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Identification of microRNAs in ecological model plant *Mimulus*

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ABSTRACT

MicroRNAs (miRNAs) are small, non-coding and regulatory RNAs about 20 to 24 nucleotides long. Their conserved nature among the various organisms makes them a good source of new miRNAs discovery by comparative genomics approach. This study resulted in 34 miRNAs belonging to 21 families in ecological model plant *Mimulus*. All the miRNA families (mir 156, 157, 159, 160, 164, 172, 319, 393, 395, 397, 399, 400, 403, 417, 419, 472, 782, 854, 858, 867 and 2112) are found for the first time in *Mimulus*. The MIR-399 is found as precursor miRNA cluster with 4 mature sequences. All 34 miRNA precursors form stable minimum free energy stem loop structure as their orthologues form and the mature miRNAs reside in the stem portion of the stem loop structure. Twenty eight are from *Mimulus guttatus* and six miRNAs belong to *Mimulus* lew*i*sii. Their targets consist of dihydroflavonol-4-reductase, cycloidea-like protein, DNA-directed RNA polymerase II, maturase (matR) and transcription factors like; squamosa-promoter binding, MYB, palmate-like pentafoliata 1.

Keywords: Comparative Genomics; MicroRNAs; *Mimulus*

1. INTRODUCTION

Mimulus is a diverse plant genus. It is also called as the monkey-flowers and musk-flowers. It belongs to the family Phrymaceae consist of about 150 species [1]. The *Mimulus* properties make this group of angiosperm plants ideal for ecological and evolutionary genomics research [2]. MicroRNAs (miRNAs) are non-coding, endogenous, small RNAs about 20 - 24 nucleotide long [3]. They are conserved in plants and animals [4,5] and play vital role in post transcriptional gene regulation [6,7]. Primary transcript of mature miRNAs (pri- miRNAs) folds into a stable hair-pin/stem-loop structure forming miRNAs precursor (pre-miRNAs). The loop of pre-miRNA is detached to create a short double-stranded RNA (dsRNA), a single strand of the dsRNA acts as mature miRNA [8]. A special RNaseIII-like endonuclease, Dicer-like enzyme (DCL) in plants involved to process the mature miRNA production [9], that also mostly integrate the mature miRNA into the RNA induced silencing complex (RISC) [8]. The RISC complex negatively regulates gene expression either by inhibiting translation elongation or by triggering messenger RNA (mRNA) destruction on the basis of the degree of complimentary of miRNA within its target [10,11]. The miRNAs execute multipurpose functions in plant and animals like; in growth [12] organogenesis [12,13], transgene suppression [14], signaling pathway [15], environmental stresses [16,17], disease development [18] and defense against the invading viruses [19].

Mostly miRNAs are conserved among animals and plants [5,20]. The conserved nature of these miRNAs becomes a logical approach for identification of new orthologues by comparative genomics in other species [21,22].

Here we report thirty four new miRNAs, belonging to twenty one miRNA families by homology search from the known *Mimulus* ESTs. All the miRNA families (mir 156, 157, 159, 160, 164, 172, 319, 393, 395, 397, 399, 400, 403, 417, 419, 472, 782, 854, 858, 867 and 2112) are reported for the first time in *Mimulus*. All 34 miRNA precursors form stable minimum free energy (mfe) stem loop structures, as their orthologues form and the mature miRNAs reside in the stem portion of the stem loop structure. Twenty eight are from *Mimulus guttatus* and six miRNAs belong to *Mimulus lewisii*.

2. MATERIALS AND METHODS

2.1. Identification of Candidate Sequences

Almost same approach as we reported earlier [21],

was used. The plant pre-miRNAs from the microRNA Registry Database (Version Rfam 16.0 released Sept 2010) [23], were BLAST against publicly available *Mimulus* ESTs at http://blast.ncbi.nlm.nih.gov/Blast.cgi using blastn [24]. Adjusted blast parameter settings were as follows: expect values were set at 1000; low complexity was chosen as the sequence filter, database (others. *Mimulus*) program selection (somewhat similar sequence) and all other parameters were used as default. The candidate sequences FASTA formats with a range of 0 - 4 mismatches with the mature sequences were saved and single tone EST was created for each miRNA after removing the repeated ESTs of the same gene.

To validate the initial candidate *Mimulus* miRNAs as non-protein coding, their sequences were subjected for protein homology search against protein database at NCBI using Blastx with default parameter [25].

2.2. Creation of Hairpin Structures

Zuker folding algorithm, MFOLD (version 3.2) [26], publicly available at http://www.bioinfo.rpi.edu/applications/mfold/rna/form1.cgi., was used to create the hairpin structure of the candidate's sequences. The parameters were adjusted same as reported earlier [21]. The stem portion of the hairpin checked for the mature sequences with at least 12 base pairs involved in Watson-Crick or G/U base pairing between the mature miRNA and the opposite strand (miRNA*).

