academicJournals

Vol. 13(1), pp. 32-43, 1 January, 2014
DOI: 10.5897/AJB2013.13359
ISSN 1684-5315 ©2014 Academic Journals
http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

In silico mining of micro-RNAs from *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae)

R. Asokan[1], H. K. Roopa[1], K. B. Rebijith[1]*, H. H. Ranjitha[1] and N. K. Krishna Kumar[2]

[1]Division of Biotechnology, Indian Institute of Horticultural Research, Hessaraghatta Lake P.O., Karnataka, Bengaluru, 560 089, India.
[2]Division of Horticulture, ICAR, Krishi Anusandhan Bhawan - II, New Delhi - 110 012, India.

Accepted 17 December, 2013

MicroRNAs (miRNAs) are small, endogenously, non-coding genes that regulate protein production either by mRNA cleavage or by translational repression in eukaryotes and viruses. miRNAs plays a key role in biological processes including growth, development and physiology of an organism. In this study, we employed insilico approaches to identify the miRNAs from *Spodoptera frugiperda*, a major pest of small grain crops. A total of seven miRNAs were identified and characterized from 67,360 expressed sequence tags (ESTs) of *S. frugiperda* with: 1) mature and pre-miRNAs sizes vary from 19 to 25 ans 61 to 95 nucleotides respectively; 2) minimum free energy ranged from -31.70 to -21.00 kcal/mol; and 3) (A + U) content varied from 27 to 60. The functional annotation of these miRNAs were identified as regulation of transcription factors, catalytic activities and signal transduction pathways. Further studies of these miRNAs will help to carryout functional analyses, which promises more towards insect pest management free of insecticides and pesticides.

Key words: MicroRNAs, translational repression, *Spodoptera frugiperda*, expressed sequence tags, minimum free energy, insect pest management.

INTRODUCTION

Central dogma, a flow of genetic information now reached a new insight into the non-coding RNA contigs of ~20 to 22 nucleotides in length (Wang, 2008). Small RNAs have burst on the scene as ubiquitous, versatile repressors of gene expression in plants, animals and many fungi. Among small RNAs, MicroRNAs (miRNAs) are a family of small, endogenously initiated non-coding RNAs that extensively regulate gene expression either by mRNA cleavage or by translational repression thus play important roles in the growth, development and physiology of plants, animals and some viruses. miRNA mediated gene regulation is an emerging post transcriptional regulation of biological process namely, cell division and differentiation (Makeyev et al., 2007), notch signaling (Lai et al., 2005), growth and development (Leaman et al., 2005), apoptosis (Stark et al., 2003) and oogenesis (Nakahara et al., 2005) by altering gene turn on and off mechanism (Wang, 2008). Lee et al. (1993) discovered the first miRNA, *lin-4*, in *Caenorhabditis elegans*, which regulate timings of larval development. A total of 21,264 miRNAs (miRBase v19.0, 2012) were reported so far from various animal species, including insects (Marco et al., 2010).

miRNAs were initially discovered in *Caenorhabditis elegans*; however, recents advancements in both bioinformatics and sequencing technologies helped in miRNA discoveries in more than 22 species of insects. Wherein miRNA transcribed by RNA polymerase-II as a long precursor transcript, which are called primary miRNA (pri-miRNAs) (Lee et al., 2004). These pri- miRNAs are then

*Corresponding author. E-mail: rebijith@gmail.com.

cleaved into 60 to 80 nt long precursor miRNA sequences (pre-miRNAs) by the Drosha, Pasha (Denli et al., 2004; Landthaler et al., 2004). These hairpin pre-miRNAs were further transported by Exportin-5 in the presence of cofactor, Ran-GTP from nucleus to cytoplasm and cleaved by Dicer (Bartel, 2004) and thus produces approximately 19 to 22 bp miRNA:miRNA duplex (Denli et al., 2004; Ghosh et al., 2007).

The mature miRNAs are then selectively loaded into a ribonucleoprotein complex, miRNP (Hutvagner and Zamore, 2002), similar to the RNA-induced silencing complex (RISC) which contains Argonaute (Ago) family proteins, the effector moleculaes of RNAi (Hammond et al., 2000). MiRNAs can mediate the downregulation of target gene activity in two ways namely, translational repression (partial complementarity of miRNA and mRNA) or by target mRNA cleavage (high complementarity) (Zeng et al., 2003; Wienholds et al., 2005). Agricultural production and productivity are limited by biotic factors such as insects and can cause severe damage through direct feeding thus defoliation. In this regard, *Spodoptera frugiperda* (Smith), is a polyphagous, ubiquitous pest on glasshouse that spreads infestation sporadically and causes damage by direct feeding. Extensive use of insecticides has resulted in developing resistance as well as causing environmental pollution, thus demanding an alternative safe and effective management strategy such as miRNA mediated gene silencing and insect transgenesis for insect pest management. Identification of miRNA involves three main approaches namely, forward genetics, bioinformatics prediction (Zhang et al., 2005) and direct cloning and sequencing (Chen et al., 2005).

In the present study, we employed insilico prediction and characterization to identify the putative microRNAs and their targets in *S. frugiperda* with the advances in the comparative genomics along with the available expressed sequence tags (ESTs).

MATERIALS AND METHODS

miRNAs and ESTs sequence dataset

A total of 21,264 known mature miRNAs from all animal species were downloaded from the miRBase sequence database (Release 19, August- 2012) (http://www.mirbase.org/) (Griffiths-Jones et al., 2006). On the other hand, 67,360 ESTs of *S. frugiperda* were downloaded from the National Centre for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/genomes). Redundancy test for both miRNAs and ESTs were cured on the basis of sequence identity and the remaining miRNAs and EST sequences were used for further analysis.

Homology search and prediction of potential microRNAs

Homology search carried out by a local BLAST search for the miRNA sequences against the EST sequences in BioEdit 9.0 (Hall, 1999), with an E-value ≤ 0.01 and other parameters kept as default. The major criteria used for screening the BLAST results were; 1) sequence identity should be >80% between the predicted and reference miRNA; 2) hits having ≤3 bp mismatches selected as a potential miRNA containing ESTs.

