SHORT COMMUNICATION

Communicative & Integrative Biology 5:6, 543–545; November/December 2012 © 2012 Landes Bioscience

High efficient of females of B-type *Bemisia tabaci* as males in transmitting the whitefly-borne *tomato yellow leaf curl virus* to tomato plant with Q-PCR method confirmation

Wen Xie,[1] Yan-xia Xu,[1] Xiao-guo Jiao[2] and You-jun Zhang[1,*]

[1]Department of Plant Protection; Institute of Vegetables and Flowers; Chinese Academy of Agricultural Sciences; Beijing, P.R. China; [2]Faculty of Life Sciences; Hubei University; Wuhan, P.R. China

Keywords: B-biotype *Bemisia tabaci*, TYLCV, female, male, quantitative real-time PCR

It has been previously recorded that TYLCV can be transmitted from viruliferous males to non-viruliferous females and from viruliferous females to non-viruliferous males, but not between insects of the same sex; female whiteflies transmit TYLCV-Is with higher efficiency than males through symptoms recognition and viral DNA identification in tomato test plants (one insect per plant, with 48 h AAP and 48 h IAP). However, it remains unclear whether non-infected female and male could obtain same virus from TYLCV-infected tomato plants, and whether TYLCV-infected female and male could transmit same virus to non-viruliferous tomato plants. To address this issue, quantitative real-time PCR were applied to detect TYLCV content in adults or tomato plant. The acquisition and transmission experiments showed that both female and male can acquire and transmit the virus and no acquisition capability difference was observed between newly emerged female and male, however, female demonstrated superior transmission capability than male. Moreover, gene expressions profilings of *GroEL* and *Hamiltonella* in non-viruliferous and viruliferous female was all higher than that in male. These results further indicated that sex is an important factor affecting TYLCV transmission efficiency in *B. tabaci*.

The whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), is a phloem-feeding insect pest that causes severe damage in both agricultural and horticultural systems worldwide. It has been previously reported that TYLCV can be transmitted between whiteflies of the B biotype in a sex-dependent manner in the absence of any other source of the virus; Meanwhile, female whiteflies transmit TYLCV-Is (the virus isolated from Israel) with higher efficiency than males (100% vs 20%) through virus-symptoms recognition and viral DNA identification in tomato test plants.[1,2] However, the reason of this difference is not clear until now. Whether existed difference in the acquisition and transmission capability of TYLCV by female and male, especially the TYLCV quantities of acquisition and transmission by female and male at different period of time are not clear and necessary to carry out more experiments.

To examine sex difference in the acquisition and transmission capability of TYLCV by female and male, quantitative real-time PCR described in Pan et al. (2012)[3] were conducted to quantify the relative gene expressions of TYLCV in *B. tabaci* female and male or in tomato plant. For acquisition comparison of TYLCV by female and male of *B. tabaci* B biotype, relative gene expressions of TYLCV in *B. tabaci* female and male were measured using a qRT-PCR after non-viruliferous whiteflies fed on the TYLCV-infected tomato plants for 3, 6, 12, 18, and 24 h. While for transmission comparison of TYLCV from viruliferous females or males to non-viruliferous tomato plant, relative gene expressions of TYLCV in tomato leaf were measured using a qRT-PCR after viruliferous whiteflies fed on the non-viruliferous tomato plants for 7, 14, 21, and 28 day. Meanwhile, the GroEL homologue seems to bind to and protect begomoviruses from degradation in the haemolymph; disturbing the GroEL-TYLCV association leads to the degradation of the virus and to a markedly decrease in transmission efficiency of the virus.[4,5] Recent research has revealed that the S-symbiont *Hamiltonella* is closely associated with the transmission efficiency of TYLCV by the whitefly vector.[6] In this study, gene expressions profiling of *GroEL* and *Hamiltonella* in non-viruliferous and viruliferous whiteflies were also obtained, after non-viruliferous whiteflies fed on the TYLCV-infected tomato plants for 0, 12, and 24 h. Primer sequences used for real-time PCR analysis are shown in **Table 1**.

