RNA interference for the control of whiteflies (*Bemisia tabaci*) by oral route

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RNA interference (RNAi)-mediated gene silencing was explored for the control of sap-sucking pest *Bemisia tabaci*, commonly known as whitefly. dsRNAs and siRNAs were synthesized from five different genes – actin ortholog, ADP/ATP translocase, α-tubulin, ribosomal protein L9 (RPL9) and V-ATPase A subunit. A simplified insect bioassay method was developed for the delivery of ds/siRNA through the oral route, and efficacy was evaluated. ds/siRNA caused 29–97% mortality after 6 days of feeding. Each insect ingested nearly 150 nl of insect diet per day, which contained a maximum of 6 ng of RNA. Knocking down the expression of RPL9 and V-ATPase A caused higher mortality with LC_{50} 11.21 and 3.08 µg/ml, respectively, as compared to other genes. Semi-quantitative PCR of the treated insects showed significant decrease in the level of RPL9 and V-ATPase A transcripts. siRNAs were found stable in the insect diet for at least 7 days at the room temperature. Phloem-specific expression of dsRNAs of RPL9 and V-ATPase A in transgenic plants for the protection against whiteflies might be an interesting application of this technology.

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1. Introduction

Cultivation of Bt-transgenic cotton has significantly reduced the usage of chemical pesticides for the control of the chewing pests. However, Bt-cotton and other crops are infested by a variety of sap-sucking insects, which were minor pests earlier. Some of them are whiteflies, aphids, leafhoppers, mealy bugs and mites. None of the Cry toxins of *Bacillus thuringiensis* is effective on these insects (Dutt 2007; Virla *et al.* 2010). Proteins such as enzyme inhibitors, chitinases and lectins can provide some degree of resistance against sap-sucking pests. However, these proteins often have low specificity and toxicity, which lead to physiological adaptations in insects. Some of the molecules such as enzyme inhibitors and lectins have anti-nutrient and toxic effects on mammals including humans (Gatehouse *et al.* 1995; Van Damme *et al.* 1998; Carlini and Grossi-de-sa 2002).

RNA interference (RNAi) technology can be a powerful tool to address the problem of sap-sucking pests and is expected to be highly target-specific. It has been reported to function against coleopteran and lepidopteran pests (Baum *et al.* 2007; Mao *et al.* 2007). Transgenic-plant-mediated silencing of the *CYP6AE14* gene is reported for the effective

Keywords. α-tubulin; *Bemisia tabaci*; RNAi; ribosomal protein L9; V-ATPase

Abbreviations used: ASAL, *Allium sativum* agglutinin; DMRT, Duncan's multiple range test; EST, expressed sequence tag; RNAi, RNA interference; RPL9, ribosomal protein L9

Supplementary figures and results of BLAST analysis pertaining to this article are available on the *Journal of Biosciences* Website at *http://www.ias.ac.in/jbrosci/Mar2011/pp153-161/suppl.pdf*

control of *Helicoverpa armigera* (Mao *et al.* 2007). Similarly, *Diabrotica virgifera virgifera* (a destructive coleopteran pest of corn) is controlled by silencing of a few genes (Baum *et al.* 2007). Mechanism of dsRNA uptake through insect gut has been comprehensively reviewed by Huvenne and Smagghe (2010).

Whiteflies have become an important pest of several crops during the past three decades (Byrne and Bellows 1991). They damage crops by feeding on phloem, excretion of honey dew and transmitting plant viruses (Brown and Czosnek 2002). Although, whiteflies are economically very important, very little is known about their genetic makeup. Some expressed sequence tag (EST) sequences of their developmental stages have been reported (Leshkowitz *et al.* 2006). RNAi in whiteflies has been demonstrated by injecting long dsRNA molecules into the body cavity (Ghanim *et al.* 2007). However, delivery of dsRNA/siRNA through the oral route for the control of whiteflies has not yet been explored. The current investigation explores the utility of RNAi in silencing a few important genes of whiteflies through the oral route and identifies the target genes for their control.

2. Materials and methods

2.1 *Isolation of RNA and cDNA synthesis*

Total RNA was isolated from adult *B. tabaci* using TRI reagent (Sigma, USA) and analysed on agarose gel. Contamination of DNA in the RNA preparations was removed with DNA-free Kit (Ambion, USA). The purified RNA was stored at −80°C for further experiments. cDNA synthesis was performed using First Strand cDNA Synthesis Kit (Invitrogen, USA) following the manufacturer's protocol.

