RNAi mediated gene knockdown in sucking and chewing insect pests

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ABSTRACT

RNA interference (RNAi) describes the ability of double-stranded RNA (dsRNA) to inhibit homologous gene expression at the RNA level. The specificity is sequence-based and depends on the sequence of one strand of the dsRNA corresponding to part or all of a specific gene transcript. In general, RNAi is a post-transcriptional control mechanism involving degradation of a target mRNA by dicers, mediated through the production of small interfering RNAs or short interfering RNAs (siRNAs). No effective Bt toxins are known against sap-sucking homopteran pests such as aphids, leafhoppers etc. With this view in mind, in the current study, RNAi has been applied to block different proteins biosynthesis by sucking insect pests. To achieve the objectives, clones were selected and transcribed to dsRNA. The transcribed dsRNAs were digested with RNase III to prepare siRNAs. Fifty micro liter volumes of test samples containing either control reagent or siRNA in varying quantities were mixed with the insect diet (1ml and 1g for sucking and chewing pests). Five different concentrations, 40, 20, 10, 5, 1 µg/ml of siRNA from a gene were applied to find out the concentration required to kill 50% of insects. The current investigation has explored the utility of RNAi as a tool for specific and strong silencing of various genes in adult sucking pest and larval chewing pest to examine their potential as candidates target genes for pest management. This report says observations of gene knockdown in sucking pests using siRNAs synthesized from different genes through RNAi technology for the first time. The same siRNA treatment resulted in specific gene silencing (not significant) and consequently brought very less mortality percentage. The present results suggest that feeding of siRNAs through an artificial diet can be exploited for the screening of siRNAs for insect pests control and functional genomic studies in both sucking and chewing insect pests.

Keywords: gene knockdown, gene silencing, insect control, RNA interference (RNAi), short interfering RNAs (siRNAs)

INTRODUCTION

RNA interference (RNAi) describes the ability of double-stranded RNA (dsRNA) to inhibit homologous gene expression at the RNA level. The specificity is sequence-based and depends on the sequence of one strand of the dsRNA corresponding to part or all of a specific gene transcript (Price and Gatehouse, 2008; Borgio, 2010). In general RNAi is a post-transcriptional control mechanism involving degradation of a target mRNA. The degradation of dsRNA by dsRNA-specific endonucleases referred to as dicers is mediated through the production of small interfering RNAs or short interfering RNAs (siRNAs) (Bernstein *et al.,* 2001). Eukaryotic organisms, including insects, possess a common machinery for sequence-specific gene silencing that is triggered by the presence of dsRNA. This process is called RNA interference (RNAi) in animals and post-transcriptional gene silencing in plants (Kennerdell and Carthew, 2000; Aouadi *et al.,* 2009). The siRNAs are 21 bp dsRNA fragments carrying two base extensions at the 3′ end of each strand; one strand of the siRNA is assembled into an RNA-induced silencing complex (RISC) in conjunction with the argonaute multi-domain protein, which contains an RNAseH-like domain responsible for target degradation (Price and Gatehouse, 2008). Microinjection of dsRNA into adults and larvae has been used to silence genes in *Tribolium castaneum* (Herbst) (Coleoptera) (Bucher *et al.,* 2002; Tomoyasu and Denell, 2004). Similarly, dsRNA induced an RNAi response in Lepidopteran, such as *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) and *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) (Rajagopal *et al.,* 2002; Turner *et al.* 2006; Baum *et al.,* 2007). No effective Bt toxins are known against sap-sucking homopteran pests such as aphids, leafhoppers, etc (Price and Gatehouse, 2008). With this view in mind, in the current study, RNAi is applied to five proteins biosynthesis in a sucking insect and *S. litura.*

MATERIALS AND METHODS
Materials and chemicals
DNA Engine (Dyad Peltier Thermal Cycler, Bio-Rad Inc. USA), NanoDrop Spectrophotometer ND-1000 (JH Bio, USA), Gel documentation system - Fluor-S™ MultiImager (Bio-Rad Inc., USA), MilliQ grade water (Miilipore Corp.,

Massachusetts, USA), Chemicals for Native PAGE, Diethylpyrocarbonate, Total RNA isolation kit (Sigma, USA), Agarose, QIAquick TM (Qiagen, USA), Primers (Chromas, Bangalore, India and MWG, Bangalore, India), Deep Vent DNA polymerase, T4 DNA ligase, LITMUS38i, pBluescript SK (+/-) (New England Biolabs, Beverly, MA, USA), T7 enzyme mix, RNase III, DNase, RNase, MEGAscript® RNAi kit, DNA-free Kit (Ambion, Austin, Texas, USA), M-MuLV Reverse Transcriptase, First strand cDNA synthesis kit (Fermentas, USA), AuPrep Kit (India Labs, India) and regular laboratory chemicals (Sigma, USA, HiMedia, India) were used in the present study.

