Silencing the ecdysone synthesis and signaling pathway genes disrupts nymphal development in the whitefly

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A B S T R A C T

Sap-sucking insects are important pests in agriculture and good models to study insect biology. The role of ecdysone pathway genes in the life history of this group of insects is largely unknown due to a lack of efficient gene silencing methods allowing functional genetic analyses. Here, we developed a new and high throughput method to silence whitefly genes using a leaf-mediated dsRNA feeding method. We have applied this method to explore the roles of genes within the molting hormone-ecdysone synthesis and signaling pathway for the survival, reproduction and development of whiteflies. Silencing of genes in the ecdysone pathway had a limited effect on the survival and fecundity of adult whiteflies. However, gene silencing reduced survival and delayed development of the whitefly during nymphal stages. These data suggest that the silencing method developed here provides a useful tool for functional gene discovery studies of sap-sucking insects, and further indicate the potential of regulating the ecdysone pathway in whitefly control.

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1. Introduction

Sap sucking insects, including whiteflies, aphids, thrips, leaf-hoppers, planthoppers and mealybugs, are important agricultural pests (Consortium, 2010; Hogenhout et al., 2008; Morse and Hoddle, 2006; Oliveira et al., 2001). This group of insects can serve as a good model to study insect biology (e.g. development, growth and reproduction) and ecology (e.g. insect interactions with plants, viruses and endosymbionts) (Brumin et al., 2012; Götz et al., 2012; Liu et al., 2007; Luan et al., 2011, 2013).

The ecdysone synthesis and signaling pathway is conserved among insect taxa (Brown et al., 2009; Fahrbach et al., 2012; Nakagawa and Henrich, 2009). The Halloween genes coding for the P450 enzymes are responsible for the synthesis of ecdysone and 20-hydroxyecdysone (20E), major insect molting hormone, often referred to informally as ecdysone, and Cyp18a1 is involved in 20E inactivation (Brown et al., 2009; Christiaens et al., 2010; Guittard et al., 2011; Rewitz et al., 2010). 20E, bound to its receptor, directly induces the ‘early’ genes. Then these genes induce ‘early—late’ genes and ‘late’ genes that play a more direct role in controlling the biological responses to the hormone (Christiaens et al., 2010; Fahrbach et al., 2012; Thummel, 2002). It has been demonstrated that the ecdysone synthesis and signaling pathway is involved in the regulation of insect growth, development and reproduction (Fahrbach et al., 2012; Kozlova and Thummel, 2003; Shirk et al., 1990; Thummel, 2001). Many studies have explored the molecular basis of ecdysteroid function during development and reproduction in holometabolous insects (Brown et al., 2009; Fahrbach et al., 2012; Rewitz et al., 2010; Thummel, 2001). In contrast, few studies have investigated the role of ecdysone synthesis and signaling pathway genes in the life history of hemimetabolous insects, especially those of agricultural importance such as sap sucking insects (Cruz et al., 2006, 2007; Erezyilmaz et al., 2006; Mané-Padrós et al., 2008; Martín et al., 2006).

Although the functional gene discovery in sap sucking insects has been recently studied (Ghanim et al., 2007; Pitino et al., 2011; Zha et al., 2011), our understanding of the molecular events underlying the biology of these insects is still scarce, likely because of a lack of high throughput methods allowing large functional genetic analyses (Li et al., 2013). Suppressing the expression of specific genes using RNA interference (RNAi) has been widely used for reverse genetics research in insects. Direct dsRNA injection and feeding through artificial diet or transgenic plants are the most commonly used methods to silence insect genes. dsRNA injection is
more suitable for large insects; while injecting small insects (the size of a few mm) causes high mortality due to physical damage during handling (Barchuk et al., 2008; Ghani et al., 2007). dsRNA fed to some insect species together with an artificial diet, for example through membranes, is effective in repressing gene expression (Araujo et al., 2006; Upadhyay et al., 2011). However, it is difficult to silence genes during the larval stages of some insects (e.g. whitefly *Bemisia tabaci*) using this method. Recently, the expression in transgenic plants of dsRNA directed against insect genes has been shown to be effective (Mao et al., 2007; Pitino et al., 2011; Zha et al., 2011). However, the long time period required to generate transgenic plants significantly delays the results, and the silencing of several genes necessitates numerous plants.

