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Full Length Article

Transmission of *Cucurbit Chlorotic Yellows Virus* (*CCYV*) by Whitefly Biotype B in Riyadh, Saudi Arabia

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Abstract

Whitefly samples were collected from several greenhouses and identified as *Bemisia tabaci* by polymerase chain reaction (PCR), followed by sequencing of mtCOI gene that revealed their identity as biotype B MEAM1. Transmission of *Cucurbit chlorotic yellows virus* (CCYV) by the identified whitefly biotype was estimated by conducting several experiments. The results showed that a single whitefly was sufficient to transmit the virus to healthy seedlings. It was also found that the vector required a 90-min acquisition and inoculation access period to successfully transfer the virus. Moreover, vector was found to be able to retain the virus for 6 days' post acquisition. Efficiency of cucumber and *D. stramonium*, plants was compared as a source of CCYV inoculum for virus acquisition by *B. tabaci* and obtained results revealed that cucumber was a more efficient source of virus infection than *D. stramonium*. Using cucumber as source of infection, 90% of the inoculated plants (from each species i.e. cucumber and *D. stramonium*), were infected while using *D. stramonium*, as a source, the percentage of infection in cucumber plants dropped to 60% (cucumber) and to 50% in *D. stramonium*. These results showed that *D. stramonium*, is a relatively less efficient source of virus for whitefly acquisition as compared to cucumber. Host range experiments for CCYV showed that all the cucurbit plants were infected and had different levels of symptoms. *Cucumis sativus, C. melo, C. amaranticolor* and *D. stramonium* showed interveinal chlorosis, whereas general yellowing and reduced growth were observed in all positively tested hosts. © 2018 Friends Science Publishers

Keywords: *Bemisia tabaci*; Biotype B; CCYV; Host range; Transmission

Introduction

Crinviruses are single-stranded RNA viruses belonging to *Closterovirus*, one of the genera in the family *Closteroviridae*. Genera included in this family are *Ampelovirus*, *Closterovirus*, *Crinivirus* and the newly proposed *Velarivirus* (Al Rwahnih *et al*., 2012; Martelli *et al*., 2012). The family *Closteroviridae* consists of a diverse group of plant viruses having distinct particle shapes, lengths, vector transmission, phloem-limitation, cytopathology, genetic makeup, and expression (Karasev, 2000). *Criniviruses* are transmitted by whiteflies, *Ampeloviruses* by mealybugs, and *Closteroviruses* by aphids; the mode of transmission for *Velariviruses* has yet to be determined (Al Rwahnih *et al*., 2012). To date, only four of the fourteen *Criniviruses* have been reported to infect cucurbits: *Cucurbit yellow stunting disorder virus* (CYSDV), *Cucurbit chlorotic yellows virus* (CCYV), *Beet pseudo-yellows virus* (BPYV) and *Lettuce infectious yellows virus* (LIYV).

Criniviruses are typically transmitted in a semi-persistent manner and are retained in their vectors for a relatively short period. CYSDV and CCYV are transmitted by the *Bemisia tabaci* MEAM1 (biotype B) and MED (biotype Q) species complexes (Célix *et al*., 1996; Berdiales *et al*., 1999), while New World *B. tabaci* (biotype A) is an

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inefficient vector of CYSDV (Wisler and Duffus, 2001). LIYV is more efficiently transmitted by New World *B. tabaci* (biotype A) as compared to MEAM1 (biotype B) (Wisler *et al*., 1998). BPYV is transmitted by *Trialeurodes vaporariorum, which* is unique among *Criniviruses* infecting cucurbits. New hosts including, *Lactuca serriola L. salligna, Tribulus terrestris* and *Calotropis procera* are being added to the host range of CCYV and *Malva parviflora* is recently added to the host range of CYSDV (Shakeel *et al*., 2017).