Isolation of RNA

Total RNA was isolated from a sucking insect using an AuPrep RNA kit (Sigma, USA). 50 mg of insects were frozen at -80°C for 10 minutes to anesthetize the live insects and was ground under liquid nitrogen to a fine powder using a prechilled mortar and pestle. The tissue powder together with liquid nitrogen was allowed to evaporate. The tissue powder together with a fraction of liquid nitrogen was mixed with 1 ml of lysis buffer containing 1% (v/v) β-Mercapto ethanol and transferred to a 1.5 ml micro centrifuge tube, followed by vigorous vortexing. The lysate was loaded onto a spin column and centrifuged at 13000 rpm for 1 min. The cleared lysate was transferred to a new tube and equal volume of 75% ethanol was added and mixed well by pipetting to create conditions, which promote selective binding of RNA to the RNeasy membrane. The sample was then applied to the RNeasy spin column. Total RNA bound to the membrane, contaminants were washed away, and RNA was eluted in RNase-free water. The integrity and size distribution of total RNA isolates was investigated by agarose gel electrophoresis and ethidium bromide staining (Sambrook and Russell, 2001).

Removal of DNA contaminants

A DNA-free Kit (Ambion) was used to remove contaminating DNA from RNA preparations, and to subsequently remove the DNase and divalent cations from the RNA sample. Less than 10 μg of RNA was treated in a single DNA-free reaction. For each reaction it was added 0.2 volumes of 10 x DNase buffer and 1 μl of DNase I (2 U/μl) was added to the RNA followed by gentle mixing and incubation at 37°C for 1 hour. Thereafter 0.1 volumes of the slurry of the DNase inactivation buffer was added to the sample, the sample was mixed and incubated for 2 minutes at room temperature. After centrifugation at 13000 rpm for about 1 minute to pellet the DNase inactivation reagent together with DNase and divalent cations, the purified RNA solution was removed and stored at -80°C for further experiments (Sambrook and Russell, 2001).

Synthesis of cDNA

First strand cDNA synthesis was primed using First strand cDNA synthesis kit (Fermentas, USA). In a 20 μl reverse transcription reaction, 1 μl of oligo $(dT)_{18}$ primer was mixed with 2 ìg total RNA and DEPC treated water was added to a final volume of 16.2 μl. The RNA mixture was denatured for 10 minutes at 65°C and quenched on ice. cDNA-synthesis reaction buffer and 10 mM dNTP were added and the primer was allowed to anneal the RNA template by incubating at 42°C for 2 minutes. Thereafter, the cDNA synthesis was initiated by adding 1 μl M-MuLV reverse transcriptase (20 U/μ) and incubated at 37°C for 60 minutes. Heating the reaction at 70°C for 10 minutes inactivated the reverse transcriptase (Sambrook and Russell, 2001).

Amplification and elution of desired genes

The synthesized cDNA was used to amplify 5 genes using degenerated forward and reverse primer tabulated in the following table 1. The annealing temperature of the primers was optimized using gradient PCR (53 to 64°C). PCR was performed with 100 ng cDNA template, 505 picomols of each primers, 8 mM $MgSO_4$, 10× PCR buffer, all four deoxynu cleotides at 10 mM and 1 U of Deep Vent DNA polymerase in 25 μl PCR reaction. PCR conditions were as follows: 95°C× 15s (1 cycle); 95°C×30s, 54°C×60 s, 72°C×60 s (35 cycles);

Table 1. List of genes and designed primers.