2.3. Sequence and Structural Features Filtration

To validate the miRNAs through the sequence and structural features filter, the GC content, Core mfe, hairpin mfe and Ch_ratio were calculated as described by Li *et al.*, (2006) [27] with a little modification for Core mfe calculation, as described by Barozai *et al.*, (2008) [21]. The mfe for core and hairpin structures were calculated by MFOLD (version3.2) [26] publicly available at http://www.bioinfo.rpi.edu/applications/mfold/rna/form1.cgi. The parameters were adjusted same as described earlier. For Ch_ratio calculation, we divided the core mfe by the hairpin mfe, and the quotient is referred to as the ch_ratio.

2.4. Conservation and Phylogenetic analysis of *Mimulus* miRNAs

The *Mimulus* miRNA family; mir-156 conservation and phylogenetic analysis with *Vitis vinifera*, *Arabidopsis thaliana*, *Oryza sativa* and *Populus trichocarpa* orthologues was done with the help of publically available weblogo: a sequence logo generator [28] and ClustalW to generate cladogram tree using neighbor joining clustering method [29] respectively. The results were saved.

2.5. Prediction of *Mimulus* miRNA Targets

We predicted the *Mimulus* miRNA targets using the NCBI Blastn program [24]. The mRNA sequences showing 75% query coverage were selected and subjected to RNA-hybrid, a miRNA target prediction tool [30] for the confirmation of the targets. The results were saved.

