Back to Basics: Are Begomoviruses Whitefly Pathogens?

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Abstract

Begomoviruses and whiteflies have interacted for geological times. An assumed long-lasting virus-vector intimate relationship of this magnitude implies that the partners have developed co-evolutionary mechanisms that insure on one hand the survival and the efficient transmission of the virus, and on the other hand the safeguard of the insect host from possible deleterious effects of the virus. Several studies have indicated that viruses belonging to the Tomato yellow leaf curl virus (TYLCVs) family from China, Israel and Italy are reminiscent of insect pathogens. TYLCVs like all begomoviruses are transmitted in a circulative manner by the whitefly Bemisia tabaci. The survival of the virus in the haemolymph of B. tabaci is ensured by a GroEL homologue produced by a whitefly secondary endosymbiont. Following acquisition and transfer to non-host plants, the virus may remain associated with the insect for its entire 4-5 wk-long adult life. During this period, the ability of the insects to inoculate plants steadily decreased, but did not disappear. The long-term presence of TYLCVs in B. tabaci was associated with a decrease in the insect longevity and fertility. Viral DNA was transmitted to progeny, but seldom infectivity. TYLCV transcripts were found associated with the insects, raising the possibility of replication and expression in the vector. TYLCVs may spread amidst whiteflies during copulation. Functional genomics tools such as microarrays, deep sequencing, quantitative PCR and gene silencing allow revisiting the proposition that TYLCVs have retained, or acquired, some characteristics of an insect pathogen.

Key words: begomovirus, Bemisia tabaci, vector, transmission

INTRODUCTION

Viruses may have host range spanning insects and plants. Viruses infect all types of cellular organisms, from bacteria to human. Some of the first viruses to be described infected insects have been isolated from Coleoptera, Hymenoptera, Diptera, and Orthoptera (Miller and Ball 1999). Contrary to many circulative mammalian viruses, circulative plant viruses by and large do not replicate in their arthropod vector and apparently do not adversely affect their insect host. Nonetheless some viruses belonging to bunyaviruses, reoviruses, rhabdoviruses, and picornavirus-like viruses have evolved, or conserved, mechanisms allowing replication in both plant and animal hosts (Black 1950; de Assis Filho et al. 2002; Ammar et al. 2007). Most of these viruses depend on their arthropod host for survival; some are deleterious to their host and impair the insect longevity and fecundity. Virus replication and deleterious effects on the insect host have been associated with transovarial transmission to insect progeny (Sylvester 1973). In most cases it is not known whether the virus genes necessary for replication in plants and in insects are the same, and whether identical strategies are implemented. It is not fully understood how vi-
ruses became pathogens of both plants and insects. It has been shown that a plant virus movement protein can facilitate the systemic movement of an insect virus (Dasgupta et al. 2000). Recombination between a virus infecting plants and another infecting animals may be the driving force enabling plant viruses to extend their host range to insects. Indeed evidence has been recently presented suggesting that a plant virus has acquired a vertebrate host by recombining with a vertebrate-infecting virus (Gibbs and Weiller 1999). Plant viruses such as luteoviruses and geminiviruses seem to use recombination as a way to broaden their host range, to invade new regions and possibly to diversify their functions (Gibbs and Cooper 1995; Padidam et al. 1999; Lefeuvre et al. 2010). In particular, geminiviruses may constitute a family of plant viruses who are in the process of acquiring, or losing abilities to interact actively with their insect vector, the whitefly Bemisia tabaci, to a point reminiscent of a host-pathogen relationship. This assumption is particularly pertinent when examining the relationship between B. tabaci and begomoviruses. Some of the last two decade investigations are presented and discussed in this review in the light of the geminivirus insect-pathogen hypothesis.

BEGOMOVIRUSES AS INSECT PATHOGENS

Whitefly-begomovirus co-adaptation during geological times

Whiteflies and begomoviruses have a long history of co-habitation, which has left various traces. On the one hand fossils anatomically similar to modern whiteflies have been found in ~120 million-year (MY)-old amber from Lebanon (Schlee 1970). On the other hand, multiple repeats of geminiviral DNA sequences highly homologous to regions of the modern bipartite Tomato golden mosaic virus seem to have integrated into the genome of some tobacco ancestors during Nicotiana speciation, about 25 MY ago (Bejarano et al. 1996). In this context it is interesting to note that the endosymbiotic bacteria that produce the GroEL homologue on which depends the survival of begomoviruses in their insect vector (Morin et al. 1999, 2000; Gottlieb et al. 2010), have been associated with whiteflies for the last 200 MY (Bauman et al. 1993).