Annotation	Forward / Reverse primer	Sequence (5' → 3')
GENE 1	LAF1	CCTAATGAAATCAATGAAATAGC
	LAR1	CTTCTCAGCCTTCTT(C/T)TTGTG
GENE 2	RP F1	AGATGCTGGTCGCATTTGTC
	RP R1	AGCATTTCTCATTCCTTG(A/G)CA
GENE 3	vAF1	TATGCTGCTATGTTGGCTGC
	vAR1	ACCTCCCTTCCTTC(G/T)GGT
GENE 4	T F1	GACAAGTCGGTGTGGTT(C/T)(C/T)TG
	TR1	TGCTCCACGCCCAC(T/C)TCC
GENE 5	ADF1	GTGTGGCATACTCAGACATG
	ADR1	CACACATAGTATCAGCAGTGA

Table 1. Concentrations of DNA, dsRNA and siRNA from respective gene

Annotation	Concentration (µg/µl)		
	DNA	dsRNA	siRNA
GENE 1	3.141	1.8296	1.7435
GENE 2	1.632	2.2512	2.1398
GENE 3	1.594	2.7312	2.4862
GENE 4	1.242	0.9300	0.9175
GENE 5	2.047	1.1678	1.1045

and 72ºC×5 mins. The amplification products were identified on agarose gel (2%) and purified using QIAquickTM (QIAGEN) columns. The eluted genes were quantified using a nano drop and visualized on 2% agarose gel and stored at -20°C for further use (Sambrook and Russell, 2001).

Selection of clones

Genes selected from sucking insect were cloned in *in-vitro* transcription phagemid vector, LITMUS38i between the *Apa*1 and *Sna*B1 restriction endonucleases recognition sites and used in the present study.

Amplification of cloned genes

The phagemid LITMUS38i contains GENE 1 fragment was amplified using forward (LF 5´-TAATACGACTC ACTATACGGGCC-3´) and reverse primer (LR 5´-CGTAATACGACTCACTATAGGCGT- 3´). The annealing temperature of the primers was optimized as 57ºC using gradient PCR (52 to 68ºC). PCR conditions for GENE 1: 94ºC × 5min, 57ºC × 1min, 72ºC × 30s (1 cycle), 94ºC × 1min, 57ºC × 5min, 72ºC × 30s (40 cycles) and 72ºC × 5min (1 cycle) (DNA Engine, Dyad Peltier Thermal Cycler, Bio-Rad). The above protocol applied for the remaining four genes. The presence of amplicons of the expected size, and the absence of other non-specific amplicons, were confirmed by 2% agarose gel analysis in submarine horizontal agarose slab gel apparatus as described by Sambrook and Russell (2001).

Purification, concentration and visualization of DNA

DNA was recovered directly from the PCR reaction by precipitation in 2 volumes of 100% ethanol and 0.1 vol of 3M sodium acetate. The DNA was collected by centrifugation and the pellet was washed in 70% ethanol, and dried. The precipitated DNA was resuspended in miiliQ water and the concentration was determined by absorbance at 260 nm (NanoDrop Spectrophotometer ND-1000, JH Bio). The purified DNA was confirmed by 2% agarose gel analysis. This DNA was used as template for *in vitro* transcription reactions (Sambrook and Russell, 2001).

In vitro transcription

All the reagents and enzymes used were from Ambion MEGAscript® RNAi kit. The reaction mixture (Nuclease-free water to 20µl, 1-2µg of template with opposing T7 promoters flanking in the transcription region, 2µl 10× reaction buffer, 2µl ATP solution, 2µl CTP solution, 2µl GTP solution, 2µl UTP solution and 2µl T7 enzyme mix) was prepared, mixed gently and incubated at 37°C for overnight. The annealed dsRNA was treated with DNase I and RNase (20µl annealed dsRNA, 2.5µl 10× digestion buffer, 1µl DNase I and 1.5µl RNase) at 37°C for one hour. The DNA and ssRNAs free annealed dsRNA was purified by assembling dsRNA binding mix (25µl dsRNA, 25µl 10× binding buffer, 175µl Nuclease-Free water and 250µl 100% ethanol) onto the filter in the filter cartridge, discarded the flow though and washed using 2× wash solution. The pure dsRNA in the filter cartridge was eluted using elution buffer. The inte grity of the purified dsRNAs was checked on 2% agarose gels, and the concentrations were measured by absorbance at 260 nm on a Nano drop (Sambrook and Russell, 2001).

Synthesis of siRNA

After *in-vitro* transcription the resulting pure dsRNAs were digested with RNase III (10µg dsRNA, Nuclease-Free Water to 50 µl, 5µl 10× RNase III reaction buffer, 3µl RNase III) to prepare siRNA at 37°C for one hour. The

Table 2. LC$_{50}$ values for GENE 2 and GENE 3 against sucking insect.