2.2 *Selection of genes*

The target genes were shortlisted on the basis of available literature (Baum *et al.* 2007). Genes, whose siRNA showed high toxicity, were selected for the study. As the target sequences were not available in the database for *B. tabaci*, they were downloaded from the *Drosophila* database of NCBI. Available EST sequences of *B. tabaci* were also downloaded. The target gene sequences were BLAST against *B. tabaci* ESTs by using local BLAST software (supplementary file 1) for preliminary identification/annotation. The EST sequences having high similarity score were selected. These were BLAST against the database of other insects for confirmation. Further, the sequence of the selected genes were aligned with the respective gene sequences of other insects including *Drosophila* by multiple sequence alignment, and conserved regions were used in the designing of primers (data not shown).

2.3 *Amplification of target genes*

The degenerate primers (table 1) containing the *Apa*I and *Stu*I restriction sites in the forward and reverse primers, respectively, were designed from the conserved regions to amplify the target genes. The amplified DNA fragments were purified, digested and cloned in pLitmus38i (Li38) between the T7 promoters at respective sites. The cloned gene fragments were sequenced and confirmed by BLASTX analysis with the NCBI database (data not shown). The desired gene fragments were amplified with forward (5′-TAATACGACTCACTATACGGGCC-3′, Li38F) and reverse (5′-CGTAATACGACTCACTATAGGCGT-3′, Li38R) primers that included T7 promoters at both ends. The amplified DNA fragments were analysed on 2% agarose gel and DNA quantity was determined by absorbance measurement at 260 nm on NanoDrop Spectrophotometer (ND-1000, JH Bio, USA). A plant gene encoding the *Allium sativum* agglutinin-(accession number AAR23523) was also amplified with suitable primers (table 1) from genomic DNA and cloned in Li38 as discussed above and was used in making dsRNA/siRNA to serve as negative control (this gene is not found in insects).

2.4 *In vitro transcription*

In vitro transcription was performed with MEGAscript® RNAi Kit (Ambion, USA), following the manufacturer's protocol. PCR products having T7 promoters at both the ends were used as templates. The transcripts from both the strands annealed automatically and generated dsRNA. After transcription, DNA and ssRNA in the reaction mixture were digested with DNase I and RNase, respectively, and dsRNAs were purified. They were analysed on agarose gel and quantified.

2.5 *Digestion of dsRNAs into siRNAs*

The dsRNAs were digested with RNase III to generate siRNAs, following the manufacturer's protocol (Ambion, USA). siRNAs were separated from undigested dsRNA on 30 kDa cut-off filtration device (Millipore, USA), precipitated with ethanol and dissolved in sterile water. siRNAs were analysed on 15% native polyacrylamide gel and quantified.

Table 1. Primers sequences used for the amplification of selected genes and control *Allium sativum* agglutinin (ASAL) gene

Sl. No.	Primer name	Forward primer (5′-3′)	Reverse primer (5′-3′)
1	Actin	ATGCGGGCCCGGCATCACA CYTTCTACAACG	GCTA AGGCCT ACG ACC AGC CAA GTC CAA AC
2	Tubulin	ATGCGGGCCCAACGCTTGY TTYGAACCAGC	GCTA AGGCCT ATT CWC CY TCT TCC ATA CCT TC
3	ADP/ATP translocase	ATGCGGGCCCGCVTTCAAR GAYRWRTACAAG	GCTA AGGCCT CAK CTS CKS MTT TR CCG AC
4	RPL9	ATGC GGGCCC GCY CA TTT CCCC AT CAA Y TGT	GCTA AGGCCT CRT CYAA RAA YTT ACG RAT ATC
5	V-ATPse A	ATGC GGGCCC CTG ARG CTY TKM GWG AAA TTT C	GCTA AGGCCT GAR AAG TCW CCA CCD GGA GG
6	ASAL	GATCGGGCCCAGGTAGGAA CCTACTCACCAACGGTG	GATCAGGCCTCTAACATCC ATGGTCATCTGGTTTATAG