Transmission factors of CCYV such as acquisition access period (AAP), inoculation access period (IAP), and persistence of virus in the vector, have never been determined; however, they are expected to resemble those exhibited by *Lettuce chlorosis virus* (LCV) based on the similarity of the transmission determinants (CP 76.9% and CPm 59.7%) (Abrahamian and Abou-Jawdah, 2014). *B. tabaci* is considered a cryptic species complex which contains several biotypes, which cannot be distinguished morphologically, but differences among members of this species complex can be studied at the molecular level, and members have full or incomplete reproductive isolation due to reproductive incompatibility (Oliveira *et al*., 2001; De Barro *et al*., 2011).This species complex has been found to have differential behavior in host range (Zang *et al*., 2006; Xu *et al*., 2011), virus transmission (Bedford *et al*., 1994; Li *et al*. 2010), insecticide resistance (Horowitz *et al*., 2005; Crowder *et al*., 2010; Luo *et al*., 2010; Wang *et al*., 2010), and interactions with viruses and host plants (De Barro and Bourne, 2010). Previous studies from Saudi Arabia showed that all tested whiteflies belonged to biotype B, except those from the Fayfa location, which were designated as biotype Q (Ragab, 2013; Shakeel *et al*., 2016). Lately, some studies have been carried out to estimate the transmission factors of CCYV by the vector using Real-Time PCR (Li *et al*., 2016) but have never been studied using the vector in greenhouse. This study aimed to identify the whitefly species and biotype in the Riyadh region, to test the efficiency of the identified biotype B to transmit CCYV, and to evaluate the efficiency of cucumber and weed plants as sources of infection.

Materials and Methods

Source of Virus and Whitefly and Biotype Identification using Molecular Methods

Plants showing typical yellowing disease symptoms accompanied by chlorotic spots and interveinal chlorosis were collected from greenhouse cucumber crops at the Al-Kharj location and tested against CCYV using specific primers (Hamed *et al*., 2011) by RT-PCR. Singly infected CCYV source plants were maintained in an insect-free isolated cage (Fig. 1) avoid introduction of any unknown viruses. Whiteflies were collected from tomato greenhouses and fed on healthy tomato plants (non-host to CCYV) for 3 months to ensure virus-free status.

Fig. 1: Aviruliferous whitefly colony established under controlled conditions at the greenhouses

To identify the whitefly biotype, DNA was isolated from single whitefly insect following the alkaline method as described by Wang *et al.* (2009). We targeted the mtCOI gene, which was amplified with specific primers (MD10: 5′-ttgatttttggtcatccagaagt-3′; MD12: 5′-tccaatgcactaatctgccatatta-3′) for identification of *B. tabaci* (Horowitz *et al*., 2005) using the KAPA2G Fast HotStart PCR Kit with dNTPs (KAPA Biosystems). The amplified product was visualized on a 1% agarose gel by electrophoresis, and 1Kb Plus DNA Ladder (Invitrogen) was used to determine the size of the fragment. To identify the whitefly biotype, three selected amplified PCR products were purified using the E.Z.N.A. Nucleic Acid Purification Kit following the manufacturer's protocol and sequenced in both directions (BGI Sanger Sequencing, Hong Kong). The obtained sequences were subjected to a BLASTX search for comparison of translated nucleotides with homologous sequences available in GenBank. Alignment and phylogenetic analyses were performed using DNASTAR software, including several amino acid whitefly isolates obtained from GenBank.

Determination of Minimum Acquisition and Inoculation Access Period

Healthy cucumber seedlings were grown in an insect-free environment until the three-leaf stage was attained. To determine minimum AAP, an average of 50 aviruliferous whiteflies were allowed to feed on RT-PCR-positive CCYV plants for a period of half, 1, 1.5, 2, 4, 8, 12, 24 or 48 h. On completion of each acquisition period, the whiteflies were transferred to 12 healthy cucumber seedlings, and were allowed to feed for an IAP of 48 h. To determine minimum IAP, on completion of 48 h of AAP, viruliferous whiteflies were transferred to test plants for an IAP of half, 1, 1.5, 2, 4, 8, 12, 24 or 48 h.

Determination of Minimum Number of Whiteflies Necessary to Transmit CCYV

Healthy plants were maintained in small plastic bottle cages with muslin cloth at the top to allow sufficient aeration and light. A single plant per pot per cage was maintained to confine the whiteflies to the leaves, and each treatment was replicated six times. Aviruliferous whiteflies were allowed to feed on CCYV-positive cucumber plants for a 48 h AAP. Healthy cucumber plants were inoculated with CCYV using 1, 5, 10, 20, 40 and 60 viruliferous whiteflies per plant for a 48 h IAP.

Determination of Retention of CCYV in Whitefly Vectors

The viruliferous whiteflies were allowed to feed on eight healthy cucumber seedlings for an IAP of 24 h. On completion of first treatment, whiteflies were transferred to a set of eight new seedlings and allowed to inoculate for another 24 h IAP. A new set of healthy seedlings was inoculated till 10th day post acquisition and labeled as D 0 to D10.