Gene name	Day of incubation	LC$_{50}$ (µg/ml)	Fiducial limits (µg/ml)		Slope	Chi^2
			Lower	Higher		
GENE 3	Two	75.19	39.02	273	2.25 (3)	0.63 ± 0.12
	Three	16.97	13.03	23.13	3.82 (3)	1.16 ± 0.16
	Six	3.08	1.55	4.81	3.72 (3)	0.68 ± 0.10
	Seven	3.03	1.83	4.35	2.69 (3)	0.92 ± 0.10
GENE 2	Two	40.63	22.80	69.46	1.72 (3)	1.12 ± 0.1
	Three	22.61	17.36	31.91	6.50 (3)	1.20 ± 0.1
	Six	11.21	9.09	13.58	1.10 (3)	1.68 ± 0.1
	Seven	8.49	6.82	10.17	9.37 (3)	1.94 ± 0.1

resulting siRNA population was quantitated using a nano drop and visualized on 15% non-denaturing acrylamide gel and stored at -20°C (Turner *et al.,* 2006).

Insect bioassay

Bioassays with sucking insects were conducted using an artificial diet according to the following procedure. Fifty microliter volumes of test samples containing either control (MilliQ water) reagents or siRNA in varying quantities were mixed with the insect diet (1ml). The diet was filtered through 2μm membrane and dispensed in 200μl aliquots into the top and sealed with parafilm nicely before sample application. We applied 200μl of test sample per treatment, with sterile water serving as the untreated check. The tops were placed on the vials after adding insect adults. White files were obtained from the Entomology Division, NBRI. Twenty to 25 five (d" 1 day old) adults were added per vial with a fine paintbrush. Vials were sealed with parafilm and ventilated using an insect pin. Forty to fifty adults were tested per treatment. The bioassay vials were incubated at 27°C, 60% relative humidity. The vials were scored for larval mortality till 7 days.

Surface coating of the siRNA on semi-synthetic diet performed insect bioassay with *S. litura* (200 μl). The siRNA was spread on the diet surface of each small Petri plate. Diet with siRNA was air-dried. First instar larvae of *S. litura* were obtained from the Entomology Division, NBRI. Ten first instar larvae of *S. litura* were released in each Petri plate. The experiment was performed in duplicate. Sterile water was used as a control. The wells were scored for larval mortality till 72 hours.

LC_{50}

Based on the percentage of mortality, the best two genes (GENE 2 and GENE 3) were selected and used for LC_{50} calculation. Five different concentrations, 40, 20, 10, 5, 1 μg/ml of siRNA from GENE 2 were applied to find out the concentration required to kill 50% of sucking insect. The same protocol was applied to find out the LC_{50} of siRNA from GENE 3.

RESULTS

The total RNA from the sucking insect was isolated and electrophoresed to visualize the presence of RNA in the isolated sample using 1% agarose gel. The gel was documented and is presented in figure 1. The purity and quantity of the isolated RNA was noted as 676.8 ng/μl and 2.02. It was stored at -80°C for further use. Ten micrograms of total RNA was treated with 2 units of DNaseI-RNase-free (Ambion) for 30 min, to remove residual DNA molecules. DNA molecule present in the isolated

RNA sample was removed to prevent the hindrance of DNA during amplification of gene, confirmed the stability of RNA and the absence of DNA on agarose gel (1%). The result is displayed in figure 1. Again the purity and quantity of DNA free RNA was measured as 416.1 ng/μl and 2.01. The pure DNA free RNA was stored at -80°C for the amplification of genes using the degenerated primers through polymerase chain reaction.
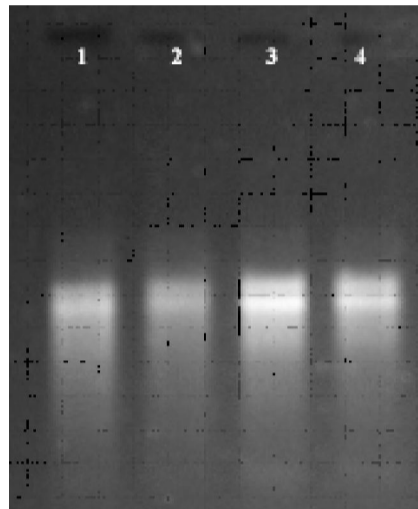

Figure 1. Isolated RNA from sucking insect. (Lane 1 and 2 - RNA after DNAse treatment. Lane 3 and 4 - RNA before DNAse treatment.