During this long-lasting virus-vector relationship begomoviruses might have optimized the conformation of their capsid to fit the receptors that mediate their circulation in the insect host and to interact with the chaperonins produced by the whitefly endosymbiotic bacteria. It is interesting to note that the adaptation of the local vector to the local begomovirus is reflected in the parameters of acquisition and transmission. Transmission of a begomovirus by an insect from the same geographical region is more efficient than in the case where virus and insect originated from two different regions (McGrath and Harrison 1995). Whiteflies may have also adapted to begomoviruses. Circulation of the virus may be one mechanism developed to avoid invasion of insect tissues by harmful viruses. In the latter case, it is clear that these efforts have been only partially successful since many begomoviruses remain associated with the insect vector for many days following a short acquisition access period (AAP) (Polston et al. 1990; Caciagli et al. 1995; Rubinstein and Czosnek 1997), and some begomoviruses are able to invade the reproductive system (Ghanim et al. 1998; Bosco et al. 2004; Wang et al. 2010) and affect vital parameters (Rubinstein and Czosnek 1997; Jiu et al. 2007; Matsuura and Hoshino 2009).

The path of begomoviruses in their whitefly host

Once ingested by whiteflies, begomoviruses translocate in the insect digestive tract, penetrate the gut membranes into the haemolymph, reach the salivary systems and finally enter the salivary duct from where they are egested with the saliva. Translocation of begomoviruses from the digestive tract to the haemolymph and from the haemolymph to the salivary gland is thought to be mediated by still unidentified receptors. PCR has been used to estimate the velocity of translocation of two begomoviruses the monopartite Tomato yellow leaf curl virus (TYLCV) and the bipartite Squash leaf curl virus (SLCV) with similar results (Rosell et al. 1999; Ghanim et al. 2001). The virus was detected in the head of whiteflies 10 min after the beginning of the AAP and in the midgut after 40 min. It crossed the midgut and reached the haemolymph 30
min later and in the salivary glands 5.5 h after it was first detected in the haemolymph, 7 h after the beginning of the AAP. Whiteflies were able to infect tomato plants, 1 h after the virus was first detected in the salivary system indicating that the threshold amount of virions necessary to obtain an efficient infection is low. The virus was found in the honeydew 7-8 h after the beginning of the AAP. Begomoviruses were visualized in the tissues involved in the circulative pathway by immunolocalization and in situ hybridization (Hunter et al. 1998; Brown and Czosnek 2002; Czosnek et al. 2002; Medina et al. 2006; Ghanim and Medina 2007; Caciagli et al. 2009; Ghanim et al. 2009).

**Virion and whitefly determinants insuring efficient transmission of TYLCV by *B. tabaci***

The virus capsid protein (CP) is the structure that is exposed to the whitefly tissues and interacts with insect chaperons and possibly with putative receptors. Vector specificity of geminiviruses is determined by the coat protein (Höfer et al. 1997) and there is no evidence for the involvement of other virus-encoded proteins in transmission. Loss of transmission by *B. tabaci* can be caused by amino acid replacements in a region between amino acids 129 and 152 of the CP of monopartite and bipartite begomoviruses (Noris et al. 1998; Caciagli et al. 2009; Kheyr-Pour et al. 2000; Hönlé et al. 2001). Mutagenesis has shown that virion formation and stability are necessary but not sufficient for begomovirus transmissibility. In addition, crossing the salivary gland barrier may not be sufficient for transmission.