The data obtained showed that no significant difference were observed in acquisition of TYLCV between newly emerged female and male of *B. tabaci* B biotype in each of the five AAP (acquisition access period) (**Fig. 1**). Through our previous report, the virus acquisition and transmission capability of Q biotype

*Correspondence to: Youjun Zhang; Email: zhangyj@mail.caas.net.cn
Submitted: 07/03/12; Revised: 08/01/12; Accepted: 08/01/12
http://dx.doi.org/10.4161/cib.21692

Table 1. Primer sequences used for real-time PCR analysis

Gene Name	Primer Name	Primer Sequence	Amplicon Size	References
TYLCV	TY-F	GTC TAC ACG CTT ACG CC	144	[3]
	TY-R	GCA ATC TTC GTC ACC C		
GroEL	Groq-F	CAT TCC GCC CAT TCC ACC	157	[4-5]*
	Groq-R	CAC GTT CTG CAT TGC AAT AT		
Hamiltonella 16S rDNA	Ham-F	GCA TCG AGT GAG CAC AGT TT	243	[7]
	Ham-R	TAT CCT CTC AGA CCC GCT AGA		
β-actin	β-actin-F	TCT TCC AGC CAT CCT TCT TG	130	[7]
	β-actin-R	CGG TGA TTT CCT TCT GCA TT		
Tomato 25S rRNA	25S-F	ATA ACC GCA TCA GGT CTC CA	113	[8]
	25S-R	CCG AAG TTA CGG ATC CAT TT		

*Designed from the full length of *GroEL*.

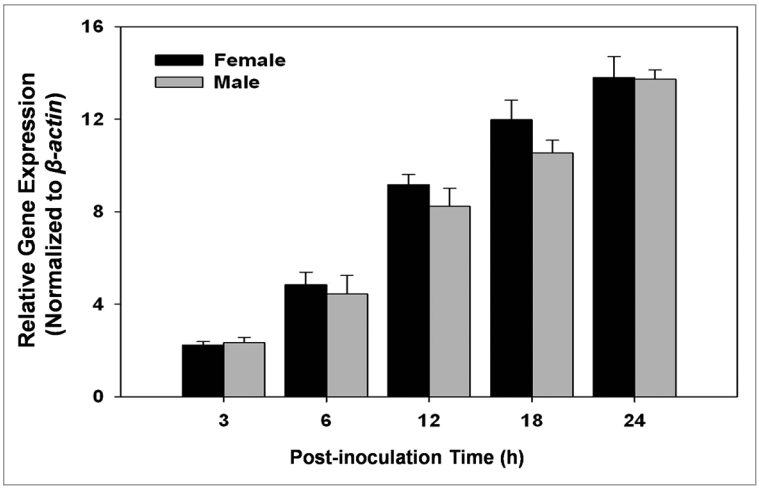
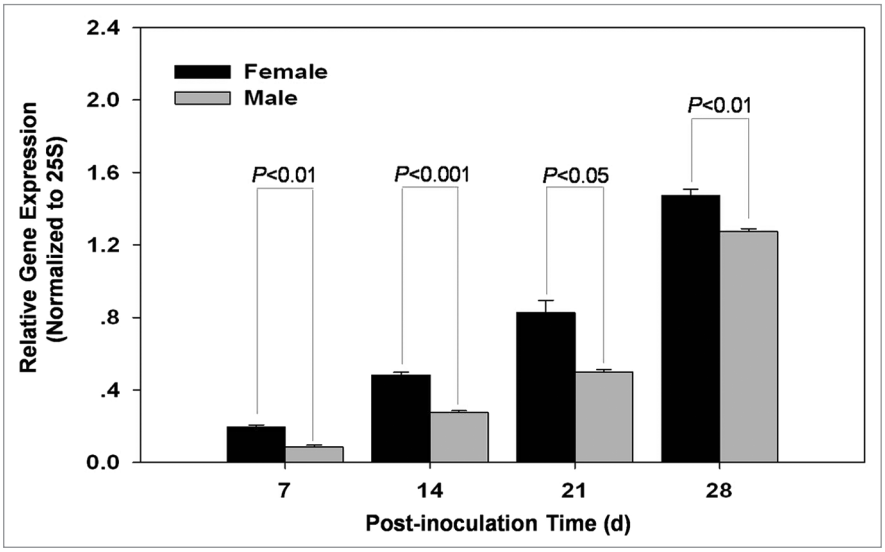


Figure 1. Acquisition of TYLCV by *B. tabaci* B biotype. Relative gene expressions of TYLCV in *B. tabaci* female and male were measured using a qRT-PCR after non-viruliferous whiteflies fed on the TYLCV-infected tomato plants for 3, 6, 12, 18, and 24 h. Three biological replicates were used and each represented 50 females or males. No differences were denoted based on two-sample unpaired *t*-test. All *p*-values at each of the five AAP are above 0.05 (0.7123, 0.6985, 0.6334, 0.4982 and 0.9882).



was significant higher than that of the B biotype through same qPCR analysis method.[3] In this study, further result indicated that virus transmission capability difference not only existed among biotypes, but also in sexes.