Efficiency of Different Hosts as a Source of Infection

RT-PCR was used to confirm CCYV infection in *D. stramonium* weed plants. A total of 400 aviruliferous whiteflies were allowed to feed on positive cucumber and *D. stramonium* source plants separately for a 48 h AAP and then transferred to a set of 10 healthy plants from each cucumber and *D. stramonium* for a 48 h IAP.

Maintenance of Inoculated Plants

Aviruliferous 60 whiteflies were allowed to feed on four healthy plants each of cucumber and *D. stramonium* seedlings for a 48 h AAP separately and then used to inoculate healthy control plants for a 48 h IAP for each experiment. On completion of all the experiment, inoculated plants were sprayed with Acetamiprid to kill the whiteflies and older leaves were cut off to avoid any hatching of whitefly eggs and unwanted virus transmission. These plants were kept in insect free cages for a period of three weeks to observe symptom development and testing by RT-PCR.

Host Range Studies of CCYV in Greenhouses

On average 50 viruliferous whiteflies were allowed to feed on each healthy seedling of the following host species: *Nicotiana glutinosa, Nicotiana occidentalis, Lagenaria siceraria, Chenopodium quinoa, Chenopodium amaranticolor, Citrullus lanatus, Cucumis sativus, Cucurbita pepo, Cucumis melo, Momordica charantia, Phaseolus vulgaris, Solanum melongena, Lactuca sativa, Amaranthus viridis, Solanum nigrum* and *Datura stramonium* for 48 h of IAP.

Results

Molecular Identification of Whitefly Biotypes

Identification of the collected whiteflies using morphological characters indicated that all the insects belonged to the *B. tabaci* species, which was further confirmed by PCR analysis. All the DNA products from the collected whitefly samples were found to be *B. tabaci*, as bands of expected size (800 bp) were amplified when specific primers were used (Fig. 2). The whitefly sequences obtained from mtCOI gene were submitted to NCBI, and the accession numbers KT946806, KT946807, and KT946805 were assigned to isolates 8TMD-SA, 9TMD-SA, and 12TMD-SA, respectively. Translated amino acid sequences of the obtained nucleotide sequences were aligned with 10 isolates obtained from NCBI (AEA92444, AGK85099, AEA92434, AEA36871, AEA36797, ADY16654, ACI41865, ACI41864, ACI41832, and ACI41722) and found to be 100% similar to *B. tabaci* MEAM1 (biotype B) by BLASTX analysis. Saudi Arabian isolates showed 100% similarity with *B. tabaci* MEAM1 biotype B. A phylogenetic tree constructed using amino acid sequences of whitefly samples (using DNASTAR software) showed that all Saudi Arabian isolates fell in the same cluster as the other isolates obtained from GenBank (Fig. 3).

Determination of Minimum Acquisition and Inoculation Access Periods

Most of the inoculated plants developed clear CCYV symptoms and had positive PCR reactions when tested three weeks after inoculation (Table 1). All the results were confirmed using RT-PCR, and fragments of expected size (760 bp) were obtained from the positive samples. The results revealed that the vector requires a minimum of 90 min to acquire CCYV from infected plants and 90 min to inoculate healthy seedlings. The incidence of CCYV in cucumber plants was found to be 0, 0, 33, 50, 50, 83, 83, 83, 100 and 100% at half, 1, 1.5, 2, 4, 8, 12, 24 and 48 h of AAP respectively. The IAP experiments showed that 0, 0, 50, 66, 50, 66, 83, 83, 100 and 100% of cucumber plants were infected by CCYV at half, 1, 1.5, 2, 4, 8, 12, 24 and 48 h, respectively.

Determination of Minimum Number of Whiteflies Required to Transmit CCYV

The results revealed that one whitefly was sufficient to transfer CCYV to healthy plants. Testing plants from different treatments showed a variable rate of infection that generally increased with increasing numbers of insects. Infected plants showed typical CCYV symptoms three weeks post inoculation. RT-PCR results also showed that 33, 66, 66, 83, 83 and 100% of the inoculated plants were infected when treated by 1, 5, 10, 20, 40 and 60 virus-carrying insects, respectively (Table 2).