During begomovirus circulative transmission, it is likely that particles interact with whitefly proteins. In order to search for insect proteins interacting with *Tomato yellow leaf curl Sardinia virus* (TYLCSV), the virus CP was used as bait in a yeast two-hybrid screen against a cDNA library constructed from *B. tabaci* biotype Q (Ohnesorge and Bejarano 2009). A 16 kDa small heat-shock protein (named BtHSP16) belonging to the HSP20/a-crystallin family was shown to bind to the TYLCSV CP. The viral CP interaction domain with BtHSP16 (CP amino acids 47 to 66) overlapped almost completely with the nuclear localization signal described for the CP of TYLCV (Kunik et al. 1998). Virions that cross the gut wall into the haemolymph on their way to the salivary gland face a particularly hostile environment. A GroEL homologue produced by *Buchnera* primary endosymbionts of aphids has been shown to play a crucial role in the transmission of luteoviruses (van den Heuvel et al. 1994). Similarly, the endosymbiotic bacteria housed in the whitefly bacteriocytes seem to have a cardinal role in safeguarding begomoviruses in the haemolymph. As demonstrated for TYLCV, the GroEL homologue seems to bind 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 (Morin et al. 1999). In the whitefly B biotype, GroEL produced by the facultative secondary endosymbiont Hamiltonella (but not by the primary symbiont Portiera or the facultative Rickettsia- or Wolbachia and Arsenophonus in the Q biotype) was shown to interact with the TYLCV CP and was associated with TYLCV transmission competence (Gottlieb et al. 2010).

The role of GroEL in luteovirus transmission by aphids was recently questioned (Bouvaine et al. 2011). It was argued that since *Buchnera* GroEL from two aphid species could not be detected in insect bacteriocyte-free haemolymph, the chaperon is unlikely to interact with *Barley yellow dwarf virus* (BYDV). An intriguing alternative scenario is that the virus interacts with GroEL derived not from *Buchnera* but from secondary symbionts, which are present in some aphids (Oliver et al. 2010). In another study, it was found that *Buchnera* protein composition differed when comparing *Cereal yellow dwarf virus-RPV* transmission competent and refractive aphids (Cilia et al. 2011b). GroEL was not differentially expressed in these two aphid populations. The *Buchnera* proteins associated with virus transmission were further validated in field-collected aphid populations that were efficient vectors (Cilia et al. 2011a).

**Begomovirus replication and transcription in the whitefly host?**

Begomovirus replication in its vector remains a controversial issue. The persistence of TYLCV/TYLCSV in *B. tabaci* as infective entities for longer than the latent
period, sometimes for the entire life of the insect, (Caciagli and Bosco 1997; Rubinstein and Czosnek 1997), raises the question of replication of the virus in the insect. Accumulation of viral DNA in B. tabaci reared on a TYLCV-non host plant after first feeding on plants infected with TYLCV has been interpreted as multiplication of TYLCV in its vector (Mehta et al. 1994; Czosnek et al. 2001). These results have been confirmed by feeding whiteflies with purified virions through membranes, and measuring the viral DNA by quantitative PCR after the insects were transferred to non-host cotton plants (Mahadav et al., unpublished). It has to be noted that following acquisition of the closely related TYLCSV, accumulation of viral DNA was not observed (Caciagli and Bosco 1997).

Begomovirus transcription in its vector was assessed by quantifying selected gene transcripts, including the CP, of the monopartite TYLCV and the bipartite Tomato mottle virus ToMoV, after feeding on virus-infected tomato plants and after subsequent transfer to cotton, a virus non-host (Sinisterra et al. 2005). The ToMoV gene transcripts rapidly became undetectable in whiteflies following transfer from tomato to cotton, probably because degradation was not accompanied by new gene transcript synthesis. On the other hand, TYLCV transcripts increased after transfer of whiteflies to cotton, and were readily detected after 7 d indicating active TYLCV transcription. RNAase-sensitive transcripts of the TYLCV CP gene were identified by in situ hybridization using short DNA oligonucleotides complementary to CP RNA. The transcripts were localized mostly to the filter chamber and the descending midgut of the whitefly digestive tract (Ghanim et al. 2009).