2.6 *Insect bioassay*

Bioassay was carried out with 0- to 1-day-old *B. tabaci*. Whitefly culture was maintained on potted cotton plant in the laboratory at 26±2°C and 80% relative humidity. Bioassays were also performed in similar conditions. Cotton plants having large number of nymphs and pupae were selected and adults were removed. The plants were kept in isolation for the emergence of new adults. Bioassays were carried out in 30 ml specimen tubes that were perforated for proper aeration. The flies were collected in tubes directly by placing the leaf containing newly emerged insects at the open end of the tube. Leaves were tapped gently to move the flies inside the tube. The process was repeated until the required number of adults was collected in each tube. Tubes were capped and kept in an inverted position. The artificial diet (with/without dsRNA/siRNAs) was filter-sterilized (0.22 µm). A 100 µl diet was sandwiched between the two layers of UV-sterilized parafilm and stretched on the inner surface of the tube cap. The entire process was carried out aseptically. The caps of insects containing tubes were replaced with the diet containing caps and tubes were kept upright. The diet was replaced on alternate days to minimize the possibilities of degradation in test samples and prevent microbial contamination (figure 1).

2.6.1 *Selection of diet:* Diet A5 developed by Febvay *et al.* (1988) was compared with the diet containing 5% yeast extract and 30% sucrose (Jancovich *et al.* 1997; Blackburn *et al.* 2005) for survival of adults whiteflies.

2.6.2 *Efficacy of siRNAs and dsRNA:* Diet A5 was mixed with different concentrations of the dsRNA/siRNA and tested for their toxicity to whiteflies. The initial insect bioassay was performed with 20 µg/ml concentration of siRNAs and dsRNAs. Subsequently, siRNAs of RPL9 and V-ATPase A were tested at different concentrations (1, 5, 10, 20 and 40 µg/ml). About 20 adults were taken in each bioassay tube and the experiment was performed in triplicate. The bioassays were carried out for 6 days, and mortality was recorded daily by counting the dead insects at the bottom of the tube. The mortality data were analysed by one-way ANOVA and the means were compared by the Duncan's multiple range test (DMRT). The LC_{50} values were calculated by the probit analysis on the SPSS program (version 10).

2.6.3 *Calculation of daily intake of dsRNA/siRNA:* Bioassay vials containing diets with (set 1) and without (set 2) whiteflies were kept for the calculation of diet intake in triplicate. The weights of the caps with the diets were measured before and after 24 h of feeding. The average volume of the diet consumed by each insect was calculated by using formula-:

$$\text{Average volume of diet consumed/day/insect} = \frac{\text{Weight difference in set 1} - \text{Weight difference in set 2}}{\text{Number of insect in set 1}} \times 0.917$$

where 0.917 is the density of diet.

2.7 *Semi-quantitative RT-PCR*

Semi-quantitative RT-PCR was performed to observe the expression patterns of RPL9 and V-ATPase A genes at different time points after respective siRNA treatments (20 µg/ml) of whiteflies. Total RNA was isolated after 0, 1, 2, 4 and 6 days of treatment, and cDNA was synthesised. Semi-quantitative RT-PCR reactions were performed with