Table 1: Determination of CCYV transmission by different numbers of whiteflies

No. of Insects	Tested Plants	Symptomatic Plants	RT-PCR-Positive reaction for CCYV
1	12	0	2
5	12	2	4
10	12	4	4
20	12	8	8
40	12	8	8
60	12	10	10

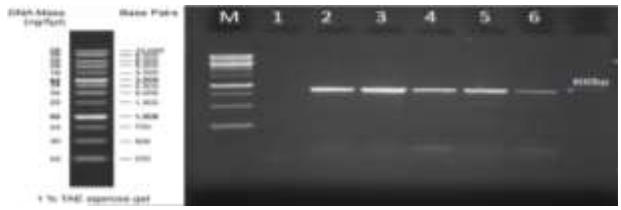

Fig. 2: Agarose gel (1%) electrophoresis showing amplification of whitefly bands with an expected size of 800 bp using *Bemisia tabaci*-specific primers (MD10/12) (Lane 2–6). Lane 1: No PCR product was amplified, with DNA extracted from *Myzus persicae* as a negative control. Lane M: 1-kb Plus DNA Ladder (Fisher Scientific)

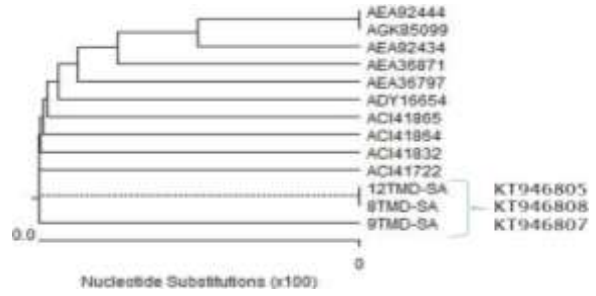

Fig. 3: Phylogenetic tree based on three Saudi Arabian amino acid sequences of *Bemisia tabaci* and 10 isolates from other countries. All isolates fell in the same cluster, showing a close relationship. Tree was constructed by DNASTAR software

Determination of Virus Retention in the Vector

Positively Infected of tested plants was expressed typical CCYV symptoms three weeks post inoculation and found to be 87, 75, 87, 75, 50 and 50% at 1, 2, 3, 4, 5 and 6 d after acquisition, respectively. All results were confirmed using RT-PCR, and fragments of expected size (760 bp) were obtained from the positive samples. All plants inoculated after 6 d neither expressed symptoms nor reacted positively to RT-PCR (Table 3).

Host Efficiency Determination

The obtained results showed that, cucumber was a more efficient source of virus than *D. stramonium*. As a source, cucumber could infect 90% (9 of infected plants/10 of inoculated plants are positive) of the seedlings of cucumber and *D. stramonium* seedlings three weeks post inoculation. While, when *D. stramonium* was used as a source of CCYV, only 60% (6/10 are positive) of cucumber and 50% (5/10 are positive) of *D. stramonium* seedlings were found to be infected with CCYV. The efficiency of the host was determined by the results of RT-PCR using CCYV specific primer that amplifies a product of expected size of 760 bp (Fig. 4).

Table 2: Determination of minimum AAP and IAP required by whiteflies to acquire CCYV from infected cucumber

AAP/IAP Duration (h)	Tested plants	Symptomatic plants		RT-PCR-positive reaction for CCYV	
		AAP	IAP	AAP	IAP
1/2	12	0/12	0/12	0	0
1	12	0/12	0/12	0	0
1.5	12	2/12	6/12	4	6
2 h	12	6/12	6/12	6	8
4	12	6/12	4/12	6	6
8	12	10/12	8/12	10	8
12	12	10/12	10/12	10	10
24	12	8/12	10/12	10	10
48	12	12/12	12/12	12	12

Table 3: Determination of CCYV retention in the whitefly vector

Day of inoculation	Tested plants	Symptomatic plants	RT-PCR-Positive for CCYV
1	8	7/8	7
2	8	6/8	6
3	8	7/8	7
4	8	6/8	6
5	8	4/8	4
6	8	4/8	4
7	8	0/8	0
8	8	0/8	0
9	8	0/8	0
10	8	0/8	0

Experimental Host Range of CCYV

Infection results of different hosts inoculated with CCYV are shown in Table 4 and Fig. 5. The plant species infected by CCYV were *N. glutinosa, C. quinoa, C. amaranticolor, L. siceraria, C. lanatus, C. sativus, C. pepo, C. melo, M. charantia, L. sativa S. nigrum,* and *D. Stramonium* maintained in isolated insect cages. RT-PCR products showing positive and negative results from different plant species were visualized on a 1% agarose gel, and the fragments of expected size (760 bp) were obtained from the positive samples (Fig. 6).