Secondary structure prediction

Generally, miRNA precursors produced from pri-miRNAs and thus produced miRNA precursor have 70 to 120 nucleotides in length. In this regard, Pre-miRNA sequences extracted from the selected EST sequences employing sliding window method described by Singh and Nagaraju (2008). Thus, extracted precursor miRNA sequences were submitted to Mfold (http://mfold.rna.albany.edu/?q=mfold/RNA-Folding-Form), which will calculate the free energy (∆G) for various possible miRNA hairpin structures. The criteria employed for selection of pre-miRNA structures were: 1) the minimal folding free energy (MFE) ≤ -18 kcal/mol; 2) mature miRNA on the stem region of the hairpin structure; 3) the A + U content should be in the range of 30 too 70%; 4) the bulge size in the mature miRNA region should not exceed 7 bases (Singh and Nagaraju, 2008; Ghosh et al., 2007). Pre-miRNAs that satisfied all the aforementioned criteria was selected for naming and target prediction.

miRNAs nomenclature

miRNAs nomenclature of predicted seven miRNAs from *S. frugiperda* in the present study has been done according to Griffiths -Jones et al. (2006). The prefix were fixed as ‘sfr’, since all miRNAs are from *S. frugiperda*. The rest of the naming convention criteria were in accordance with miRBase (Griffiths-Jones et al., 2006).

Conservation of pre-miRNA sequences

Predicted pre-miRNAs classified into different families according to Rfam (Griffith et al., 2003) and compared with the other pre-miRNAs in the corresponding family inorder to analyse the evolutionary relationship. Our alignment file created by BioEdit (Hall, 1999) was used to find out the areas of conservation in the pre-miRNA sequences.

miRNA target prediction and functional annotation

The imperfect complementarity of animal miRNAs with their target sequences on mRNA makes it more difficult to judge the accuracy of prediction as against the plant counterparts. However, target prediction is important in understanding the biological functions of miRNAs in a given species. In this connection, potential targets for the newly identified miRNAs were predicted employing TargetScan (Lewis et al., 2003), an online software works on the thermodynamic modelling of RNA-RNA duplex along with the complementarity at the seed region. Functional annotation carried out employing the *D. melanogaster* genome in FlyBase (Crosby et al., 2007).

RESULTS AND DISCUSSION

Seven miRNAs were newly identified and characterized from *S. frugiperda* employing *In silico* approach that primarily depends on the sequence homology and secondary structure verification. The various steps involved in the present study is depicted in Figure 1.

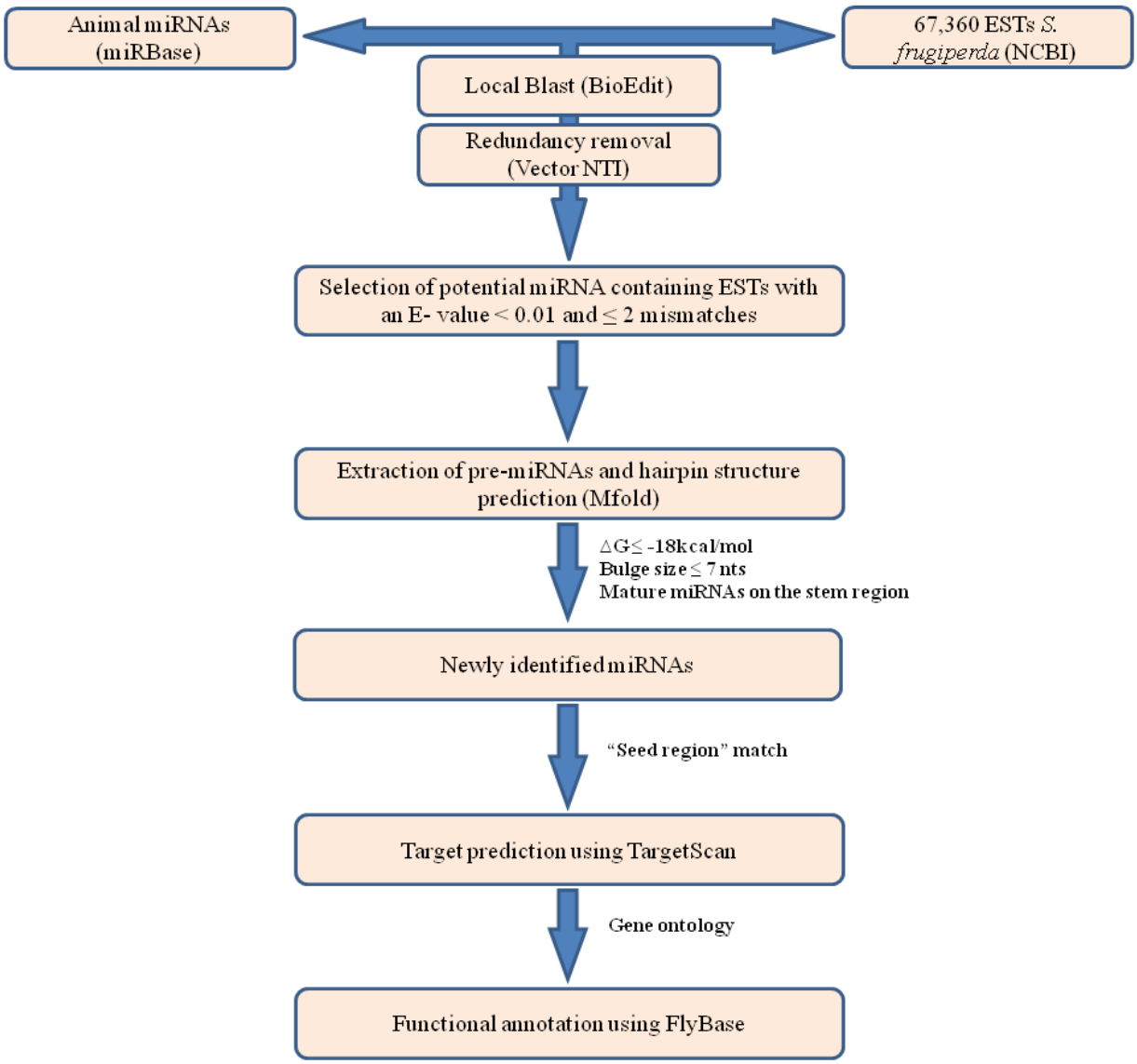

Figure 1. Flowchart showing various steps involved in miRNA prediction and functional annotation

Homology search and secondary structure prediction

Homology search was carried out employing 21,264 mature miRNAs from all animals against the EST sequences of *S. frugiperda* resulted in 37 potential miRNA containing ESTs. The pre-miRNAs sequences were further subjected to secondary structure prediction and resulted in seven pre-miRNAs with various possible hairpin structures (Figure 2). Based on previously mentioned criteria, a total of seven mature miRNAs (Table 1) with their pre-miRNA sequences (Table 2) were identified in the current study and none of them were reported previously from *S. frugiperda*.