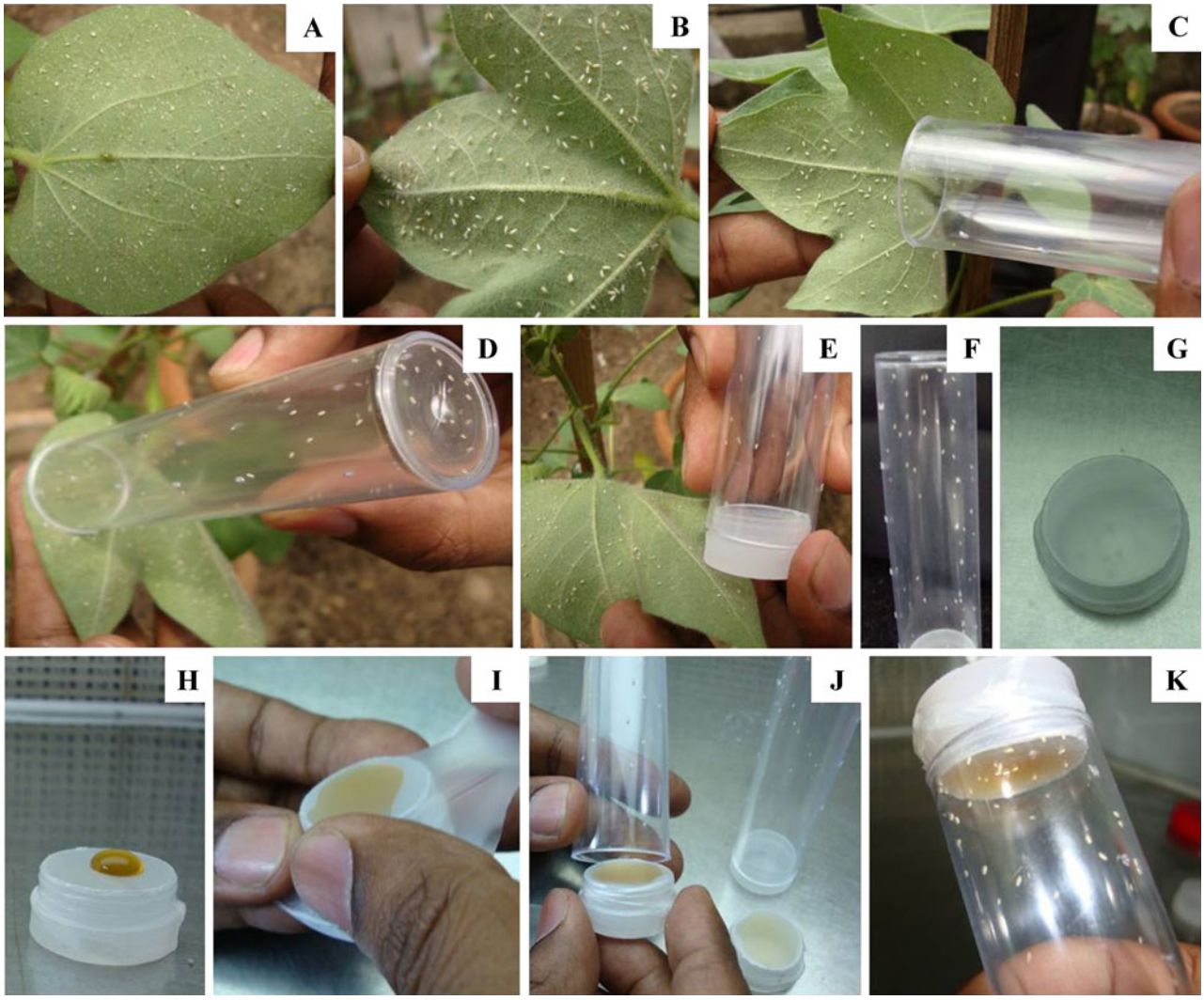

Figure 1. Collection of whiteflies and bioassay. (**A**) Cotton leaf showing whiteflies nymph; (**B**) adult whiteflies; (**C**, **D** and **E**) collection of adult whiteflies in bioassay vial from cotton leaves; (**F**) collected whiteflies in bioassay vial; (**G–I**) preparation of diet pouch using sterilized stretched parafilm on the cap of bioassay vial; (**J**) replacement of vial cap with cap containing diet; (**K**) whiteflies feeding on the diet.

respective primers (table 2), and actin served as the internal control. Each reaction mixture of 100 µl contained 100 ng cDNA template, 20 pmol primers (forward and reverse), 1.5 mM $MgCl_2$, 400 µM dNTPs, 1 × Taq buffer and 1 U Taq DNA polymerase. PCR steps were as follows: 1st cycle – 95°C for 5 min, 60°C for 1 min and 72°C for 1 min; 2nd–30th cycle –95°C for 1 min, 60°C for 1 min and 72°C for 1 min. 10 µl samples were taken from each reaction after 10, 15, 20, 25 and 30 cycles and analysed on agarose gel. The experiment was performed in three independent biological replicates.

Table 2. Primer sequences used for semi-quantitative PCR analysis of V-ATPase A, ribosomal protein L9 (RPL9) and the internal control actin

Sl. No.	Primer name	Forward primer (5′-3′)	Reverse primer (5′-3′)
1	V-ATPse A	TTTCCGGACGTTTGGCAGAGAT	GAGAAGTCACCACCAGGAGGC
2	RPL9	GCTCATTTCCCCATCAACTGTAT	CGTCCAAGAACTTACGAATATCC
3	Actin	ACGACCAGCCAAGTCCAAACG	GGCATCACACTTTCTACAATGAG