Deleterious effects of begomoviruses in their whitefly host

TYLCV is associated with the whitefly vector (B biotype) for the entire life of the vector (Rubinstein and Czosnek 1997) while TYLCSV is undetectable after approximately 20 d (Caciagli and Bosco 1997). The long-term association of TYLCV with female B. tabaci was correlated with a decrease in longevity compared with non-viruliferous insects (Rubinstein and Czosnek 1997). Following a 48-h AAP on TYLCV-infected tomato plants insects reared on eggplant, a TYLCV non-host, the life span of the viruliferous insects was shorter by 5 to 7 d compared to that of non-viruliferous whiteflies (out of 28 to 32 d). Similarly the long-term association of TYLCV with female B. tabaci was correlated with a decrease in fertility (Rubinstein and Czosnek 1997). Following a 48-h AAP on TYLCV-infected tomato plants, the mean number of eggs laid either on tomato or on eggplant during a 7 or 20 d long period significantly decreased by 25 to 50% (depending on the age of the adult). The decrease in fertility was not observed during the first 24 h following AAP. The percentage of eggs that developed into instars was similar, whether they were laid by infected or non-infected insects. Therefore TYLCV influenced the number of eggs laid but not the emergence of the instars. Confirmation for these findings came from field observations where TYLCV infection of tomato plants had a deleterious effect on the reproduction of B. tabaci (Lapidot et al. 2001).

In a similar experiment, the effect of a TYLCV isolate from China (Tomato yellow leaf curl China virus TYLCCNV) on two B. tabaci biotypes (invasive B and local ZHJ1) was evaluated (Jiu et al. 2007). Following a 48-h AAP on TYLCCNV-infected tobacco plants longevity and fertility of viruliferous B and ZHJ1 biotypes on cotton decreased by 40 and 35% respectively. In the same study, the effect of another monopartite geminivirus, the Tobacco curly shoot virus (TbCSV) on the two biotypes was appraised following a 48-h AAP on TbCSV-infected tobacco and transfered to cotton plants. The results were just the opposite of those obtained with TYLCCNV. Viruliferous B biotype whiteflies exhibited higher longevity and fertility than non-viruliferous whiteflies, while the effect of TbCSV on ZHJ1 insects was minor. The performance of the B and ZHJ1 whitefly biotypes on uninfected, TYLCCNV-infected (together with its DNAβ) and TYLCV-infected tomato plants were studied (Liu et al. 2009). The infection of tomato plants by either of the viruses had no or only marginal effects on the development, survival and fecundity of the B biotype. In contrast, survival and fecundity of the ZHJ1 biotype were significantly reduced on virus-infected plants, compared to those on uninfected plants. Populations of the B biotype on uninfected and TYLCCNV-infected plants increased at
similar rates, whereas population increase of the ZHJ1 biotype on TYLCCNV-infected plants was affected adversely. These asymmetric responses to virus infection of tomato plants between the B and ZHJ1 biotypes are likely to offer advantages to the alien biotype in its invasion and displacement of the indigenous biotype (Hu et al. 2011).

The effect of TYLCV from Japan on the biology of the Q biotype has been studied lately (Matsuura and Hoshino 2009). In these experiments, the insects were constantly raised on infected or healthy tomato. There were no differences in the survival rate and fecundity of the Q biotype of B. tabaci between TYLCV-viruliferous and non-viruliferous individuals. Moreover, the proportion of 3rd- and 4th-instar nymphs did not differ between infected and healthy tomato plants, suggesting that TYLCV symptom expression probably does not have a deleterious effect on the development of nymphal instars on infected tomato plants.

In contrast to TYLCV, the bipartite begomovirus ToMoV does not affect whitefly fertility (McKenzie 2002). Whiteflies of the B biotype infected with ToMoV deposited significantly more eggs on healthy tomato leaves than non-viruliferous whiteflies. There was no significant difference between viruliferous and non-viruliferous whiteflies for the number of adults emerged or the proportion of those adults surviving from the egg stage. There was no significant correlation between the number of eggs deposited per female and progeny survival rates on healthy tomato for whitefly infected with or without the virus. These observations indicate that some begomoviruses have deleterious effects on their insect host while others do not.

The possible reasons for these differences, sometimes dramatic, have been discussed in length (Czosnek et al. 2001; Bosco et al. 2004; Sinisterra et al. 2005; Wang et al. 2010). Several factors may be involved, acting alone or in concert. The genetic make-up (monopartite vs. bipartite) of the virus, its regional origin and adaptation to the local whitefly population, its virulence, all may affect the fitness of B. tabaci. The chemical composition of the phloem of the infected plant on which whiteflies feed may also play a role on the interactions between virus and vector. The gender and the age of the insect at the time of virus acquisition may also be a major factor influencing these interactions.

The whitefly endosymbiotic fauna has certainly an effect on virus acquisition and long-term retention. And, also, different experimental conditions may result in different results.