Characteristics of novel miRNAs

According to the study of Qiu et al. (2007), it is estimated that the probability of finding a new miRNA ranges from 1 to 1.67 in every 10,000 ESTs. However, in the present study, we identified seven miRNAs from 67,360 ESTs with an average of 0.89, which is slightly lesser with the aforementioned estimation, due to the fact that many of the ESTs were removed during the redundancy test. Length of newly identified mature and pre-miRNAs ranged from (19 to 25) and (61 to 95) nucleotide respectively and (A + U) content ranged from 27 (sfr-miR-1895) to 60% (sfr-miR-716b). The (A + U) content of miRNA is always high compared to other RNAs (Zhang et al., 2006) in regulating post-transcriptional events, which bind more strongly to certain proteins like oncoproteins, cytokines and transrcription factors (Gupta et al., 2010). The minimum free energy (MFE) values of the newly identified seven miRNAs ranged from -31.70 to -21.00 kcal/mol. Newly identified seven miRNAs were classified

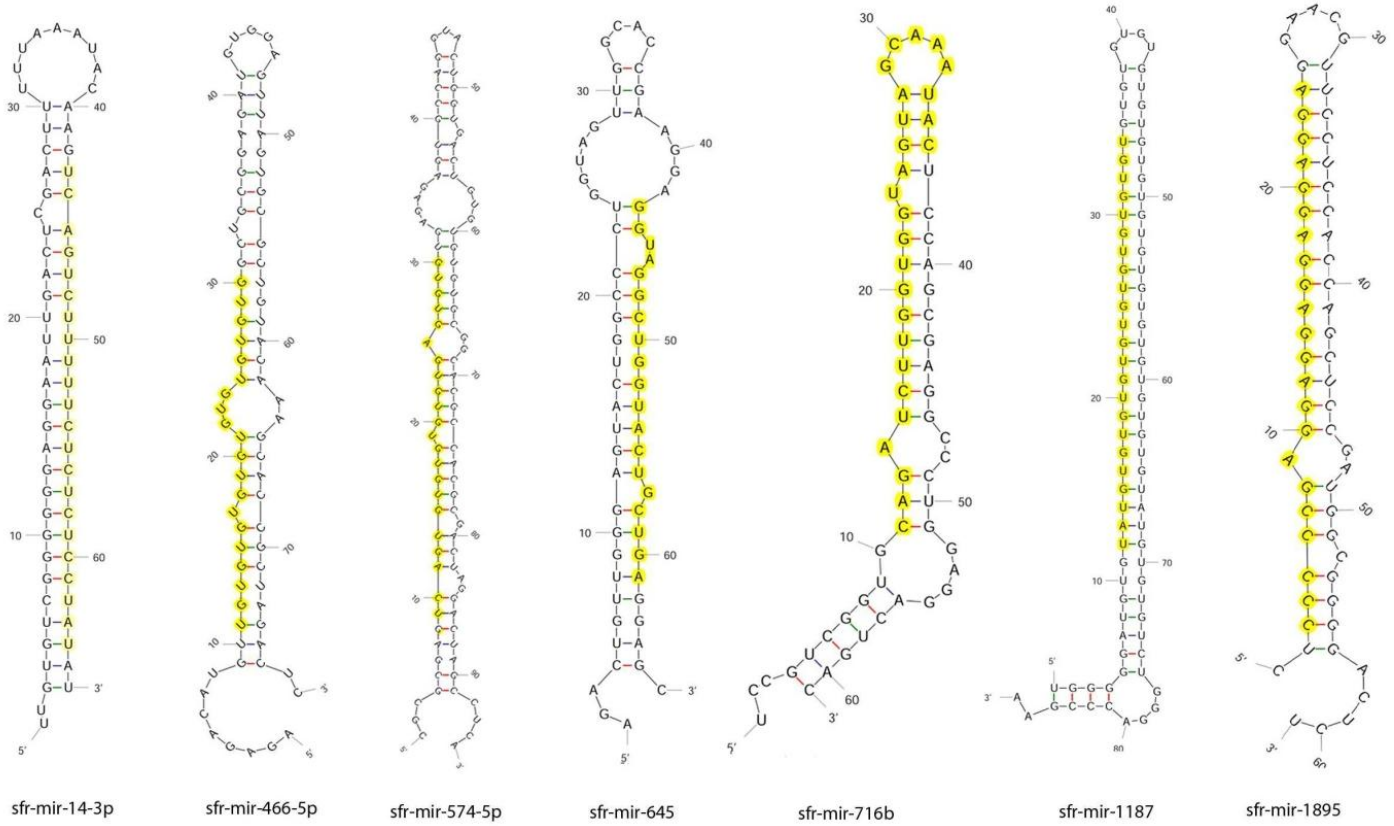

Figure 2. Various hairpin secondary structures of seven newly identified premiRNAs of *Spodoptera frugiperda*.

Table 1. Details of the seven newly identified mature miRNAs from *S. frugiperda.*

miRNA	EST ID	Start position	End position	Strand	Match extent	MFE (Kcal/Mol)	A + U content (%)	Predicted miRNA sequence	MFEI
sfr-miR-645	FP374527	265	281	-	17/19	-21.00	40.91	GGUAGGCUGGUACUGCUGA	0.54
sfr-miR-14-3p	FP355748	603	624	-	22/22	-27.30	60.61	UCAGUCUUUUUCUCUCUCCUAU	1.05
sfr-miR-574-5p	FP374665	138	158	+	21/23	-31.70	40.00	UCAGUGUGUGUGUGUGAGUGUGU	0.56
sfr-miR-466i-5p	FP374357	49	68	-	20/20	-22.50	47.44	UGUGUGUGUGUGUGUGUGUG	0.55
sfr-miR-716b	DY795104	433	456	+	24/25	-20.70	42.62	AGAUCUUGGUGGUAGUAGCAAAUAC	0.59
sfr-miR-1895	DY793599	586	607	+	21/22	-29.40	32.79	CCCCCGAGGAGGAGGAGGAGGA	0.72
sfr-miR-1187	FP364853	70	92	+	20/23	-21.20	48.84	UAUGUGUGUGUGUGUGUGUGUGU	0.48

Table 2. Details of the seven newly predicted pre-miRNA sequences from *S. frugiperda*.