Here, we have developed an efficient system to silence genes of the whitefly *B. tabaci* through leaf-mediated dsRNA feeding. We have applied this method to silence genes of the ecdysone synthesis and signaling pathway and examined the effect of its silencing on the whitefly development. We show that our method is efficient in silencing genes in the ecdysone synthesis and signaling pathway of whiteflies. Down-regulation of these genes affected the development and survival of whitefly nymphs.

2. Materials and methods

2.1. Plant and whitefly

Tomato plants (*Solanum lycopersicum* cv. Daniella) were grown in a greenhouse under controlled conditions. All plants were grown to the 5–6 true leaf stage for experiments. The population of whitefly *B. tabaci* Middle-East-Asia Minor 1 species (previously known as the ‘B biotype’), as defined recently (De Barro et al., 2011; Liu et al., 2012), was maintained on tomato plants (*S. lycopersicum* cv. Daniella) in insect-proof wooden cages in climate-controlled rooms at 24–27 °C and a 14L:10D light cycle as described before (Ebyshitz et al., 2009). Newly emerged whiteflies were used in the following experiments related to whitefly infestation that were conducted in climate-controlled rooms under the same conditions as described above.

2.2. Identification of ecdysone synthesis and signaling pathway genes in whiteflies

To identify the ecdysone synthesis and signaling pathway genes in whiteflies, all the known genes involved in this pathway were compiled from reports on other insects (Brown et al., 2009; Christiaens et al., 2010; Guittard et al., 2011; Rewitz and Gilbert, 2008; Rewitz et al., 2007, 2010; Yamazaki et al., 2011). Then, these genes were detected in whiteflies based on the functional annotation in the transcriptome database of three whitefly species: *B. tabaci Mediterranean* (SRX018661), *B. tabaci* Middle-East-Asia Minor 1 (SRX022878) and *B. tabaci* Asia I 3 (SRX002575), and by searching the literature (Graham et al., 2007; Wang et al., 2010, 2011, 2012). Cyp314 was not found in the transcriptome database of three whitefly species of *B. tabaci*; however this gene was represented in the transcriptome of the greenhouse whitefly *Trialeurodes vaporariorum* (Karatolos et al., 2011). Finally, these genes were further analyzed by association with terms in the Kyoto Encyclopedia of Genes and Genomes database via KOBAS 2.0 (http://kobas.cbku.edu.cn/home.do). The ecdysone synthesis and signaling pathway in whiteflies was then summarized referring to previous reports on other insects including *Drosophila*, honeybee and aphid (Brown et al., 2009; Christiaens et al., 2010; Guittard et al., 2011; Ritter and Beckstead, 2010; Yamazaki et al., 2011). Totally 22 genes were found to be involved in 20E synthesis, inactivation and response (Fig. 1A and Table S1). Among them, the 20E synthesis gene Cyp315A1, the 20E degradation gene Cyp18A1, and 20E response genes EcR and E75 were selected for the silencing experiments.

2.3. RNA isolation and quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from whitefly samples with TRI-reagent method (Sigma—Aldrich, USA). The RNA was then subjected to gel electrophoresis and quantified using a NanoDrop spectrophotometer (Wilmington, DE, USA). RNAs were first strand reverse transcribed using the EZ-First Strand cDNA synthesis Kit (Biological Industries, Israel). RNA levels were measured using SYBR® Premix Ex Taq™ II (Perfect Real Time) (Takara, Japan). The expression of each gene was tested in two technical replicates for each of three biologically independent experiments. qRT-PCRs were performed using the Rotor-Gene 6000 machine (Corbett Robotics Pty Ltd, Brisbane, Australia) with SYBR-Green detection. The accompanying software was used for qPCR data normalization and quantification. The tubulin gene was used as internal control. qPCR assay showed that the transcript of the tubulin gene is at the same level in both the dsRNA-treated and control whiteflies (data not shown), indicating that the expression of the tubulin gene was not affected by dsRNA treatment. The primers used in this study are shown in Table S2. All the primers generated a single peak in the real-time
2.4. Transcription analysis of ecdysonic synthesis and signaling pathway genes in different development stages of whiteflies

For each of three replicates, approximately 500 adult whiteflies that emerged from tomato plants were released and allowed to infest one tomato plant in an insect cage. Five days after feeding, the adult whiteflies were discarded and the progeny was allowed to develop. Ten days after the initiation of emergence, whiteflies at all developmental stages including eggs, 1st–3rd instar nymphs, 4th instar nymphs, female adults and male adults were collected. RNA was isolated and first strand reverse transcribed. The transcripts of four genes involved in the ecdysonic synthesis and signaling pathway were detected using qRT-PCR as described above.