The DNA free cDNA was used as template to amplify the five desired genes, and the products were visualized on 2% agarose gel. From the result we found many artifacts in the gel, hence, the suitable annealing temperature for respective pair of primers for the individual genes were performed using gradient PCR. The annealing temperature of the primers was optimized for all the five genes using gradient PCR. The gradient PCR (52 to 68ºC) was performed to optimize the annealing temperature of the primers (LF and LR) for five genes cloned separately in phagemid LITMUS38i. From the result (figure 2), the annealing temperature was selected as 57ºC.
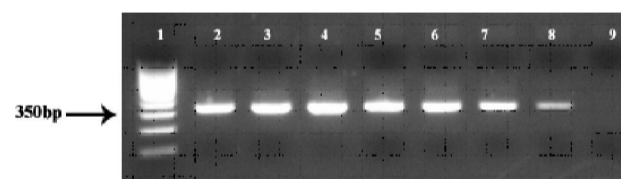

Figure 2. Agarose gel analysis of DNA molecules generated using the gradient PCR of sucking insect. Lane designations: lane 1, 100bp DNA Ladder; lane 2, 52°C; lane 3, 54.6°C; lane 4, 56.5°C; lane 5, 58.8°C; lane 6, 61.5°C; lane 7, 63.8°C; lane 8, 65.5°C; lane 9, 68°C.

Amplification of desired genes, of sucking insect cloned separately in phagemid LITMUS38i were performed using LF and LR primers with annealing temperature, 57ºC. The amplicons were confirmed by 2% agarose gel and is presented in figure 3.
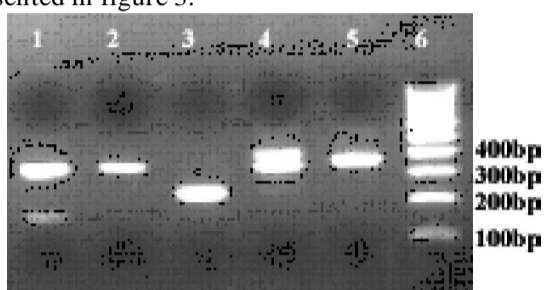

Figure 3. The products from amplification with LF and LR primers resolved on an ethidium bromide-stained agarose gel (2%) are shown. Lane designations: lane 1, GENE 5; lane 2, GENE 4; lane 3, GENE 3; lane 4, GENE 2; lane 5, GENE 1; lane 6, 100bp DNA Ladder.

The result indicates that the size of the product from the clones, GENE 1 to 5 were ~350bp, ~300bp, ~250bp, ~350bp and ~350bp respectively. Amplified DNAs were purified from the PCR reaction mixture by ethanol precipitation followed by 70% ethanol wash. The purified DNAs were resuspended in miiliQ water and the concentration was determined by NanoDrop Spectrophotometer as tabulated (table 1). The purified DNA were confirmed by 2% agarose gel analysis and used as the template for in vitro transcription reactions.

In vitro transcription of the amplified gene with opposing T7 promoters flanking in the transcription region were carried using Ambion MEGAscript® RNAi kit with overnight incubation at 37°C. The template and the ssRNA were removed using DNase I and RNase and the dsRNA was purified using filter cartridge. The integrity of the purified dsRNAs were checked on 2% agarose gels and is presented in figure 4, the concentrations were measured as tabulated in table 1.
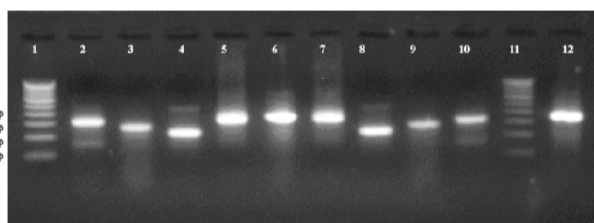

Figure 4. Agarose-gel analysis of dsRNA synthesized by *in-vitro* transcription. Lane designations: lane 1&11. 100bp DNA Ladder; lane 2 & 10, GENE 1; lane 3 & 9, GENE 2; lane 4 & 8, GENE 3; lane 5 & 7, GENE 4; lane 6 & 12, GENE 5.

