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

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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 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*.
was significant higher than that of the B biotype through same qPCR analysis method. 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. To our knowledge, this is the first report of sex acting as an important factor for TYLCV

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

| Gene Name | Primer Name | Primer Sequence | Amplicon Size | References |
|-----------|-------------|----------------|--------------|------------|
| TYLCV     | TY-F        | GTC TAC AGC CTT AGC C | 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       | CGG 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).

**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 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.

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![Figure 3](image)

**Figure 3.** Gene expression 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.

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