PremiRNA	EST ID	Start - end position	PremiRNA sequence
sfr-miR-645	FP374527	259-324	AGACUGUUGGGAGUACUGGCCCUGGUAGUUGGCACCGAAGGAGGUAGGCUGGUACUGCUGAGGAGC
sfr-miR-14-3p	FP355748	68-133	UUGUGUCGGGGGGAGGAAUUGACUCGACUUUUUAAAUACAAGUCAGUCUUUUUCUCUCUCCUAUAU
sfr-miR-574-5p	FP374665	128-222	CGCGCGAGUCAGUGUGUGUGUGUGAGUGUGUGAGAGAGUGCCAGGUACUGGUGACUGUGUGUGUGCGGCACGCCACGCGACUAGGACUAGCCUCA
sfr-miR-466i-5p	FP374357	1-78	AGAGACAUGUUGUGUGUGUGUGUGUGUGUGGCUGCGGAGAUGUGGAGUUAGUGCGCUGUACAAAGCACCGCUAGACUC
sfr-miR-716b	DY795104	422-482	UCCGUCGGUGCAGAUCUUGGUGGUAGUAGCAAAUACUCCAGCGAGGCCCUGGAGGACUGAC
sfr-miR-1895	DY793599	584-644	CUCCCCCGAGGAGGAGGAGGAGGAGGAACGUUCCUCCACCAGCUCCGAUGGCGGGGACUCU
sfr-miR-1187	FP364853	25-110	UGGGGGAUGUGUAUGUGUGUGUGUGUGUGUGUGUGUGUGUGUGUGUGUGUGUGUGUGUGUGUAUGUGUGUCUGGGACCC

into four Rfam (Griffith-Jones et al., 2005) families namely, miR 467, miR14, miR 574, miR 645 and miR 716 and their corresponding pre-miRNA sequences were aligned using BioEdit (Table 3). Mature and pre-miRNAs are highly conserved and are considered as evolutionarily conserved regulators of gene expression among various species of organism (Zhang et al., 2005).

In this regard, the alignment of pre-miRNA sequences of miR 467, miR14, miR 574, miR 645 and miR 716 showed conservative sites in the aligned sequences (Figure 3).

Target prediction

Recent studies demonstrated that the miRNAs can bring
about miRNA cleavage or translational repression by binding to 3' UTRs (untranslated regions), 5' UTRs and even to coding regions on the mRNA (Lytle et al., 2007). However, animal miRNAs primarly target the 3' UTRs; hence, we confined our target search only to 3' UTRs of *D. melanogaster* in the target scan fly (Releas 6.2); and the predicted targets are given in Table 4. In contrast to plants, animal miRNAs shows lower degree of complementarity with miRNA suggesting translational repression is more prevalent in animals compared to RISC mediated miRNA cleavage in plants (Asokan et al., 2013). In the target prediction, only the seed region, that is, 2nd to 7th position of 5' end of miRNA (Lewis et al., 2005) considered to be crucial for target repression (Kim et al., 2006). In this regard, we selected only those miRNA:mRNA complex having strong seed region complementarity, without allowing the gap introduction in the alignment. According to Ghosh et al. (2007), the minimal complementarity at the seed region can be compensated by the strong pairing on the 3' end of the miRNA to confer the regulatory functions.

A total of 128 targets were predicted in Target ScanFly and all of our miRNA:mRNA duplex has strong seed region complementarity because of the absence of gaps and mismatches.

Functional annotation

The functional annotation were carried out employing the *D*. *melanogaster* genome available in the FlyBase (http://flybase.org/). The aforementioned identified 128 targets for seven miRNAs were involved in transcription regulatory factors, signal transduction pathways, catalytic activity and protein or nucleic acid binding on par with the recent studies on other species of insects (Singh and Nagaraju, 2008; Sathyamurthy and Swamy, 2009). Of which, sfr-miR-645 targets involved in the functioning of ligand-gated ion channel activity. Whereas, sfr-miR-466i-5p targets maximum number of gene targets involved in the regulation of signaling proteins like DNA-binding transcription factor activity, water transmembarne transporter activity, voltage gated calcium ion channel, Notch binding, protein kinase-A binding, sodium-potassium exchangeing ATPase activity, G-protein coupled receptor activity by GTpase. It also paly role in regulating transcription factors like RNA- polymerase- II transcription factors, RNA helicase activity, DNA topoisomersae, satellite DNA binding: mRNA 3'- UTR binding, odorant binding and most importantly it regulates ubiquitin-protein ligase activity. It also targets major receptor and in blocking nerve signals which includes GABA-A, glutamate, transmembrane signaling, SNAP receptors, Dopamine beta-monooxygenase activity and 3'-5'-cyclic nucleotide phosphate activity.

sfr-miR-14-3p targets involved in governorning various enzymatic activities, transcriptional activities etc. are given in Table 4. sfr-miR-1187 targets the genes functioning in transcription factors like RNA polymerase-II factors activity, heterodimerization activity, GABA receptor, microtubule motor activity, transmembrane

Table 3. Pre-miRNAs belonging to different Rfam family.