dsRNA was synthesized *in vitro* and was diluted in milliQ water. Agarose gel electrophoresis showed that the dsRNAs used in the present study were ~275bp, ~250bp, ~200bp, ~275bp and ~275bp for GENE 1 to 5 respectively. After synthesis, the dsRNA were digested into siRNAs with RNase III. The siRNA diluted in nuclease free water and quantitated using a nano drop as tabulated (Table 1). Later the siRNAs fragments were visualized on 15% non-denaturing acrylamide gel as shown in the figure 5, to ensure that longer dsRNA were completely eliminated.
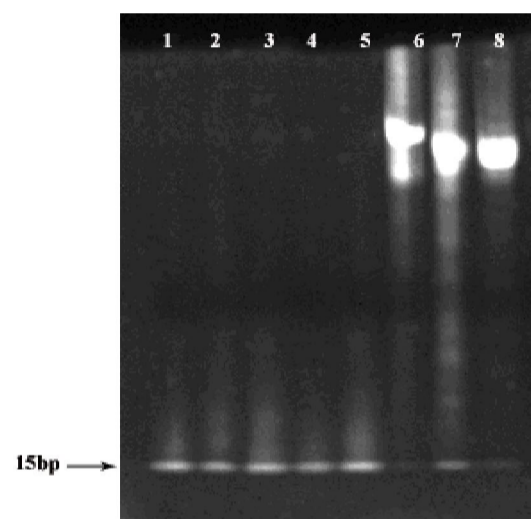

Figure 5. Native polyacrylamide gel analysis of different siRNA molecules generated using the RNase III**.**

Approximately 40ng of each siRNA was analyzed on a 15% non-denaturing PAGE gel with TAE running buffer. Following electrophoresis, the gel was stained with 0.5µg/ml ethidium bromide. Lane designations: lane 1, siRNA synthesized using GENE 1; lane 2, siRNA synthesized using GENE 2; lane 3, siRNA synthesized using GENE 3; lane 4, siRNA synthesized using GENE 4; lane 5, siRNA synthesized using GENE 5; lane 6, dsRNA from GENE 1; lane 7, dsRNA from GENE 2; lane 8, dsRNA from GENE 3. Native polyacrylamide gel electrophoresis showed that the siRNAs synthesized in the present study was ~15 for all the genes, according to Sambrook and Russell (2001), the synthesized siRNA migrated along with the bromophenol dye front. Native polyacrylamide gel electrophoresis clearly indicated the absence of dsRNA in the siRNA mixture (figure 5). Insect bioassay indicated that the highest mortality (88.55%) by GENE 3, followed by GENE 2 (88.46) after seven days of incubation. On the

same of incubation, the lowest mortality was recorded in the GENE 4. In initial days (3, 4 and 5th days) the mortality was higher in GENE 2 by in later days (6 and 7th days) it was overcame by the GENE 3 (figure 6). Interestingly the LC_{50} values were higher during initial days in GENE 3 but in later days it was the lowest (figure 9).
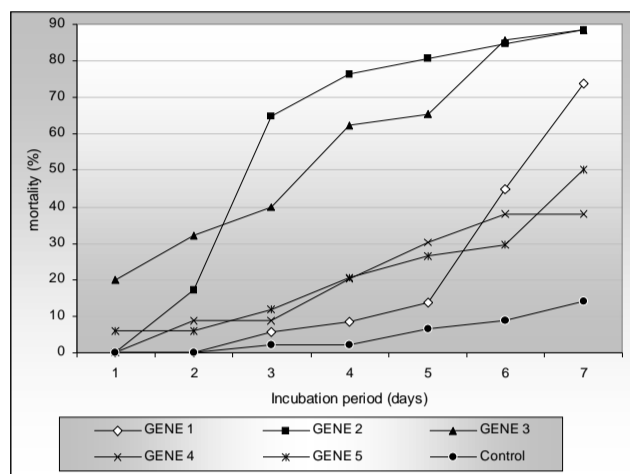

Figure 6. Mortality values determined from the insect bioassay with sucking insects.