Moreover, two sets of experiments were carried out for the sex-virus combinations (viruliferous female or male with non-viruliferous tomato plants, respectively, during a 48 h IAP (inoculation access period) following a 24 h AAP). The qPCR results of TYLCV in tomato leaf in each of post-inoculation time, indicated that female exhibited significantly higher tomato-transmission frequency than male (**Fig. 2**). In the meanwhile, higher gene expression of *GroEL* and *Hamiltonella* were observed in female than that in male, no matter infected TYLCV or not (**Fig. 3**). Higher tomato-transmission frequency in female than male might be related with the higher *GroEL* product in female.

Evidently, the results of this study and previous report of female whiteflies transmit TYLCV-Is with higher efficiency than males through symptoms recognition and viral DNA identification in tomato test plants, indicate that sex bias in transmission TYLCV of *B.tabaci* were obvious, especially female show higher capacity in obtain virus from matting and transmitting virus to healthy tomato plants.[1,2] To our knowledge, this is the first report of sex acting as an important factor for TYLCV

Figure 2. Transmission of TYLCV from viruliferous females or males of *B. tabaci* B biotype to non-viruliferous tomato plant. Relative gene expressions of TYLCV in tomato leaf were measured using a qRT-PCR after viruliferous whiteflies fed on the non-viruliferous tomato plants for 7, 14, 21, and 28 day. 50 viruliferous females or males were used to infect a tomato plant with non-TYLCV, during a 48 h IAP, following a 24 h AAP in order to obtain identical virus content whiteflies. Three biological replicates were used and 50 whiteflies per plant. Significant differences were denoted based on two-sample unpaired *t*-test.

transmission in *B.tabaci*. Sex bias in gene expression is being analyzed and documented in this insect species recently (unpublished data), which may provide more information aimed to explain this phenomenon.

Declaration of Potential Conflicts of Interest

The authors have declared that no conflict of interest exists.

Acknowledgments

This research was supported by the National Science Fund for Distinguished Young Scholars (31025020), the 973 Program (2009CB119200 and 2009CB119004), the National Natural Science Foundation of China (No. 30900153), and the Key Laboratory of Biology and Genetic Improvement of Horticultural Crops, Ministry of Agriculture, P.R. China.

