*Probability of single aphids for transmission BYMV isolate.

**Number of infected plants/number of treated.

Table (2): Transmission (%) of BYMV by different numbers of aphids.

Aphids species	No. of aphid/plant		
	1	5	10
<i>Aphis craccivora</i>	12	73	92
<i>Muzus persicae</i>	36	84	100

Table (3): Effect of different acquisition and inoculation access periods on the transmission of BYMV by *M. persicae*.

Period	Transmission (%)	
	Acquisition	Inoculation
10 min	0.0	0.0
20 min	0.0	18.0
30 min	0.0	27.0
45 min	3.0	32.0
1 hr	5.0	48.0
1.5 hr	11.0	53.0
2 hr	12.0	86.0
4 hr	15.0	88.0
6 hr	38.0	95.0
12 hr	75.0	100
24 hr	81.0	100
48 hr	100	100

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