Discussion

All whitefly samples collected from several hosts from the Riyadh region were analyzed at the amino acid level and

Table 4: Incidence of CCYV infection in different hosts tested by RT-PCR

Species	Tested Plants	Symptomatic Plants	RT-PCR-positive reaction for CCYV
N. glutinosa	10	2/10	4/10
N. occidentalis	10	5/10	5/10
C. quinoa	10	0/10	6/10
C. amaranticolor	10	2/10	5/10
C. lanatus	10	7/10	7/10
C. sativus	10	9/10	9/10
C. pepo	10	6/10	7/10
C. melo	10	8/10	9/10
M. charantia	10	6/10	6/10
P. vulgaris	10	0/10	0/10
S. melongena	10	0/10	0/10
L. sativa	10	6/10	7/10
*A. viridis*L.	10	0/10	0/10
S. nigrum	10	0/10	0/10
D. stramonium	10	6/10	6/10
L. siceraria	10	9/10	9/10

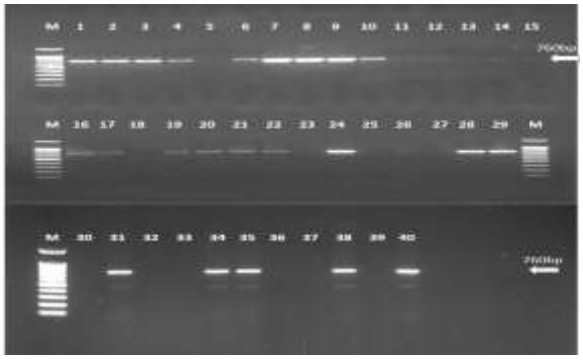

Fig. 4: Agarose gel (1%) electrophoresis to determine host efficiency as a source of CCYV infection. Amplification of the CCYV-specific band (760 bp) in cucumber and *D. stramonium* plants three weeks post inoculation using cucumber and *D. stramonium* plants as a source of virus. Lane M: Ladder, Lanes 1–10: CCYV-positive cucumber as source to infect healthy cucumber (9/10 positive), Lanes 11–20: CCYV-positive cucumber as a source to infect healthy *D. stramonium*(9/10 positive), Lanes 21–30: CCYV-positive *D. stramonium*as a source to infect healthy cucumbers (6/10 positive), Lanes 31–40: CCYV-positive *D. stramonium*as a source to infect healthy *D. stramonium*(5/10 positive)

were identified as biotype B (MEAM1). All sequences were 100% similar to *B. tabaci* biotype B obtained from NCBI. During 2011–2012, several whitefly samples were collected from 22 localities in Saudi Arabia as well as from Qatar, Bahrain, Kuwait, and Sudan. This extensive survey and testing showed that all samples from all locations belonged to *B. tabaci* biotype B except those from the Fayfa location, which belonged to biotypes A and Q (Ragab, 2013). Another study from the Al-Ahsa region reported that no biotype of *B. tabaci* was identified other than biotype B (Alhudaib *et al*., 2015).

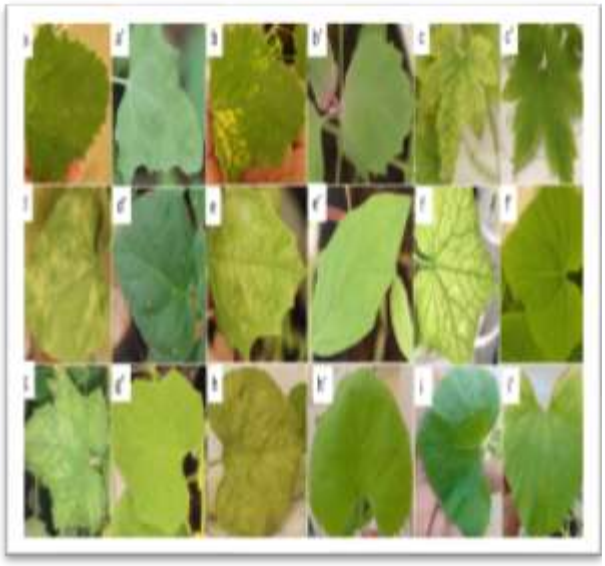

Fig. 5: Symptoms produced on different plant species included in the CCYV host range experiment. Symptoms on the leaves of different species of plants including (a) *C. quinoa*, (b) *C. amaranticolor*, (c) watermelon, (d) *N. glutinosa*, (e) *D. stramonium*, (f) cucumber, (g) bottle gourd, (h) melon, (i) *S. nigrum*, and (j) pumpkin. All captions with apostrophes are controls of the same species for comparison