Further studies were conducted to find out the LC_{50}, for GENE 2 and GENE 3, the experimental observations are presented in the table 2, figure 7, 8 and 9. The various concentrations of siRNA used for the bioassay with sucking insects were 40, 20, 10, 5, 1 µg/ml for both GENE 2 and GENE 3 separately. The LC_{50} were calculated as presented in table 2, the lowest concentration (3.03 µg/ml) needed to kill the sucking insects in laboratory conditions were noted in the GENE 3 after 7 days of bioassay. Insect bioassay against the first instar *S. litura* was not more than 30 percent for all the siRNAs (data not presented here).
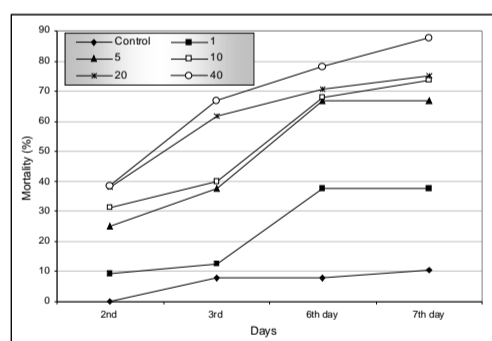

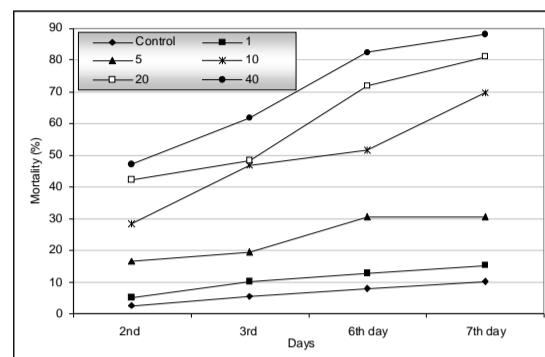Figure 7. Effect of different concentration of GENE 3 on adult sucking insect.

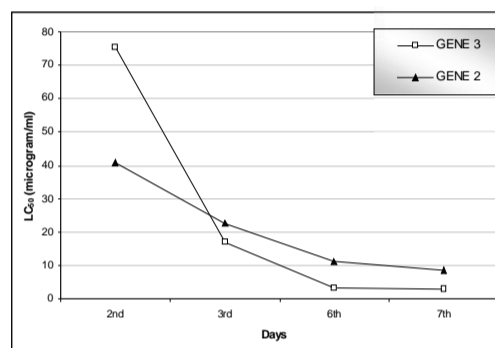Figure 8. Effect of different concentration of GENE 2 on adult sucking insect.

Figure 9. Comparative efficacy (LC_{50}) of siRNA (GENE 3 and GENE 3).

The siRNA recovered from the insect diet and checked on 15% native PAGE to confirm the stability (figure 10). The study indicates it was stable even after the insect bioassay at room temperature for 7 days.
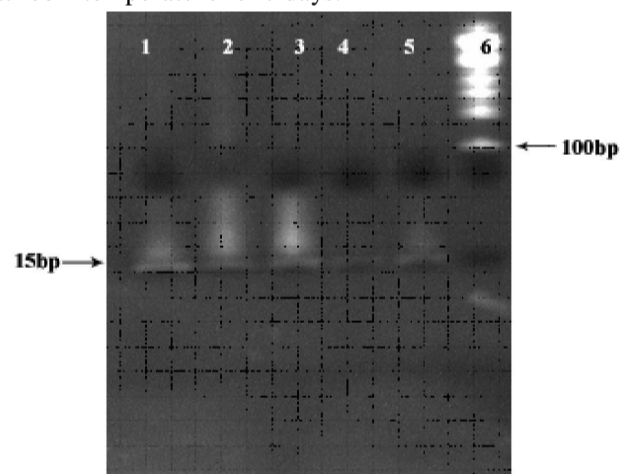

Figure 10. Recovered siRNA from insect diet (Lane 1-5 - siRNA from insect diet; lane 6 - 100bp ladder.

DISCUSSION

RNA interference is an effective means of regulation of gene expression both *in vitro* and *in vivo*. The results presented here show that siRNAs are powerful agents for gene silencing, even at low concentrations, and mediate post-transcriptional degradation of GENE 2 and GENE 2 transcripts selectively as confirmed through LC_{50}.