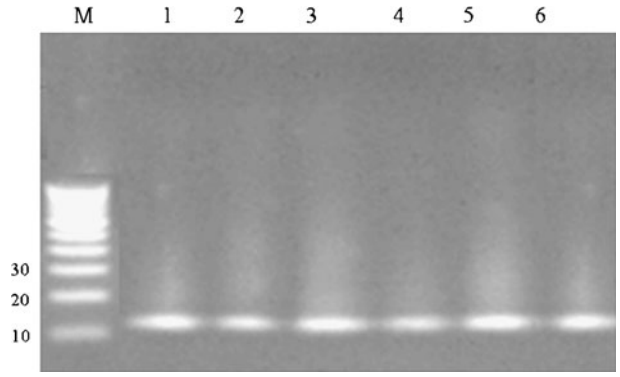

Figure 2. Native polyacrylamide gel analysis of siRNA. Approximately 50 ng of each siRNA was analysed on a 15% native PAGE gel with TAE running buffer. Following electrophoresis, the gel was stained with 0.5 µg/ml ethidium bromide. Lane M, 10 bp ladder; lane 1, actin ortholog; lane 2, ADP/ATP translocase; lane 3, V-ATPase A subunit; lane 4, ribosomal protein L9; lane 5, α-tubulin.; lane 6, *Allium sativum* agglutinin (ASAL) gene siRNA (used as control).

2.8 *Integrity of siRNAs*

The integrity of siRNAs was observed after 7 days of incubation in the diet under conditions similar to the insect bioassay. The siRNAs were recovered by precipitation with ethanol, dissolved in water and analysed on 15% native polyacrylamide gel.

3. Results

3.1 *Selection and amplification of the target genes*

A total of 10 genes were selected for targeting. We could not obtain sequence information for all the selected genes because of the limitations of the whitefly sequence database.

Table 3. Survival of whiteflies on artificial diet

	Diet A5	Yeast extract (5%) + Sucrose (30%) [pH 7.5]
	Percentage mortality (Mean ± Std. Error)	
Day 1	3.18±2.86	4.84±5.0
Day 2	6.03±1.77	7.75±3.13
Day 3	9.40±1.57	7.75±3.13
Day 4	12.58±3.82	15.50±6.27
Day 5	12.58±3.82	15.50±6.27
Day 6	13.91±4.19	18.28±1.66
Day 7	17.28±4.43	21.61±7.30

Therefore, only 5 genes were selected for the amplification (supplementary file 1). These genes were – actin ortholog, ADP/ATP translocase, α-tubulin, RPL9 and V-ATPase A subunit. DNA fragments of 293, 244, 189, 283 and 184 bp, respective were amplified with suitable primers. The amplified DNA fragments were cloned in Li38 vector and confirmed with sequencing (supplementary figure 1) and BLASTX analysis with NCBI database (data not shown). The cloned gene fragments were further amplified with suitable primers which included T7 promoter at both the ends (supplementary figure 2). The amplified DNA fragments were used as template for in vitro transcription.

3.2 *In vitro transcription and siRNA synthesis*

In vitro transcription was carried out and double stranded transcripts were analysed on 2% agarose gels (supplementary figure 3). dsRNAs were digested with RNAse III which generated 12–15 bp siRNAs as seen on native polyacrylamide gel. siRNAs were separated from undigested dsRNAs by passing them through 30 kDa cut-off filtration device; siRNAs were collected in flow through and purity was analysed on gel (figure 2). Some smear was observed above the bands of siRNAs, showing incomplete separation of siRNA from dsRNA. Further, it is noteworthy that the manufacturer claims that the siRNA produced by the digestion with RNAse III shows equal potential in knocking down the expression of the genes as compared to siRNA produced by dicer (*http://www.biocompare.com/Articles/ApplicationNote/951/Dicer-Vs-RNase-III-ForPreparation-Of-SiRNA-Cocktails.html*).

3.3 *Insect bioassay*

Diet A5 was found to be relatively better than the diet containing yeast extract and sucrose (table 3). Whiteflies survived well for more than 7 days on A5. Insect bioassay results are shown in tables 4 and 5. dsRNA/siRNA caused significant mortality as compared with the control within 6 days of treatment. In the siRNA treatment, high mortality was observed in the case of V-ATPase A and RPL9 (85.62 and 84.61%, respectively, at 20 µg/ml concentration). The lowest mortality was recorded in the α-tubulin treatment. In the dsRNA treatment, 97.5% mortality was recorded in the case of V-ATPase A, which was significantly higher as compared with all other treatments. dsRNA treatment of V-ATPase A and α-tubulin showed marginally higher mortality as compared with the corresponding siRNA treatments. siRNA of RPL9 showed significantly higher mortality in comparison with dsRNA on days 4 and 6. RNA of ADP/ATP translocase caused similar mortality in both the forms. Control dsRNA/siRNA did not cause any