PremiRNA members	Rfam family
miR-645	mir-645
miR-14-3p	mir-14
miR-574-5p	mir-574
miR-466i-5p	mir-467
miR-716b	mir-716
miR-1895	-(doesn't belong on any family)
miR-1187	-(doesn't belong on any family)

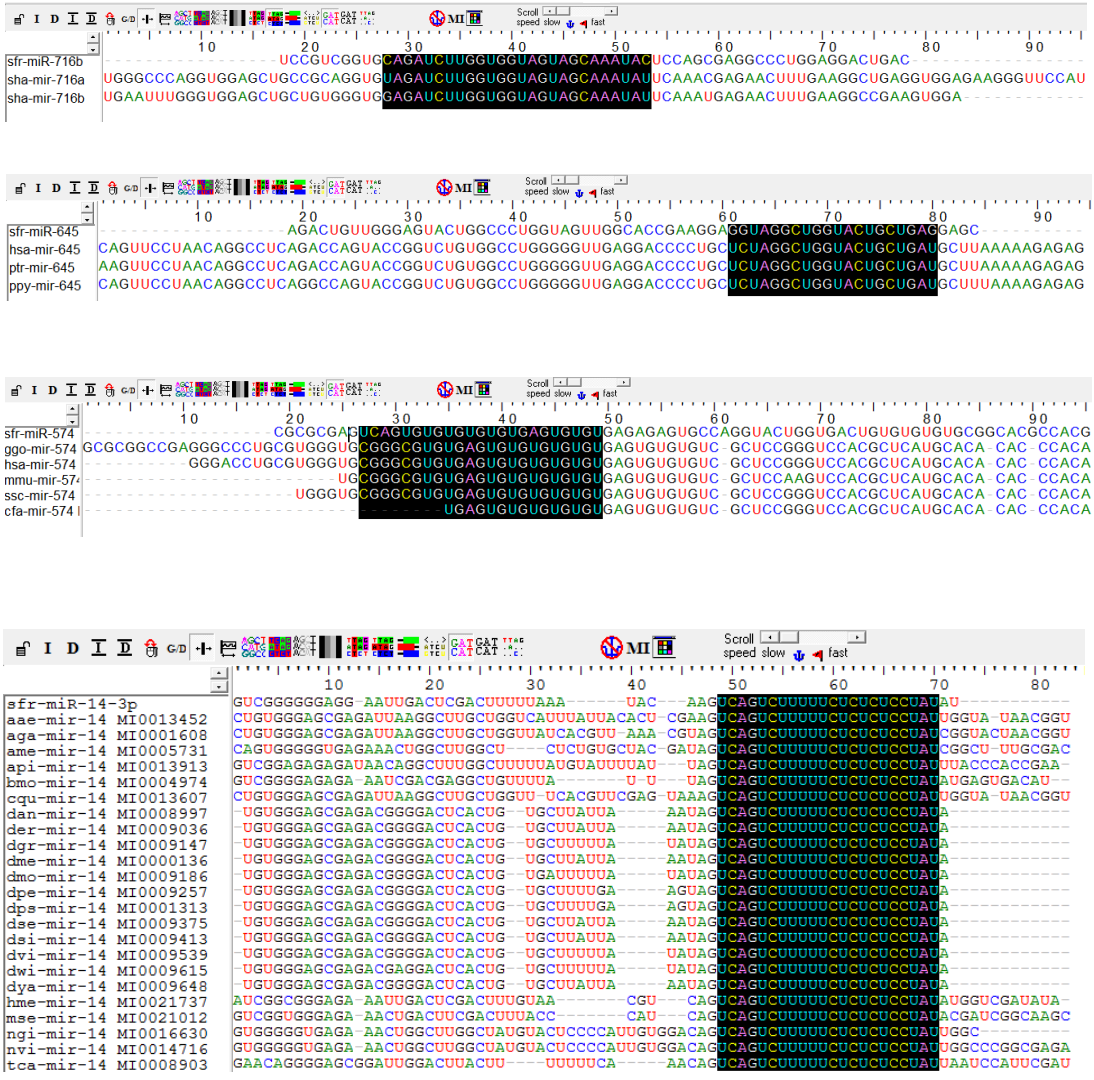

Figure 3. Comparison of the conserved region (dark box) of the newly identified *Spodoptera frugiperda* miRNAs namely: sfr-miR-716, sfr-miR-645 and sfr-miR-574 with miRNAs of other animal species belongs to the same miRNA family. sfr, *Spodoptera frugiperda*; ggo, *Gorilla gorilla*; sha, *Sarcophilus harrisii*; ppy, *Pongo pygmaeus*; ptr, *Panvtroglodytes*; mmu, *Mus musculus*; ssc, *Sus serofa*; cfa, *Canis familiaris*; has, *Homo sapiens* and sfr-miR-14-3p belongs to insects: aae, *Aedes aegypti*, aga, *Anophele gambiae*; ame, *Apis mellifera*; api, *Acyrthosiphon pisum*; bmo, *Bombyx mori*; cqu, *Culex quinquefasciatus*; dan, *Drosophila ananassae*; der, *Drosophila erecta*; dgr, *Drosophila grimshawi*; dme, *Drosophila melanogaster*; dmo, *Drosophila mojavensis*; dpe, *Drosophila persimilis*; dps, *Drosophila pseudoobscura*; dse, *Drosophila sechellia*; dsi, *Drosophila simulans*; dvi, *Drosophila virilis*; dwi, *Drosophila willistoni*; dya, *Drosophila yakuba*; hme, *Heliconius melpomene*; mse, *Manduca sexta*; ngi, *Nasonia girault*i; nvi, *Nasonia vitripennis*; tca, *Tribolium castaneum.*

Table 4. Functional annotation of mature miRNAs from *S. frugiperda*.