2.5. dsRNA synthesis

dsRNAs were synthesized using the AmpliScribe™ T7-Flash™ Transcription Kit (Epicentre Biotechnologies, Madison, USA) as described previously (Ghanim et al., 2007). The obtained dsRNA was isopropanol precipitated and diluted with nuclease-free water. The dsRNA quality was determined by electrophoresis in agarose gel. The dsRNA was kept at −80°C before use.

2.6. Development of the method to silence genes by leaf-mediated dsRNA feeding

A new method was developed to silence genes by dsRNA feeding through a plant leaf. Briefly, one tomato leaflet was cut off from a tomato plant and placed in a 1.5 ml Eppendorf tubes containing distilled water for a two-days recovery period. Then the leaf was transferred to a 1.5 ml Eppendorf tube containing 1 ml of dsRNA or distilled water. The leaf and tube were transferred into a 50 ml plastic tube and covered with a piece of paper towel tightly held with a rubber band (Fig. 2A). Whiteflies were released onto the leaf through a hole in the middle of tube wall. The solution in the Eppendorf tube was replenished every five days.

To test the stability of dsRNA in the tubes and leaves, dsRNAs of whitefly genes Cyp315a1, Cyp18a1, EcR and E75 were synthesized as described above. One ml of dsRNA solution at the concentration of 0.5 μg/ml for each gene was placed in a 1.5 ml Eppendorf tube and one leaf (third leaf from the top) was inserted into the tube. Then the tubes containing the dsRNA and leaves were transferred into the 50 ml plastic tube. 5 μl of solution in each tube was collected every day. In addition, a piece of leaf (about 0.04 g) was cut, collected, frozen immediately in liquid nitrogen, and then stored at −80°C. Five days later, all five leaf samples in each of four dsRNA treatments were homogenized in the liquid nitrogen, and total RNA was isolated from plants (Eybishtz et al., 2009). Primers for dsRNA synthesis with the RNA samples were heated in the boiled water for 5 min, and quenched on ice (Smith et al., 1992). In this way, two strands of dsRNA were separated at high temperature, and after quenching on ice, the primer can bind one strand of dsRNA. Then the RNA was reverse transcribed and cDNA was used for RT-PCR. The dsRNA solution in the tubes and PCR products of dsRNAs in plant leaves were subjected to electrophoresis. The size and integrity of dsRNAs was examined.

2.7. dsRNA treatment of whitefly adults

To test the feasibility of the newly developed method to silence genes of adult whiteflies, dsRNAs for each of four genes were adjusted to the concentration of 0.5 μg/ml and added into the tubes. Water was added into the tube as the control. For each replicate, five mated female adult whiteflies two days after emergence were collected from tomato plants and released into the silencing system. Five replicate infestations were established in each of the treatments and the control. One leaf was included within each biological replicate. Six days after infestation, the adult survival and the numbers of eggs laid by whiteflies were determined. Moreover, adult whiteflies were collected for gene expression analysis through qRT-PCR as described above.

2.8. dsRNA treatment of whitefly nymphs

To test the feasibility of the new system to silence genes of nymphal whiteflies, six mated female adult whiteflies two days after emergence were collected from tomato plants and released into the silencing system. At the beginning, only distilled water was used to sustain leaves in each tube. Whiteflies were allowed to oviposit for two days and then removed. dsRNAs for each of four genes were adjusted to the concentration of 0.5 μg/ml and then added into the tubes. Water was added to the tube as the control. Three replicate infestations were established in each of the Cyp315a1 and Cyp18a1 dsRNA treatments and control. Five replicate infestations were established in each of the EcR and E75 dsRNA treatments and control. One leave was included within each biological replicate. Two days after the initiation of emergence, whiteflies at all developmental stages (1st–4th instar nymphs and adults) were counted. The percentage of survival of total nymphs, and the percentage of nymphs at each developmental stage (first through fourth instar) and adults in all individuals of progeny were counted for the Cyp315a1 and Cyp18a1 dsRNA treatments. The percentage of individual instar nymphs and adults were counted for EcR and E75 dsRNA treatments. Moreover, 4th instar nymphs of
whiteflies were collected for gene expression analysis through qRT-PCR as described above.