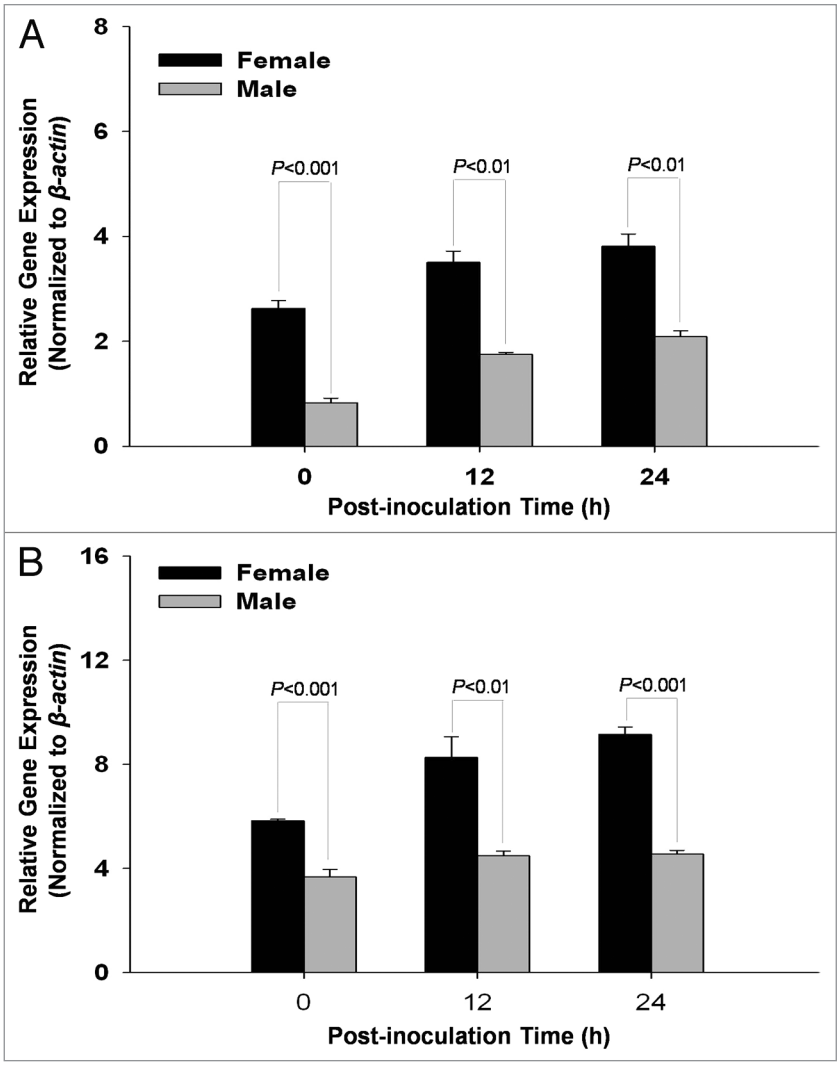


Figure 3. Gene expressions profiling of *GroEL* **(A)** and *Hamiltonella* **(B)** in non-viruliferous and viruliferous whiteflies. Relative gene expressions after non- viruliferous whiteflies fed on the TYLCV-infected tomato plants for 0, 12, and 24 h. Three biological replicates were used and each represented 50 females or males. Significant differences were denoted based on two-sample unpaired *t*-test.

References

1. Cohen S, Nitzany FE. Transmission and host range of the tomato yellow leaf curl virus. Phytopathology 1966; 56:1127-31.
2. Czosnek H, Ghanim M, Rubinstein G, Morin S, Fridman V, Zeidan M. Whiteflies: vectors - or victims? - of geminiviruses. In: Maramorosch K (ed.) Advances in virus research. Academic, New York; 2001.
3. Pan HP, Chu D, Yan WQ, Su Q, Liu BM, Wang SL, et al. Rapid spread of tomato yellow leaf curl virus in China is aided differentially by two invasive whiteflies. PLoS One 2012; 7:e34817; PMID:22514670; http://dx.doi.org/10.1371/journal.pone.0034817.
4. Morin S, Ghanim M, Sobol I, Czosnek H. The GroEL protein of the whitefly *Bemisia tabaci* interacts with the coat protein of transmissible and non-transmissible begomoviruses in the yeast two-hybrid system. Virology, 2000; 276: 404 - 416; PMID: 11040131; http://dx.doi.org/10.1006/viro.2000.0549.
5. Morin S, Ghanim M, Zeidan M, Czosnek H, Verbeek M, van den Heuvel JFJM. A GroEL homologue from endosymbiotic bacteria of the whitefly *Bemisia tabaci* is implicated in the circulative transmission of tomato yellow leaf curl virus. Virology, 1999; 30: 75 - 84; PMID: 10087228; http://dx.doi.org/10.1006/viro.1999.9631.
6. Gottlieb Y, Zchori-Fein E, Mozes-Daube N, Kontsedalov S, Skaljac M, Brumin M, et al. The transmission efficiency of *tomato yellow leaf curl virus* by the whitefly *Bemisia tabaci* is correlated with the presence of a specific symbiotic bacterium species. J Virol 2010; 84:9310-7; PMID:20631135; http://dx.doi.org/10.1128/JVI.00423-10.
7. Brumin M, Kontsedalov S, Ghanim M. *Rickettsia* influences thermotolerance in the whitefly *Bemisia tabaci* B biotype. Insect Sci 2010; 18:57-66; http://dx.doi.org/10.1111/j.1744-7917.2010.01396.x.
8. Mason G, Caciagli P, Accotto GP, Noris E. Real-time PCR for the quantitation of *Tomato yellow leaf curl Sardinia virus* in tomato plants and in *Bemisia tabaci*. J Virol Methods 2008; 147:282-9; PMID:17980920; http://dx.doi.org/10.1016/j.jviromet.2007.09.015.