miRNA	Target gene	Annotation symbol	Molecular function
sfr-miR-645	FBgn0029961	CG12663	Ligand-gated ion channel activity
sfr-miR-14-3p	FBgn0264294	CG2140	Electron carrier activity; heme binding
	FBgn0003254	CG7230	DNA binding
	FBgn0011300	CG8224	Activin receptor activity
	FBgn0026263	CG7574	Nucleic acid binding
	FBgn0014135	CG4608	Fibroblast growth factor receptor binding
	FBgn0004876	CG6027	ATP binding; protein kinase activity;
	FBgn0010105	CG17943	Protein binding
	FBgn0001168	CG6494	DNA binding
	FBgn0015239	CG7199	RNA polymerase II activity
	FBgn0053147	CG33147	Heparan sulfate-glucosamine 3-sulfotransferase 1 activity
	FBgn0032147	CG4026	Inositol-1,4,5-trisphosphate 3-kinase activity
	FBgn0085388	CG34359	Calmodulin binding
	FBgn0013469	CG12296	Metal ion binding; nucleic acid binding
	FBgn0002543	CG5481	Protein homodimerization activity
	FBgn0066101	CG31094	Calcium ion binding
	FBgn0034590	CG30388	Guanylate kinase activity
	FBgn0002945	CG11614	Calcium ion binding
	FBgn0003892	CG2411	Hedgehog receptor activity;
	FBgn0004635	CG1004	Serine-type endopeptidase activity
	FBgn0003285	CG4125	PDZ domain binding
	FBgn0024921	CG7398	Ran GTPase binding
	FBgn0004009	CG4889	Morphogen activity; Notch binding
	FBgn0026313	CG5675	Cell adhesion molecule binding
	FBgn0036316	CG10960	Transmembrane transporter activity
	FBgn0034763	CG12190	Zinc ion binding
	FBgn0036725	CG18265	Metal ion binding
	FBgn0034158	CG5522	Ralguanyl-nucleotide factor activity
	FBgn0034527	CG9945	Unknown
sfr-miR-574		**Unknown target and functions**	
sfr-miR-466i-5p	FBgn0011760	CG6998	Dynein intermediate chain binding
	FBgn0015903	CG5393	DNA binding transcription factor activity
	FBgn0011766	CG6376	DNA binding transcription factor activity
	FBgn0036323	CG14118	Metal ion binding; hydrolase activity

Table 4. Contd.

	FBgn0029871	CG42340	Potassium channel activity
	FBgn0033635	CG7777	Water transmembrane transporter activity
	FBgn0029764	CG3249	Protein kinase A binding
	FBgn0017418	CG5659	Ubiquitin-protein ligase activity
	FBgn0004635	CG1004	Serine-type endopeptidase activity
	FBgn0028734	CG6203	Protein, mRNA and RNA binding; protein self-association
	FBgn0002921	CG5670	Sodium:potassium-exchanging ATPase activity
	FBgn0004595	CG17228	RNA polymerase II transcription factor activity
	FBgn0086376	CG42344	Calcium channel activity
	FBgn0030766	CG4521	G-protein coupled receptor activity
	FBgn0030391	CG1900	GTPase activity.
	FBgn0004910	CG15855	Calcium ion binding.
	FBgn0039561	CG4963	Iron ion transmembrane transporter activity
	FBgn0003463	CG9224	Growth factor activity.
	FBgn0016041	CG12157	Protein transmembrane transporter activity.
	FBgn0029979	CG10777	RNA helicase activity.
	FBgn0027492	CG5643	Protein serine/threonine phosphatase activity.
	FBgn0004244	CG10537	GABA-A receptor activity.
	FBgn0004652	CG14307	DNA binding transcription factor activity.
	FBgn0011754	CG1830	Phosphorylase kinase activity.
	FBgn0030421	CG3812	1-acylglycerol-3-phosphate O-acyltransferase activity.
	FBgn0034603	CG9480	Glycogeninglucosyltransferase activity.
	FBgn0028428	CG8585	Voltage-gated ion channel activity.
	FBgn0031446	CG15398	DNA binding transcription factor activity.
	FBgn0033730	CG8511	Structural constituent of chitin-based cuticle.
	FBgn0052771	CG42594	Potassium channel activity.
	FBgn0022768	CG2984	Protein serine/threonine phosphatase activity.
	FBgn0010329	CG1543	Dopamine beta-monooxygenase activity.
	FBgn0030182	CG43902	Magnesium ion binding; inorganic diphosphatase activity.
	FBgn0037589	CG11732	Odorant binding.
	FBgn0003415	CG9936	RNA polymerase II transcription cofactor activity.
	FBgn0035023	CG13586	Hormone activity; neuropeptide hormone activity.
	FBgn0036672	CG42679	DNA binding transcription factor activity.
	FBgn0029708	CG3556	Cobalamin binding.
	FBgn0029805	CG42264	Carboxypeptidase activity.
	FBgn0052318	CG32318	ATP binding; microtubule motor activity.
	FBgn0052648	CG42276	3',5'-cyclic-nucleotide phosphodiesterase activity

Table 4. Contd.

	FBgn0061355	CG43164	Carbohydrate binding.
	FBgn0030089	CG9113	Protein transporter activity.
	FBgn0052447	CG32447	Glutamate receptor activity.
	FBgn0003319	CG4316	Serine-type endopeptidase activity.
	FBgn0010460	CG42281	Protein homodimerization activity.
	FBgn0030796	CG4829	Protein-glutamine gamma-glutamyltransferase activity.
	FBgn0031298	CG4428	Cysteine-type endopeptidase activity.
	FBgn0033504	CG18408	Vinculin binding.
	FBgn0036030	CG6767	Ribose phosphate diphosphokinase activity.
	FBgn0034020	CG43729	Diacylglycerol binding.
	FBgn0001168	CG6494	RNA polymerase II transcription factor activity
	FBgn0003134	CG6593	Protein serine/threonine phosphatase activity.
	FBgn0052529	CG32529	Transcription regulatory region sequence-specific DNA binding.
	FBgn0010415	CG10497	Transmembrane signaling receptor activity.
	FBgn0000286	CG11924	RNA polymerase II transcription factor activity
	FBgn0000633	CG17716	Unknown
	FBgn0015622	CG11958	Unfolded protein binding.
	FBgn0030672	CG9281	ATPase activity, Transporter activity.
	FBgn0028550	CG11405	Protein binding; sequence-specific DNA binding.
	FBgn0003888	CG3401	Structural constituent of cytoskeleton.
	FBgn0028888	CG4168	G-protein coupled receptor activity.
	FBgn0000259	CG15224	Protein serine/threonine kinase activity
	FBgn0000299	CG4145	Extracellular matrix structural constituent.
	FBgn0004924	CG6146	DNA topoisomerase activity
	FBgn0024277	CG18214	Rho guanyl-nucleotide exchange factor activity.
	FBgn0029123	CG18024	Chromatin DNA binding; protein binding
	FBgn0035802	CG33275	Guanyl-nucleotide exchange factor activity.
	FBgn0000275	CG42341	cAMP-dependent protein kinase regulator activity.
	FBgn0001320	CG4717	RNA polymerase II transcription factor activity
	FBgn0003659	CG43770	Poly-pyrimidine tract binding; growth factor activity
	FBgn0011582	CG9652	Dopamine receptor activity, coupled via Gs.
	FBgn0026061	CG4123	Phosphoprotein phosphatase activity.
	FBgn0038181	CG9297	Calcium ion binding.
	FBgn0004572	CG15113	G-protein coupled amine receptor activity
	FBgn0013343	CG31136	SNAP receptor activity.
	FBgn0026611	CG6671	Enzyme binding; protein binding; miRNA binding.
	FBgn0034822	CG9873	Structural constituent of ribosome.