2.9. Data analysis

Statistical significance was evaluated using one-way ANOVA at a 0.05 level followed by LSD tests. Data in percentages were transformed by arcsine square root before analysis. For the gene expression analysis, the Wilcoxon test was used to detect the statistical significance at a 0.05 level (Yuan et al., 2006). All data analyses were conducted using the software STATISTICA 6.1 (StatSoft, Inc., Tulsa, USA).

3. Results

3.1. Ecdysone synthesis and signaling pathway genes in whitefly and developmental expression pattern

Forty four sequences representing 22 genes involved in ecdysone and 20-hydroxyecdysone metabolism and response were retrieved (Fig. 1A, Table S1). Most genes were from B. tabaci transcriptome; EcR and Usp genes were retrieved from cDNA libraries of four insect species including B. tabaci (Graham et al., 2007); Cyp314 was from the transcriptome data of the greenhouse whitefly T. vaporariorum (Karatolos et al., 2011). The 20E synthesis gene Cyp315a1, 20E degradation gene Cyp18a1, and 20E response genes EcR and E75 were selected as silencing targets to characterize their function (Fig. 1A, red arrows).

The expression pattern of the four genes from the ecdysone synthesis and signaling pathway during whitefly development, from egg to flying adult was studied by qRT-PCR. The 20E synthesis gene Cyp315a1 and response gene E75 were highly expressed in the 4th instar nymphs as compared to their expression in eggs and 1st–3rd instar nymphs, while the 20E inactivation gene Cyp18a1 and response EcR were expressed at a lower level in the 4th instar nymphs. Interestingly, the gene Cyp315a1 was highly expressed at the adult stage, while the Cyp18a1 was expressed at a lower level; EcR and E75 in adults and in 4th instar nymphs presented opposite expression patterns (Fig. 1B).

3.2. An efficient gene silencing system based on feeding insects with dsRNA through leaves

A new method was developed to silence whitefly genes by dsRNA feeding through a plant leaf (Fig. 2A). The uptake of the silencing dsRNA by the tomato leaflet and its stability in both the Eppendorf tubes and the tomato tissues was assayed for five days. Results showed that dsRNAs of the whitefly target genes Cyp315a1, Cyp18a1, EcR and E75 were stable in the tubes and in the leaves during the five days-long experiment (Fig. 2B, C). Hence, our gene silencing system is stable and was subsequently used to silence genes in whiteflies.

3.3. Silencing of genes from the ecdysone synthesis and signaling pathway has little effect on adult performance

Five mated female adult whiteflies two days after emergence were released into the silencing system consisting of detached tomato leaflets soaked in solution of dsRNA directed against the genes Cyp315a1, Cyp18a1, EcR and E75 (water was used as control). Six days after feeding on the leaf containing the dsRNAs, the gene Cyp315a1 was down-regulated by approximately 90% and Cyp18a1, EcR and E75 were down-regulated by 55%–65% (Fig. 3A; P < 0.05). The survival of adults was not significantly affected by dsRNAs feeding (Fig. 3B; F_{4,20} = 0.94, P = 0.461). Likewise, the fecundity of adult whiteflies fed with Cyp315a1, Cyp18a1 and E75 (measured as the number of eggs laid) remained unchanged as compared to control (Fig. 3C; F_{1,8} = 0.07–1.23, P = 0.3–0.794). In contrast, whiteflies fed with dsRNA targeting the EcR gene laid significantly fewer eggs compared to control whiteflies (Fig. 3C; F_{1,8} = 20.08, P = 0.00205).