**Horizontal and vertical transmission of begomoviruses**

Using PCR, Southern blot hybridization and transmission tests, it was found that TYLCVs were transmitted to the progeny of viruliferous insects with various efficiency. Moreover the progeny of viruliferous insects was able to infect tomato test plants. Both the ovaries and the maturing eggs of viruliferous whiteflies contained TYLCV DNA (Ghanim et al. 1998). DNA from the closely related TYLCCNV was also found in ovaries (Luan et al. 2011). TYLCSV DNA was also detected in eggs and nymphs as well as in adults of the first generation progeny (Bosco et al. 2004). It has to be noted that no specific labeling of the TylCSV CP in ovaries was detected (Caciagli et al. 2009). In contrast to TYLCV, the adult progeny of viruliferous insects were unable to infect tomato plants. It is interesting to note that the same scientists found that TYLCV was detected neither in instars nor in adult progeny of viruliferous females. These divergent results may be due to intrinsic differences in the highly inbred insect colonies raised in the laboratory and used in these experiments. The way in which TYLCV and TylCSV enter the whitefly reproductive system is unknown. Invading TYLCV may affect the development of some of the eggs, causing a decrease in fertility (Rubinstein and Czosnek 1997; Jiu et al. 2007; Liu et al. 2009). The vertical transmission of TYLCV and TylCCNV by the B and Q biotypes of B. tabaci was studied using virus isolates and whitefly colonies established in China (Wang et al. 2009). Virus DNA was detected in eggs and nymphs but not in the adults of the first generation progeny, except in the combination of TYLCV and Q biotype whitefly where only about 3% of the adults contained the virus DNA. The offspring adults produced by viruliferous females did not transmit the viruses to plants. These results differed from those reported previously (Ghanim et al. 1998; Bosco et al. 2004).

TYLCV can be transmitted between whiteflies of the
B biotype in a gender-dependent manner, in the absence of any other source of the virus (Ghanim and Czosnek 2000). TYLCV was transmitted from viruliferous males to non-viruliferous females and from viruliferous females to non-viruliferous males, but not between insects of the same sex. Transmission took place when insects were caged in groups or in couples, in a feeding chamber or on TYLCV non-host cotton plants. Both viruliferous male and female whiteflies can transmit TYLCV to their counterparts; there was no significant difference in the efficiency of viral transmission between the two sexes. Transmission of TYLCV in a gender-related manner was not exclusive to the B. tabaci B biotype, but was also shared with the Q biotype, indicating that this biological feature might be widely shared among whiteflies (Ghanim et al. 2007). The bipartite begomoviruses SLCV and Watermelon chlorotic stunt virus (WmCSV) were shown also to be transmitted horizontally among whiteflies of the B biotype with an efficacy similar to that of TYLCV. The horizontal transmission of TYLCV and TYLCCNV by the B and Q biotypes of B. tabaci was studied (Wang et al. 2010). Both TYLCV DNA and TYLCCNV DNA were shown to be transmitted horizontally by each of the two biotypes of the whitefly, but frequency of transmission was usually low. The overall percentage of horizontal transmission for either TYLCCNV or TYLCV in each of the two whitefly biotypes was below 5%. Neither virus species nor whitefly biotypes had a significant effect on the frequency of transmission.

The haemolymph plays a primary role in the transmission of TYLCV amongst B. tabaci individuals of opposite gender. TYLCV was first detected in the haemolymph of the recipient insects about 1.5 h after caging, but was detected neither in the midgut nor in the head at this time. From there, TYLCV followed the pathway associated with acquisition from infected plants and did not cross the gut membranes back into the digestive system (Ghanim et al. 2001, 2007). TYLCV transmission. The virus ingested by B. tabaci was not detected in T. vaporariorum, and the virus ingested by T. vaporariorum was not found in B. tabaci. It has to be noted that while TYLCV is found in the haemolymph of B. tabaci after feeding on infected tomato plants, the virus is ingested by T. vaporariorum, but it is unable to cross the gut/haemolymph barrier (Czosnek et al. 2002) probably because the latter insect does not possess the begomoviral receptors that allow virus to cross the gut wall. Interestingly, TYLCV was not transmitted when individuals from the B biotypes where caged with individuals from the Q biotype (Ghanim et al. 2007) indicating that B and Q biotypes do not mate (Pascual and Callejas 2004).