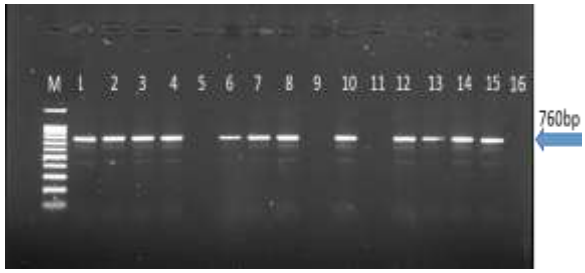

Fig. 6: Agarose gel (1%) electrophoresis showing the CCYV-specific band (760 bp) amplified from different plant species included in the experimental host range studies. Lane 1:*N. glutinosa;* Lane 2: *C. quinoa*; Lane 3: *C. amaranticolor;* Lane 4: *L. sativa;* Lane 5: *S. nigrum;* (negative); Lane 6: *C. lanatus*; Lane 7: *C. sativus*; Lane 8: *C. pepo;* Lane 9: *A. viridis*(negative); Lane 10: *C. melo;* Lane 11: *P. vulgaris* (negative); Lane 12: *N. occidentalis;* Lane 13: *N. occidentalis;*Lane 14: *D. stramonium*; Lane 15: *M. charantia;* Lane 16: *S. melongena*(negative). Lane M: 100-bp DNA ladder molecular weight marker (Promega)

The results of this study showed that one *B. tabaci* biotype B (MEAM1) individual was able to efficiently transmit CCYV to healthy cucumber plants. Whitefly population experiments revealed that the percentage of

infection gradually increased as the number of whiteflies used for inoculation increased, reaching 100% when 60 insects were confined per plant. None of the cucumber plants inoculated with one whitefly per plant showed symptoms, regardless of infection, until five weeks post inoculation. In two similar studies, CYSDV showed a transmission efficiency that ranged between 85 and 100% with a higher number of whiteflies (Célix *et al*., 1996; Berdiales *et al*., 1999). Previous studies showed that under controlled conditions, CYSDV is transmitted with similar efficiency by biotypes B and Q of *B. tabaci* (Guirao *et al*., 1997), but biotype A was found to be less efficient (Wisler *et al*., 1998). Detailed investigation showed that one and half hour was sufficient for the whitefly to acquire the virus from infected cucumber plants and another one and half hour was sufficient to infect healthy cucumber seedlings. The percentage of infection increased gradually and reached 100% when the AAP and IAP were maintained at 48 h.

Transmission of CCYV by whitefly vectors is thought to resemble that of LCV, as it has a higher level of genomic similarity in the CP and CPm regions (Célix *et al*., 1996; Berdiales *et al*., 1999). With regard to the transmission efficiency of LCV by *B. tabaci* biotype A, a single whitefly is capable of transmitting the virus with a 24 h AAP. In separate experiments conducted to estimate AAP and IAP, it was found that 1 h is sufficient to acquire and inoculate the virus. In addition, the level of infection and severity of symptoms are highly correlated with the number of insects and duration of AAP and IAP. Studies to estimate the persistence of virus in the vector were performed by daily serial transfer of virus-carrying vectors to healthy plants and they showed that *B. tabaci* biotype A loses its ability to cause LCV infection on day 5 (Duffus *et al*., 1996). In a recent study, the retention of CCYV virus particles in *B. tabaci* biotype Q (MED) was estimated to be 6 d, while at least 1 h was required to acquire and 1 h to transmit the virus. This study included testing the presence of virus in the vector foregut using RT-PCR (Li *et al*., 2016). A few other viruses from the same family showed variable retention in their vectors, including BPYV, 7 d; CYSDV, 9 d; TICV, 3 d (Wisler *et al*., 1996); and ToCV, 1 d (Wisler *et al*., 1997, 1998). Variation in vector transmission efficiency could be related to environmental conditions, the whitefly biotype involved, or crop variety.