3.4. Silencing of genes of the ecdysone synthesis and signaling pathway affects survival and development of nymphs

The effect of silencing the genes Cyp315a1, Cyp18a1, EcR and E75 on whitefly nymph survival and development was appraised by
inserting detached whitefly-infested leaflets in a solution of dsRNA directed against each of four genes (water was used as control). Cyp315a1 was down-regulated by approximately 80% and Cyp18a1 was down-regulated by 46% in 4th instar nymphs (Fig. 4A; \( P < 0.05 \)). The percentage of survival of Cyp315a1 and Cyp18a1 RNAi-treated nymphs was lower than controls (Fig. 4B; \( F_{1,4} = 19.74–24.24, P = 0.00791–0.0113 \)). Moreover, among all individuals of progeny, the proportion of early stage nymphs (1st–3rd instar) was higher in Cyp315a1 or Cyp18a1 RNAi-treated nymphs, however, the difference was only significant for Cyp315a1 RNAi-treated 1st instar nymphs and Cyp18a1 RNAi-treated 2nd instar nymphs (Fig. 4B; for Cyp315a1 RNAi-treated 1st instar nymphs, \( F_{1,4} = 28.85, P = 0.0058 \); for Cyp18a1 RNAi-treated 2nd instar nymphs, \( F_{1,4} = 8.31, P = 0.049 \); for other dsRNA-treated 1st–3rd instar nymphs, \( F_{1,4} = 0.077–1.54, P = 0.283–1.00 \)). In contrast, the proportion of 4th instar nymphs and newly emerged adults was lower in dsRNA-treated nymphs as compared to control although the difference was not significant for 4th instar nymphs (Fig. 4B; for RNAi 4th instar nymphs, \( F_{1,4} = 3.0–4.58, P = 0.099–0.158 \); for RNAI adults, \( F_{1,4} = 7.22–15.01, P = 0.0179–0.049 \)).

Similarly, after whiteflies fed on leaves containing dsRNA directed against EcR or E75, EcR and E75 were down-regulated by 41%–48% in 4th instar nymphs (Fig. 4A; \( P < 0.05 \)). Among all individuals of progeny, the proportion of early stage nymphs (1st–3rd instar) was significantly higher in EcR and E75 dsRNA-treated nymphs as compared to control (Fig. 4C; \( F_{1,8} = 4.53–29.06, P = 0.000653–0.0415 \)). In contrast, the proportion of 4th instar nymphs and newly emerged adults were significantly lower in dsRNA-treated nymphs as compared to controls (Fig. 4C; for dsRNA-treated 4th instar nymphs, \( F_{1,8} = 3.51–78.43, P = 0.000021–0.042 \); for dsRNA-treated adults, \( F_{1,8} = 32.18–64.15, P = 0.000043–0.000469 \)).

4. Discussion

We describe a new method to silence genes of whiteflies that could be efficiently used to down-regulate genes of sap sucking insects at various developmental stages. Using this method, we revealed for the first time, the role of ecdysone synthesis and signaling pathway genes in the development of this hemimetabolous insect.

20E titer is high during 4th instar nymphs of whiteflies (Gelman et al., 2002). As Cyp315a1 is responsible for 20E synthesis and Cyp18a1 is involved in 20E degradation, the higher expression of Cyp315a1 and lower expression of Cyp18a1 in 4th instar nymphs correlates well with the 20E level of whiteflies. Similarly, expression of 20E synthesis genes Cyp306a1 and Cyp307a1 is highly expressed and the 20E level is very high in final larval stages of the hemimetabolous insect, the desert locust Schistocerca gregaria (Marchal et al., 2011). Previous work shows that the earlier initiation of the adult stage in the whiteflies is accompanied by the drop in ecdysteroid level (Gelman et al., 2002). Moreover, the 20E level is usually lower in the adults of many insects (Marchal et al., 2011; Winbush and Weeks, 2011). Thus, the 20E level is likely not high in the adults of whiteflies. If this is true, the high expression of Cyp315a1 and lower expression of Cyp18a1 in adults reflect a feedback regulation of steroid levels (Rewitz et al., 2010). That is to say, when 20E level is lower in adults, the induced expression of Cyp315a1 and repressed expression of Cyp18a1 is required to keep the 20E level. For EcR and E75, low 20E concentrations induce expression of EcR and higher 20E concentrations repress EcR expression in Drosophila, however, higher 20E concentrations induce E75 expression and E75 induction in this cascade is coincident with the 20E pulses (Fahrbach et al., 2012). The expression profiles of EcR and E75 in whiteflies seem to match the expression patterns of these genes in Drosophila.

The gene silencing method we described here has several advantages over other methods: (1) easy handling. This system is easy to set up in most of molecular biology labs. (2) high efficiency. Once the system is established, it can readily be used to silence genes of sap sucking insects. Moreover, compared to the long term period to generate the transgenic plants producing dsRNA directed against
insect genes (Zha et al., 2011), the effect of silencing can be observed after a short time period using our system. Thus, this method can be used efficiently to assay a number of target genes.