Table 4. The mortality values determined from the insect bioassay with whiteflies at 20 µg/ml concentration of dsRNA and siRNA

	Day 2 Mean ± Std. Error	Day 4 Mean ± Std. Error	Day 6 Mean ± Std. Error
		dsRNA	
Control (ASAL-dsRNA)	0.0[a]	13.33±3.33[a]	13.33±3.33[ab]
Actin	17.50±2.50[ab]	25.00±5.00[ab]	35.00±0.0[c]
ADP/ATP translocase	15.00±5.00[ab]	27.50±7.50[ab]	32.50±2.50[bc]
α-tubulin	33.88±6.11[bc]	46.94±8.05[bcd]	49.72±5.28[cd]
Ribosomal protein L9	36.87±6.87[c]	45.62±10.62[bc]	56.25±6.25[d]
V-ATPase A	70.00±10.00[d]	92.50±2.50[e]	97.50±2.50[e]
		siRNA	
Control (ASAL-siRNA)	0.0[a]	0.0[a]	4.16±4.16[a]
Actin	0.0[a]	8.05±3.05[a]	44.72±0.27[cd]
ADP/ATP translocase	7.14±2.16[a]	27.44±1.12[ab]	37.96±9.39[cd]
α-tubulin	5.88±1.68[a]	20.58±2.94[ab]	29.41±0.0[bc]
Ribosomal protein L9	17.09±5.98[ab]	76.92±23.07[de]	84.61±15.38[e]
V-ATPase A	31.20±7.67[bc]	62.41±15.35[cde]	85.62±3.26[e]

Means compared using the Duncan multiple range test at $P=0.05$; means superscripted with same letter within a column are not significantly different.

ASAL, *Allium sativum* agglutinin.

Table 5. The mortality values (%) determined from the insect bioassay with whiteflies at different concentrations of siRNAs of V-ATPase A subunit and ribosomal protein L9

Concentration (µg/ml diet)	Day 2 Mean ± Std. Error	Day 4 Mean ± Std. Error	Day 6 Mean ± Std. Error
		V-ATPase A	
Control	2.77±2.77[a]	5.55±5.55[a]	8.05±3.05[a]
40	47.22±2.77[e]	64.23±7.98[cd]	82.29±1.04[f]
20	42.27±4.77[de]	48.34±4.59[cd]	71.96±1.37[ef]
10	27.25±13.92[bcd]	47.25±6.07[cd]	51.47±1.47[de]
5	25.38±5.38[bcd]	39.74±6.41[bcd]	66.38±13.62[ef]
1	10.63±4.75 [ab]	14.47±8.59[ab]	39.81±1.35[cd]
LC_{50} (µg/ml) (fiducial limits)	–	–	3.08 (1.55–4.81)
		Ribosomal protein L9	
40	38.55±3.55[de]	66.31±13.68[d]	80.36±11.94[f]
20	37.84±6.59[de]	60.76±17.01[cd]	67.43±5.89[ef]
10	31.17±0.40[cde]	37.65±0.80[bc]	70.13±7.63[ef]
5	16.71±.92[abc]	19.34±1.70[ab]	30.80±4.48[bc]
1	5.13±0.13[a]	10.26±0.26[a]	12.76±2.23[ab]
LC_{50} (µg/ml) (fiducial limits)	–	–	11.21 (9.09–13.58)

Means compared using the Duncan multiple range test at $P=0.05$; means superscripted with same letter within a column are not significantly different.

significant mortality. The mortality of whiteflies showed positive correlation with the concentrations of siRNA for RPL9 and V-ATPase A treatments (table 5). V-ATPase A caused higher toxicity than RPL9 in all the tested concentrations (LC_{50} 3.08 and 11.21 µg/ml, respectively). The average per-day consumption of diet was 152±23 nl per adult whitefly. Thus, approximately, 6.08 ng dsRNA/siRNA was fed to each insect per day in the 40 µg/ml treatment.