As per as our knowledge is concerned, no reports were available for gene silencing against sucking pests, hence the present study reports for the first time, the gene knockdown using siRNAs synthesized from five genes of a sucking insect through RNAi technology. Since the discovery that dsRNA can silence genes (Fire *et al.,* 1998), RNA interference (RNAi) has been developed as an effective tool in plants and animals (Wesley *et al.*, 2001; Tabara *et al.,* 1999) also gene silencing by double-stranded RNA, denoted as RNA interference, represents a new paradigm for rational drug design (Aouadi *et al.*, 2009). Recently in insect, the functional analysis of the circadian clock gene period by RNA interference in nymphal crickets *Gryllus bimaculatus* has been reported (Moriyama *et al.,* 2009).

Although silencing observed through RNAi in the present study is significant it is not cent percent. This could be due to the degradation of siRNA in the diet or body of the insect larvae according to Kumar *et al.* (2009). To confirm the stability of the siRNA in insect diet we recovered the siRNA from the diet used for the insect bioassay and checked the quality on 15% native PAGE. This present observation confirms that the siRNA is very stable in insect diet. This results contrast to the view of Kumar *et al.* (2009). Hence, it can be say that less mortality in some treatments may be because of physiology of the larvae.

However, the demonstrated efficacy of targeting GENE 3 could easily be extended to other insect species. Although RNAi is unlikely to have an immediate effect on crop protection against lepidopteran and coleopteran plant pests, for which Bt-based strategies offer a high degree of protection, the technology is likely to be taken up for applications where Bt-based approaches have proved difficult, for example protection against flies (dipterans), or where no effective Bt toxins are known, for example protection against sap-sucking homopteran pests such as aphids, leafhoppers and sucking insects (Price and Gatehouse, 2008). This study provides strong evidence for the view of Price and Gatehouse (2008).

Microinjection of dsRNA into adults and late-instar larvae has been used to silence genes and study gene function in the coleopteran *Tribolium castaneum* (Bucher *et al.,* 2002; Tomoyasu and Denell, 2004). Similarly, dsRNA induced an RNAi response in the lepidopteran *Spodoptera litura* upon injection, but not after ingestion (Rajagopal *et al.,* 2002). In contrast, oral delivery of highly concentrated (4,000 ppm) dsRNA reduced target mRNA levels in the lepidopteran *Epiphyas postvittana*, but did not cause mortality (Turner *et al.,* 2006). In contrast to all these studies explored the role of RNAi technology for the control of sucking pest.

The ability to trigger RNAi in coleopterans by oral delivery of dsRNAs has obvious commercial implications (Baum *et al.,* 2007). RNAi provides a unique mode of action for the control of insect pests that could complement the current strategy of expressing *B. thuringiensis* (Bt) insecticidal proteins in crops such as corn, cotton and soybeans. As adult of the sucking insects are devastating pests of cotton in India and are refractory towards most Bt insecticidal proteins, it is especially noteworthy that sucking insects are highly sensitive to an oral RNAi approach. Accordingly, RNAi has the potential to increase both the efficacy and durability of insect-protected crops designed to control this important hemipteran pest.

The current report deals with the effect of siRNA on adult by selective targeting of the five genes, of sucking insect. The synthesized siRNA molecules were directly fed to sucking insect adult along with the artificial diet. The siRNA treatment resulted in specific gene silencing of protein from GENE 2 and GENE 3 and consequently brought about mortality, as compared to control larvae. This observation correlates with the recent report of Kumar *et al.,* (2009) on *Helicoverpa armigera* by acetylcholinesterase gene of siRNA. Gene silencing clearly reduced the oviposition rates of adult females of *Spodoptera frugiperda*, the authors reported 16% mortality after 6 days of insect bioassay (Griebler *et al.,* 2008), but on sucking insects we observed 84.61 and 85.61% mortality from siRNA of GENE 2 and GENE 3 respectively. This indicates a great difference for the control of sucking insects using siRNA rather than the *Spodoptera* spp.

In conclusion, our findings present insights on the functional genes of sucking insects the GENE 2 and GENE 3 in various metabolic pathways in insects. The present results suggest that feeding of siRNAs through an artificial diet can be exploited for the screening of adult insect pests for control also functional genomic studies in insects. Further, our results strongly suggest that the GENE 2 and GENE 3 can be potential target for insect-control, and insect-resistant transgenic plants may be obtained through plant RNAi-mediated silencing of insect GENE 2 and GENE 3. Further studies are needed to find out the nature of gene.

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