(3) minimum damage to insects during handling. Unlike direct dsRNA injection (Ghanim et al., 2007), this system does not cause physical damages to whiteflies. Hence it is suitable to studying gene expression in small insects (e.g. whiteflies, aphids or planthoppers).

(4) wide range of application. With this system gene silencing can be achieved for various development stages of phloem-feeding insects since the leaves can be kept for more than 20 days allowing continuous observation over a large span of the insect life cycle; by comparison, feeding dsRNA through an artificial diet limits the insect development (Upadhyay et al., 2011). Collectively, this system can be widely used in reverse genetics studies of sap-sucking insects.

The Halloween genes (e.g. Cyp315a1 and Cyp18a1) are responsible for the 20E metabolism (Brown et al., 2007, 2009; Guittard et al., 2011, 2013; Revitz et al., 2010). Binding of 20E to the EcR-Usp/RXR complex starts the ecdysone signaling with the expression of the ‘early’ genes such as E75, ‘early–late’ genes and ‘late’ genes (Fig. 1A), thereby regulating insect development and reproduction (Christiaens et al., 2010; Fahrbach et al., 2012; Thummel, 2002). Our gene silencing method revealed that the function of these genes in whiteflies was similar to that of other insects, suggesting the conserved functional role of these genes. First, the Cyp315a1 metabolizes 2-deoxyecdysone to ecdysone in Drosophila S2 cells (Revitz et al., 2006; Warren et al., 2002). Expression of Cyp315a1 increases during the final larval instar in Bombyx mori, Tribolium castaneum and Manduca sexta, which correlates with the increased ecdysteroid production necessary for molting, indicating its role in the growth and development of insects (Hentze et al., 2013; Niwa et al., 2005; Revitz et al., 2006). For Cyp315a1 mutants, there is a failure of head involution and dorsal closure, the embryos become compacted, and the hindgut undergoes abnormal looping (Warren et al., 2002). Our gene silencing experiments provide the first evidence that Cyp315a1 is responsible for the development of whitefly nymphs. Second, silencing Drosophila Cyp18a1 similarly delayed larval development and resulted in pupal mortality (Guittard et al., 2011; Revitz et al., 2010). Third, the function of EcR in nymphal development and adult reproduction in whiteflies was similar to that in other hemimetabolous insects. Knockdown of the cockroach EcR-A gene inhibited nymphal molting which was arrested before adult eclosion; none of EcR-A knockdown females produced the ootheca in cockroach (Cruz et al., 2006). Similarly, EcR mediates egg chamber maturation in Drosophila (Buszczak et al., 1999). Finally, E75 is required for successful metamorphosis in cockroaches; E75 mutants resulted in developmental delay and molting defects in Drosophila (Bialecki et al., 2002; Mané-Padrós et al., 2008). Collectively, this is the first functional characterization of this developmental pathway in a hemipteran sap sucking insect. The data also indicate that regulating ecdysone (synthesis and signaling) pathway genes has the potential for whitefly control.

Analysis of mutant phenotype reveals that ecdysone and 20E is required for proper oogenesis in the adult females of some insect species (Ono et al., 2006; Petryk et al., 2003). Silencing of Cyp315a1, Cyp18a1 and E75 did not affect the survival and fecundity of adult whiteflies. There are two possible reasons. First, dsRNA-induced gene silencing is not as drastic as a complete knockout, the remaining enzyme activities may still be sufficient to play their roles in adults. Therefore, increasing the silencing efficiency may help us to reveal the role of these genes in the survival and reproduction of adult whiteflies. Second, there are several isoforms of some nuclear receptors including E75 in insects (Bialecki et al., 2002; Fahrbach et al., 2012). A variety of possible physiological compensatory mechanisms (such as feedback) may exist in whitefly adults (Marchal et al., 2011). Thus, after silencing one of the isoforms, others may be induced to perform the same functions. In summary, our gene silencing method can be used efficiently to down-regulate genes of whiteflies. It remains interesting to know how other genes in the ecdysone (synthesis and signaling) pathway function during the growth and development of whiteflies. The new gene silencing method described here will be a useful tool to characterize the function of these important genes in sap-sucking insects.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ibmbh.2013.05.012.

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Tomato yellow leaf curl China virus