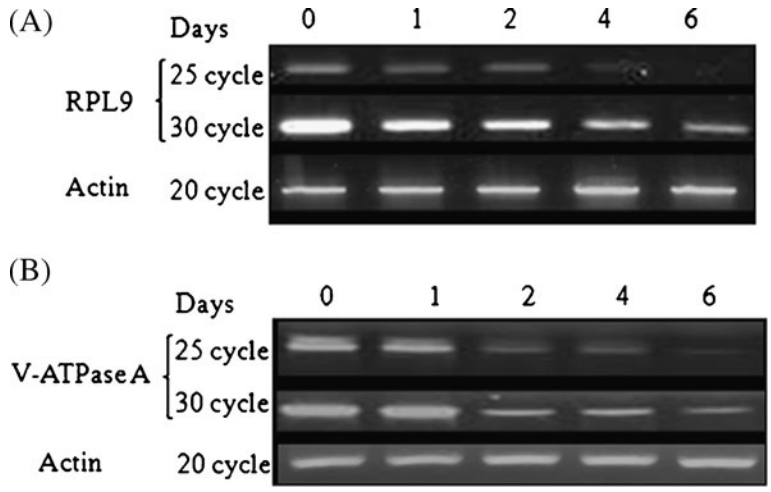

Figure 3. Semi-quantitative PCR analysis of ribosomal protein L9 and V-ATPase A genes. (**A**) Semi-quantitative PCR analysis of ribosomal protein L9 gene, (**B**) semi-quantitative PCR analysis of V-ATPase A gene. Actin gene was taken as internal control. A decrease in expression of respective gene was observed with increase in days of treatment.

3.4 *Expression profiling*

Semi-quantitative RT-PCR analysis showed progressive decrease in the expression of the target genes with the increase in the duration of treatment, maximum suppression being observed after 6 days. V-ATPase A showed higher suppression than RPL9 during later days of treatment. The result was in positive correlation with the results of insect bioassay (figure 3).

3.5 *Integrity of siRNAs*

siRNAs were recovered from the insect diet and analysed on 15% native polyacrylamide gel (figure 4). The siRNA in diet was found stable for a minimum of 7 days at room temperature.

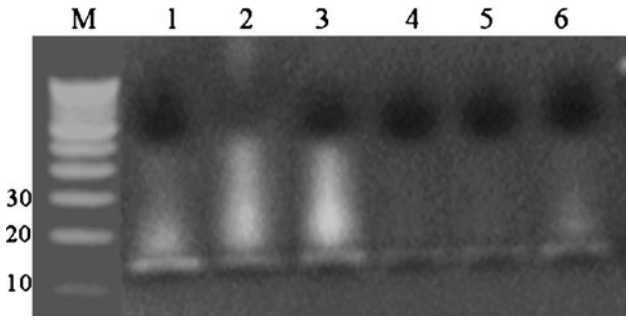

Figure 4. Native polyacrylamide gel analysis of different siRNA molecules recovered from insect artificial diet after 7 days of treatment. Approximately 40 ng of each siRNA was analysed analysed on a 15% native PAGE. Lane M, 10 bp ladder; lane 1, actin ortholog; lane 2, ADP/ATP translocase; lane 3, V-ATPase A subunit; lane 4, ribosomal protein L9; lane 5, α-tubulin; lane 6, *Allium sativum* agglutinin (ASAL) gene.

4. Discussion

RNAi is an effective method of regulating the gene expression in *in vitro* and *in vivo* conditions (Fire *et al.* 1998; Newmark *et al.* 2003; Baum *et al.* 2007) and has been used in the study of gene function relationship for the past decade now. Although such regulations have been reported in model insects (Vermehren *et al.* 2001; Mutti *et al.* 2006; Turner *et al.* 2006; Tian *et al.* 2009), little work has been done on crop insects. Mao *et al.* (2007) and Baum *et al.* (2007) have demonstrated that the physiology of the insects can be disturbed with siRNA, which generally leads to the reduction in larval growth and sometimes death. In other words, siRNA against critical genes might function as toxins against the target insects. The present study aimed to control whiteflies through RNAi, which damage many crops by phloem feeding and transmitting viruses. RNAi has been reported to suppress the expression of genes in this insect to an extent of 70% after injecting dsRNA into the body cavity (Ghanim *et al.* 2007). As injection of dsRNA is not possible in field conditions, oral delivery and uptake of dsRNA in the gut becomes very critical. Oral delivery of siRNA/dsRNA, uptake in the gut, knocking down the expression of the target genes and mortality of whiteflies have not been reported earlier.