Testing of different weeds or cultivated crops as a source of CCYV to infect cucumber or melon plants has not been performed. Further studies are needed to evaluate the role of weeds or non-crop plants as infection sources of CCYV (Okuda *et al*., 2010). In the present study, infected *D. stramonium* and cucumber plants were used as the source of CCYV infection, and cucumber was found to be a more efficient source of virus than *D. stramonium.* Infection percentage was 90% in both cucumber and *D. stramonium*, whereas the infection level dropped to half when Datura was used as a source. A possible reason for the enhanced efficiency of cucumber as a source of CCYV is the higher accumulation of viral particles.

A recent study demonstrated the relationship between CYSDV virus titer in different hosts and transmission efficiency (Wintermantel *et al*., 2016). Accumulation of the virus, the relationship between virus titer in source plants and transmission by whiteflies, and the subsequent accumulation in inoculated cucurbit plants were studied in several host plants. Cucurbits were found to be the most efficient source of virus and had the highest titer of CYSDV, whereas non-cucurbit plant species had a significantly lower virus titer and hence acted as relatively inefficient sources of infection. Factors other than virus titer, such as physical and environmental conditions, have also been demonstrated to affect the efficiency of plants as virus sources; e.g., CYSDV titer was not positively correlated with transmission efficiency of whitefly in *P. vulgaris* and *Capsella bursa-pastoris* (Wintermantel *et al*., 2016). Similarly, tomato plants infected with *Tomato chlorosis virus* (ToCV) were found to be a more efficient source than *S. nigrum* based on the number of infected plants and estimated concentration of ToCV in source plants using RT-PCR. The efficiency was attributed to the higher concentration of ToCV in tomato or possible host preferences of the vector (Orfanidou *et al*., 2016).

Host range experiments other than determination of CCYV transmission efficiency by the identified *B. tabaci* biotype B were also conducted. In the host range experiments, 12 different hosts showed different levels of infection. Although all cucurbit species were found to be infected systemically, most of them exhibited unclear symptoms. Cucumber, melon, *C. amaranticolor* and *D. stramonium* showed interveinal chlorosis and general yellowing, while reduced growth was found in all positively tested hosts. Few new arable weed species are also added to the natural host range of this virus, lately (Shakeel *et al*., 2018).

In a study by Okuda *et al.* (2010), systemically infected plants of *C. pepo* remained asymptomatic and other infected species showed varying levels of infection and symptom expression. Among the other plant species tested, *L. sativa, D. stramonium, Nicotiana benthamiana* and plants in the family *Chenopodiaceae* (*Beta vulgaris, C. amaranticolor, C. quinoa,* and *Spinacia oleracea*) were all infected with CCYV at a high rate. In *C. amaranticolor, C. quinoa,* and *S. oleracea,* CCYV infection led to yellowing or leaf-rolling symptoms. Systemically infected plants of *D. stramonium, N. benthamiana*, and *L. sativa* showed no symptoms in the inoculated and upper leaves. In the present study, *N. benthamiana* remained negative, while other two species showed mild symptoms that could be attributed to a variant local strain. The measured parameters supported the ability of the vector to acquire and transmit the virus quickly, which is supported by a number of cultivated and arable weed hosts. A single whitefly is capable of transmitting the virus within a 90 min acquisition and 90

min inoculation access period. In combination with vector management, weed eradication from the greenhouse vicinity can significantly lower the probability of developing yellowing disease in crops.

Conclusion

B. tabaci (MEAM1) was found to be an efficient vector of CCYV as a single whitefly was able to transmit the virus and it required an acquisition time as little time as one and half hour for acquisition and another one and a half hour of inoculation to spread the disease. Also, the vector can retain the virus for six days after acquisition. The efficient vector helps the virus to get established in any area very quickly and makes it difficult to control. Apart from the role of vector, the arable weeds are working as a virus reservoir during the off-season and become a source on the onset of planting time. Although, the efficiency of weed plants was found to be as little as half of the efficiency of cultivated crops but it is significantly sufficient to spread CCYV to the cultivated crop in a short period. These attributes add up to the chances of getting an infection by this virus before symptoms get noticed and managed. Along with vector management, weed eradication from greenhouse vicinity can significantly lower the possibility of developing the yellowing disease in the crops. In the case of the farming pattern followed in Riyadh region, distant location of greenhouses is a natural advantage in avoiding the yellowing disease.

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