Table 4. Contd.

	FBgn0035142	CG17090	Beta-catenin binding; transcription factor binding
	FBgn0040697	CG18676	Structural constituent of ribosome.
	FBgn0050420	CG30420	RNA polymerase II transcription factor activity
	FBgn0001235	CG17117	Transcription factor binding;
	FBgn0003205	CG9375	GTPase activity.
	FBgn0004103	CG5650	Protein serine/threonine phosphatase activity.
	FBgn0004611	CG4574	Phosphatidylinositol phospholipase C activity.
	FBgn0010905	CG16757	Protein phosphatase 1 binding.
	FBgn0027835	CG5170	Satellite DNA binding; mRNA 3'-UTR binding
	FBgn0000575	CG1007	Protein binding transcription factor activity
	FBgn0001148	CG3388	RNA polymerase II regulatory region DNA binding.
	FBgn0003984	CG10491	Epidermal growth factor receptor binding.
	FBgn0004644	CG4637	Cell surface binding; protein binding.
	FBgn0011666	CG5099	RNA binding; mRNA binding.
	FBgn0020307	CG5799	DNA binding transcription factor activity
	FBgn0020367	CG3762	Hydrogen-exporting ATPase activity
	FBgn0022960	CG3572	RalGTPase binding.
	FBgn0031995	CG8475	Phosphorylase kinase regulator activity.
	FBgn0032351	CG42403	Voltage-gated calcium channel activity.
	FBgn0033355	CG13748	Serine-type endopeptidase inhibitor activity.
	FBgn0034282	CG5784	Phosphatase inhibitor activity; microtubule binding.
	FBgn0034903	CG9850	Metalloendopeptidase activity.
	FBgn0035381	CG43128	Delayed rectifier potassium channel activity.
	FBgn0035420	CG14967	Unknown
	FBgn0035938	CG43782	mRNA 3'-UTR binding; translation repressor activity.
	FBgn0036341	CG11278	SNAP receptor activity.
	FBgn0036621	CG16807	Zinc ion binding; nucleic acid binding.
	FBgn0039632	CG1401	Ubiquitin protein ligase binding.
	FBgn0051665	CG31665	Notch binding.
	FBgn0051741	CG31741	Scavenger receptor activity.
	FBgn0052062	CG32062	Transcription factor binding
	FBgn0053171	CG42543	Carbohydrate binding
sfr-miR-716b	FBgn0034802	CG3800	Nucleic acid binding.
	FBgn0000359	CG1478	Structural constituent of chorion.
	FBgn0026077	CG10287	Chitin binding;
	FBgn0031426	CG18641	Lipase activity.

Table 4. Contd.

	FBgn0037227	CG14640	Structural constituent of chitin-based cuticle.
sfr-miR-1895		**Unknown target and functions**	
	FBgn0024555	CG9351	Protein binding
	FBgn0000448	CG33183	RNA polymerase II transcription factor activity
	FBgn0000490	CG9885	Protein heterodimerization activity; protein binding
	FBgn0004244	CG10537	GABA-A receptor activity.
	FBgn0010460	CG42281	Protein homodimerization activity.
sfr-miR-1187	FBgn0019968	CG8183	Microtubule motor activity
	FBgn0020497	CG13387	Protein binding; protein transporter activity.
	FBgn0027339	CG11352	DNA binding transcription factor activity.
	FBgn0030872	CG6492	Transmembrane transporter activity
	FBgn0033609	CG13213	SAM domain binding.
	FBgn0034158	CG5522	Guanyl-nucleotide exchange factor activity.

transporter activity, oxidative phosphorylation uncoupler activity and guanyl nucleotide exchange factor activity. Another miRNA namely, sfr-miR-716b involved in the regulation of nucleic acid binding, chitin binding, lipase activity, structural constituent of chitin-based cuticle. However, two miRNAs namely, sfr-miR-574 and sfr-miR-1895 did not find any target, this may be due to the incomplete coverage of mRNA in the FlyBase database. Thus, our study pave way for further functional validation experiments which can prove the multiple levels of effective gene regulation by these “miRNAs- Master regulator of the cell” do in a wide range of cellular and mole-cular process (Singh and Nagaraju, 2008).

Target multiplicity and cooperativity

Target multiplicity and cooperativity are two conse-quences that arise due to miRNA interaction to the target site (John et al., 2004). When single miRNA have more than one target site, it is know as target multiplicity and on the other hand, one particular transcript can be targeted by many miRNAs which is known as cooperativity. Ghosh et al. (2007) also identified single miRNA that can target multiple genes; thus, multiple binding sites on the 3’UTRs brings the effective silencing. Our study showed that most of the miRNAs have more than one target with miRNAs sfr-miR-466i-5p with maxi-mum of 110 target mRNAs showing target multiplicity (Table 4).

Conclusion

In a nutshell, the present study identified seven new miRNA candidates from *S. frugiperda* employing EST sequences of the target organism. Sequence analysis revealed both mature and pre-miRNAs are highly consereved in nature and are involved in various signal transduction pathways, transcription regulatory factors, catalytic activity and protein or nucleic acid binding on par with the recent studies on other insect species. Thus, our results will help in future studies on functional analyses of the identified candidate miRNAs, which will unravel the importance of their targets which plays an immense role in understanding host-insect interaction and develop-mental biology of *S. frugiperda.*

ACKNOWLEDGEMENTS

Financial support received for the “Out Reach Programme on Management of Sucking pests in Horticultural Crops”, from the Indian Council for Agricultural Research (ICAR), New Delhi is gratefully acknowledged. We are thankful to the Director, IIHR for the support and facilities.