The bioassay method developed in the present study was simple. Whiteflies were collected into the bioassay vials directly, and this did not involve cumbersome steps like aspiration, anesthetization and transfer. As the diet was placed in caps, it could be replaced easily. The ease made the method superior to earlier reported methods (Davidson *et al.* 1996; Jancovich *et al.* 1997; Blackburn *et al.* 2005). However, maintaining a fixed number of insects for each treatment continued to remain a concern.

Reasonable to significant mortality was observed in dsRNA/siRNA treatments. There were some differences in the toxicity in both the forms of RNAs, although the pattern of the toxicity remained the same. The RNA developed from V-ATPase A was found to be most effective in both the forms, followed by RLP-9. α-Tubulin dsRNA was the least toxic and caused mortality up to 30% only. The reported effects of siRNA-mediated gene silencing is limited to reduced larval growth in *H. armigera* (Kumar *et al.* 2009), reduction in the oviposition rates in *Spodoptera fugiperda* (Griebler *et al.* 2008) and decrease in the target mRNA levels in *Epiphyas postvittana* (Turner *et al.* 2006) and *Tribolium castaneum* (Bucher *et al.* 2002; Tomoyasu and Denell 2004). Contrary to the earlier reports, we observed more than 80% mortality of whiteflies by feeding them siRNA/dsRNA of RPL9 and V-ATPase A subunit. A remarkable high sensitivity of whiteflies to siRNA as compared with other insects was one of the major finding of this study.

Higher mortality of whiteflies was observed in the treatment of siRNA of highly active genes (RPL9 and V-ATPase A) rather than the structural and less active genes (actin ortholog and α-tubulin). This might be due to the fact that most of the growth and development had already been completed in adults. It is quite logical why structurally important genes became major target for the control of lepidopteran larvae in most of the previous studies (Price and Gatehouse 2008). The efficacy of insect-specific siRNAs of V-ATPase A and RPL9 needs to be studied in other insects also.

Some difference in the mortality was observed in the treatment of siRNA of RPL9 and V-ATPase at 20 µg/ml concentration between two independent bioassays (tables 4 and 5). Both the bioassays were conducted at different point of time although in similar conditions. The difference in efficacy of siRNA might be due to heterogeneity in freshly emerged adults and variation in the quality and the quantity of siRNA synthesized in different batches.

The silencing of the target genes had a greater impact at higher concentrations; the mortality of whiteflies increased with the increasing concentration of siRNA. This has also been observed in case of southern corn rootworm (Baum *et al.* 2007). Every target gene requires an optimal concentration of siRNA to achieve nearly complete silencing. Further increase is not likely to result in any more silencing (Meyering-Vos and Müller 2007). Gene silencing requires efficient uptake of dsRNA/siRNA. It is always higher in injection followed by feeding and topical application. Rajagopal *et al.* (2002) observed dsRNA-mediated gene silencing only through injection and not ingestion. Several known barriers such as feeding physiology and behavioural factors determine efficacy of a toxin given though the oral route (Baum *et al.* 2007). The siRNA could be degraded in the diet, gut or in insect tissues (Kumar *et al.* 2009). Although siRNA was very stable in the artificial diet, the stability might differ in the gut as several detoxifying/digestive enzymes degrade part of siRNA and reduce its availability for gene silencing. Another possibility is partial uptake of siRNA in insect gut. A higher concentration of siRNA was helpful in overcoming such limitations. The siRNA used in the present study was prepared from a long stretch of the target genes. Most sensitive regions in the target genes need to be identified in future, which could be used in developing transgenic plants.

RNAi technology is unlikely to become an immediate crop protection strategy against lepidopteran and coleopteran pests, as Bt-based strategy offers a high degree of protection in field conditions. The technology can be useful for protecting the crop plants against sap-sucking homopteran pests such as aphids, leafhoppers, whiteflies and others, if siRNA against critical genes of target insects are expressed in transgenic plants (Price and Gatehouse 2008). Most of the sap-sucking insects nourish on phloem sap flowing in sieve tubes. Therefore, it is essential to express siRNA/dsRNA in phloem under tissue specific promoters. Since the presence of miRNA, siRNA and dsRNA in sieve tube has been reported (Buhtz *et al.* 2007; Kehr and Buhtz 2008; Pant *et al.* 2008; Kragler 2010), it seems feasible to express desired dsRNA/siRNA in phloem and delivery in sieve tubes. In conclusion, our research opens up the possibility of controlling sap-sucking pests such as whiteflies by means of RNAi technology.

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