CONCLUSIONS, IN THE ERA OF WHITEFLY FUNCTIONAL GENOMICS

A puzzling question that remains to be answered is why begomoviruses are circulating in their insect vector? Whiteflies are able to transmit Carlavirus and Potyvirus families in a non-persistent manner and the need for circulation is not mandatory (Brown and Czosnek 2002). As the begomoviral capsid evolved toward a better adaptation of the virus to the insect, some whitefly species may have developed receptors in their digestive and salivary systems that facilitate and optimize begomovirus translocation. The question remains why whiteflies have developed a system that allows the circulative transmission of potentially harmful begomoviruses instead of confining the virus to the stylet or destroying the virus in the digestive system. Indeed, the data discussed above suggest that some members of the begomovirus family infecting tomato are reminiscent of insect pathogens.

It is unlikely that the passage of a begomovirus in the whitefly body and the long-term association with the insect is neutral. Even if the vector is immune to the virus, this immunity should be the result of the activation of virus-triggered gene networks. In plants immunity to pathogens is provided by well-adapted signaling cascades that specifically recognize the pathogen. Immunity has a negative effect on plant fitness. Balancing the effect of immunity on fitness is a key driver in evolution of the immune network in plants (Katagiri
and Tsuda 2010). It is possible that in order to contain the invasion of begomoviruses, whiteflies tap into resources that prove to be deleterious on their longevity and fertility.

Once begomoviruses have crossed the gut barrier, they require binding an endosymbiotic bacterial GroEL chaperon to survive in the haemolymph (Morin et al. 1999). There are no other known roles for this GroEL molecule. Hence it is puzzling that the insect is protecting the virus from destruction, unless the GroEL-based system is a safe way to trap and shuttle a potentially deleterious virus out of the insect body, via the salivary duct.

Plants react to virus invasion by inducing a gene silencing mechanism that renders them immune. As a reaction, some viruses encode proteins that silence gene suppression (Roth et al. 2004). Whether whiteflies induce the production of microRNAs in response to begomovirus acquisition is not known. However, the RNA interference mechanism discovered in many organisms, including in insects (Bellés 2010), is active in B. tabaci. Following injecting into the body cavity of long dsRNA molecules specifically directed against genes uniquely expressed in the midgut, salivary glands and ovaries, the target genes were inhibited (Ghanim et al. 2007). It is possible – but not proven – that the RNAi machinery is responsible for the inhibition of viral gene expression observed following acquisition of ToMoV, and to a lesser extent of TYLCSV (Sinisterra et al. 2005).

A functional genomics approach has been taken to understand the patterns of gene expression during the association of whiteflies with begomoviruses (Czosnek and Brown 2010). Three cDNA libraries for non-viruliferous whiteflies (eggs, immature instars and adults) have been constructed and two from adult insects that fed on tomato plants infected by TYLCV and ToMoV. The sequence of approximately 20000 clones has been determined (Leshkowitz et al. 2006). Comparisons with public databases indicated that the libraries contained genes involved in cellular and developmental processes. A cDNA microarray was constructed, which represents about 6000 contigs and singletons. The microarray has been used to study resistance to insecticides (Ghanim and Kontsedalov 2007), the immune response accompanying parasitization by the wasp Eretmocerus mundus (Mahadav et al. 2008) and differential heat response of B and Q whitefly biotypes (Mahadav et al. 2009). Genome-based functional genomics will be instrumental in 1) studying the interactions underlying the circulative transmission of begomoviruses within vector and non-vector whitefly species, 2) identifying the cellular determinants involved in transmission, and 3) deciphering the evolutionary history of begomovirus-whitefly complexes. Recently, Illumina sequencing was used to analyze the whitefly transcriptome during the insect development (Wang et al. 2010). Similarly transcriptome analyses have shown that TYLCCNV suppressed whitefly immune responses by down-regulating the expression of genes involved in Toll-like signaling and MAPK pathways (Luan et al. 2011), confirming that the association of whiteflies and begomoviruses is not neutral. Mass-sequencing of transcripts and proteins supported by a thorough understanding of the whitefly biology will greatly enhance our understanding of whitefly-virus interactions and will enrich our knowledge on gene expression in this insect, providing a most useful and up-to-date database for further studies.

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