REFERENCES

Asokan R, Rebijith KB, Ranjitha HH, Roopa HK, Ramamurthy VV (2013). Prediction and characterization of novel microRNAs from brown plant hopper, *Nilaparvata lugens*

(Stål) (Hemiptera: Delphacidae). Entomol. Res. 43:224-235.
Bartel DP (2004). MicroRNAs: genomics biogenesis mechanism and function. Cell 116:281-297.
Chen CF, Ridzon DA, Broomer AJ, Zhou ZH, Lee DH (2005). Real-time quantification of microRNAs by stem-loop RT-PCR. Nucleic Acids Res. 33:e179.
Crosby MA, Goodman JL, Strelets VB, Zhang P, Gelbart WM (2007). FlyBase: genomes by the dozen. Nucleic Acids Res. 35:486-491.
Denli AM, Tops BB, Plasterk RH, Ketting RF, Hannon GJ (2004). Processing of primary microRNAs by the microprocessor complex. Nature 432:231-235.
Ghosh Z, Chakrabarti J, Mallick B (2007). miRNomics-the bioinformatics of microRNA genes. Biochem. Biophys. Res. Commun. 363:6-11.
Griffiths-Jones S, Bateman A, Marshall M, Khanna A, Eddy SR (2003). Rfam: an RNA family database. Nucleic Acids Res. 31, 439-441.
Griffiths-Jones S, Grocock RJ, van Dongen S, Bateman A, Enright AJ (2006). miRBase: microRNA sequences, targets and gene nomenclature. Nucleic Acids Res. 34 (Database Issue):140-144.
Griffiths-Jones S, Moxon S, Marshall M, Khanna A, Eddy SR, Bateman A (2005). Rfam: annotating non-coding RNAs in complete genomes. Nucleic Acids Res. 33:121-124.
Gupta R, Soni N, Patnaik P, Sood I, Singh R, Rawal K, Rani V (2010). High AU content: a signature of unregulated miRNA in cardiac diseases. Bioinformation 5(3):132-135.
Hall TA (1999). BioEdit: a user-friendly biological sequence alignment 291 editors and analysis 292 programme for Windows 95/98/NT. Nucl. Acid S. 41:95-98.
Hammond SM, Bernstein E, Beach D, Hannon GJ (2000). An RNA-directed nuclease mediates post-transcriptional gene silencing in Drosophila cells. Nature 404:293-296.
Hutvagner G, Zamore PD (2002). A microRNA in a multiple-turnover RNAi enzyme complex. Science 297:2056-2060.
John B, Enright AJ, Aravin A, Tuschl T, Sander C, Marks DS (2004). Human microRNA targets. PLoS Biol. 2:e363
Kim SK, Nam JW, Rhee JK, Lee WJ, Zhang BT (2006). miTarget: microRNA target gene prediction using a support vector machine. BMC Bioinformatics 18:7:411.
Lai EC, Tam B, Rubin GM (2005). Pervasive regulation of Drosophila Notch target genes by GY-box-, Brd-box-, and K-box-class micro-RNAs. Genes Dev. 19:1067-1080.
Landthaler M, Yalcin A, Tuschl T (2004). The human DiGeorge syndrome critical region gene 8 and its *D. melanogaster* homolog are required for miRNA biogenesis. Curr. Biol. 14:2162-2167.
Leaman D, Chen PY, Fak J, Yalcin A, Pearce M, Unnerstall U, Marks DS, Sander C, Tuschl T, Gaul U (2005). Antisense-mediated depletion reveals essential and specific functions of microRNAs in Drosophila development. Cell 121:1097-1108.
Lee RC, Feinbaum RL, Ambros V (1993). The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 75:843-854
Lee Y, Kim M, Han J, Yeom KH, Lee S, Baek SH, Kim VN (2004). MicroRNA genes are transcribed by RNA polymerase II. EMBO J. 23:4051-4060.
Lewis BP, Burge CB, Bartel DP (2005). Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 120 (1):15-20.
Lewis BP, Shih IH, Jones-Rhoades MW, Bartel DP, Burge CB (2003). Prediction of mammalian microRNA targets. Cell 115:787-798.
Lytle JR, Yario TA, Steitz JA (2007). Target mRNAs are repressed as efficiently by microRNA-binding sites in the 5′ UTR as in the 3′ UTR. Proc. Natl. Acad. Sci. USA. 104:9667-9672.
Makeyev EV, Zhang J, Carrasco MA, Maniatis T (2007). The MicroRNA miR-124 promotes neuronal differentiation by triggering brain specific alternative pre-mRNA splicing. Mol. Cell. 27:435-448.
Marco A, Hui JHL, Ronshaugen M, Griffiths-Jones S (2010). Functional shifts in insect microRNA evolution. Genome Biol. Evol. 2:686-696.
Nakahara K, Kim K, Sciulli C, Dowd SR, Minden JS, Carthew RW (2005). Targets of microRNA regulation in the Drosophila oocyte proteome. Proc. Natl. Acad. Sci USA. 102:12023-12028.
RNA Technologies in Cardiovascular Medicine and
Sathyamurthy G, Swamy NR (2009). Computational Identification of MicroRNA Homologs from *Acyrthosiphon pisum* (Pea Aphid). J. Comput. Intel. Bioinfo. 2:109-119.
Singh J, Nagaraju J (2008). In silico prediction and characterization of microRNAs from red flour beetle (*Tribolium castaneum*). Insect Mol. Biol. 17:427-436
Stark A, Brennecke J, Russell RB, Cohen SM (2003). Identification of Drosophila MicroRNA targets. PLoS Biol. 1(3):e60.
Wang E (2008). An Overview of MicroRNA. Erdmann, V.A., et al (eds). Research. doi: 10.1007/978-3-540-78709-9.
Wienholds E, Kloosterman WP, Miska E, Alvarez-Saavedra E, Berezikov E, de Bruijn E, Horvitz RH, Kauppinen S, Plasterk RH (2005). MicroRNA Expression in Zebrafish Embryonic Development. Science 309:310-311.
Zeng Y, Yi R, Cullen BR (2003). MicroRNAs and small interfering RNAs can inhibit mRNA expression by similar mechanisms. Proc. Natl. Acad. Sci. USA 100:9779-9784.
Zhang BH, Pan XP, Cannon CH, Cobb GP, Anderson TA (2006). Computational identification of microRNAs and their targets. Comput. Biol. Chem. 30:395-407.
Zhang BH, Pan XP, Wang QL, Cobb GP, Anderson TA (2005). Identification and characterization of new plant microRNAs using EST analysis. Cell Res. 15:336-360.