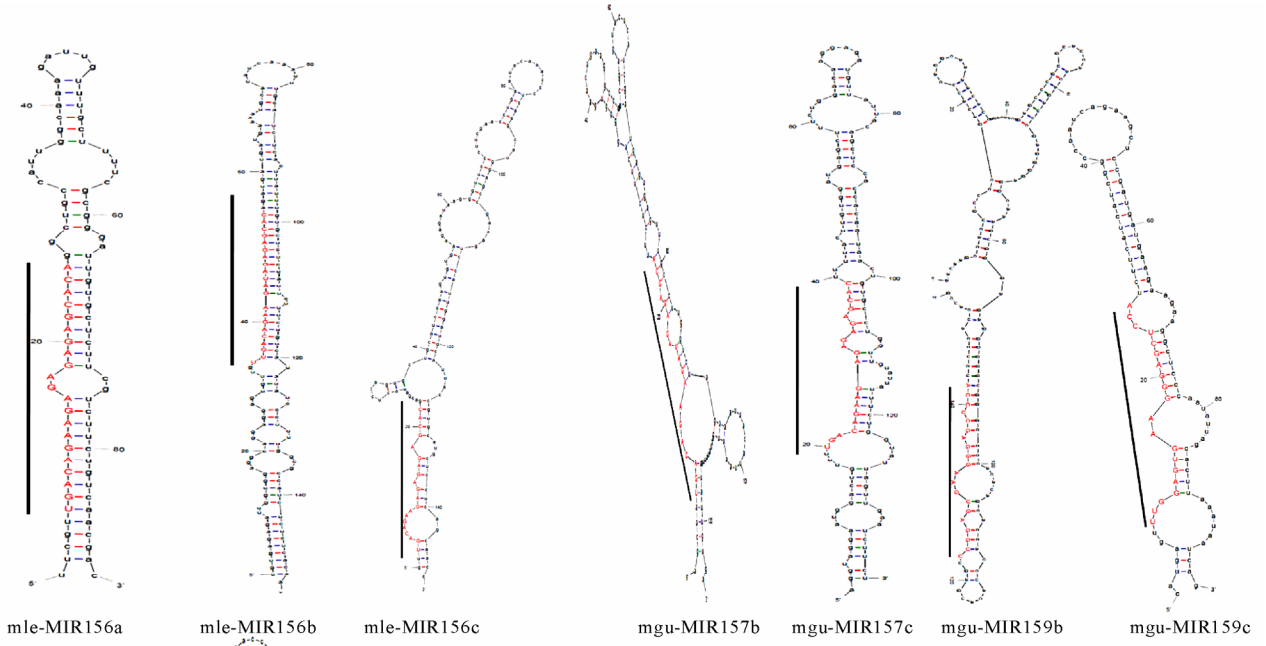
3. RESULT AND DISCUSSION

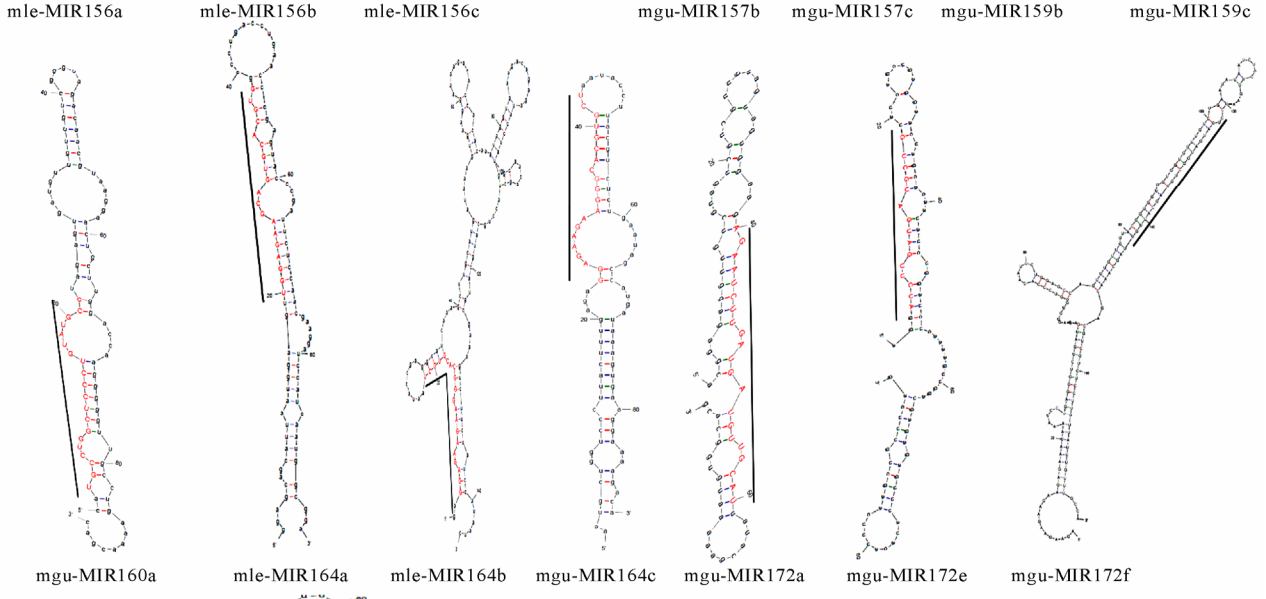
3.1. The Novel *Mimulus* miRNAs

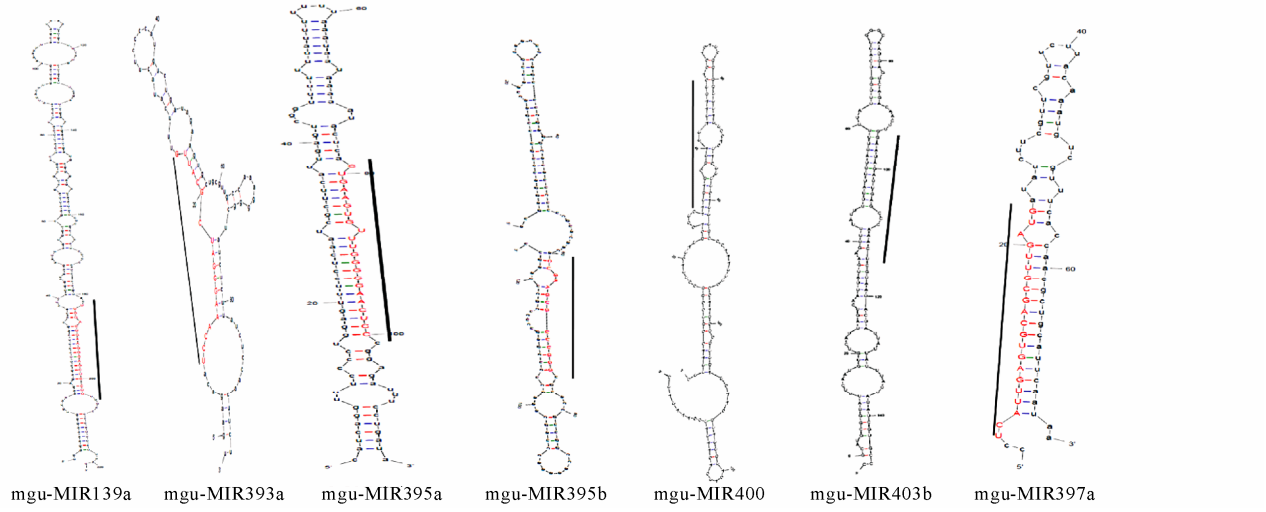
Total thirty four new *Mimulus* pre-miRNAs were identified after filtration and completion of the process. The thirty four potential *Mimulus* miRNAs belong to twenty one families (mir 156, 157, 159, 160, 164, 172, 319, 393, 395, 397, 399, 400, 403, 417, 419, 472, 782, 854, 858, 867 and 2112). Twenty eight are from *Mimulus guttatus* and six miRNAs belong to *Mimulus lewisii*. All the thirty four novel *Mimulus* miRNAs considered as a valid candidate after fulfilling the empirical formula for biogenesis and expression of the miRNAs, suggested by Ambros *et al.*, (2003) [31]. The thirty four novel *Mimulus* pre-miRNAs fulfilled the criteria B, C and D. According to Ambros *et al.*, (2003) [31] only the criterion D is enough for homologous sequences to validate as new miRNAs in different species. Meyers *et al.*, (2008) further confirmed it in favor of plants miRNA annotation [32].

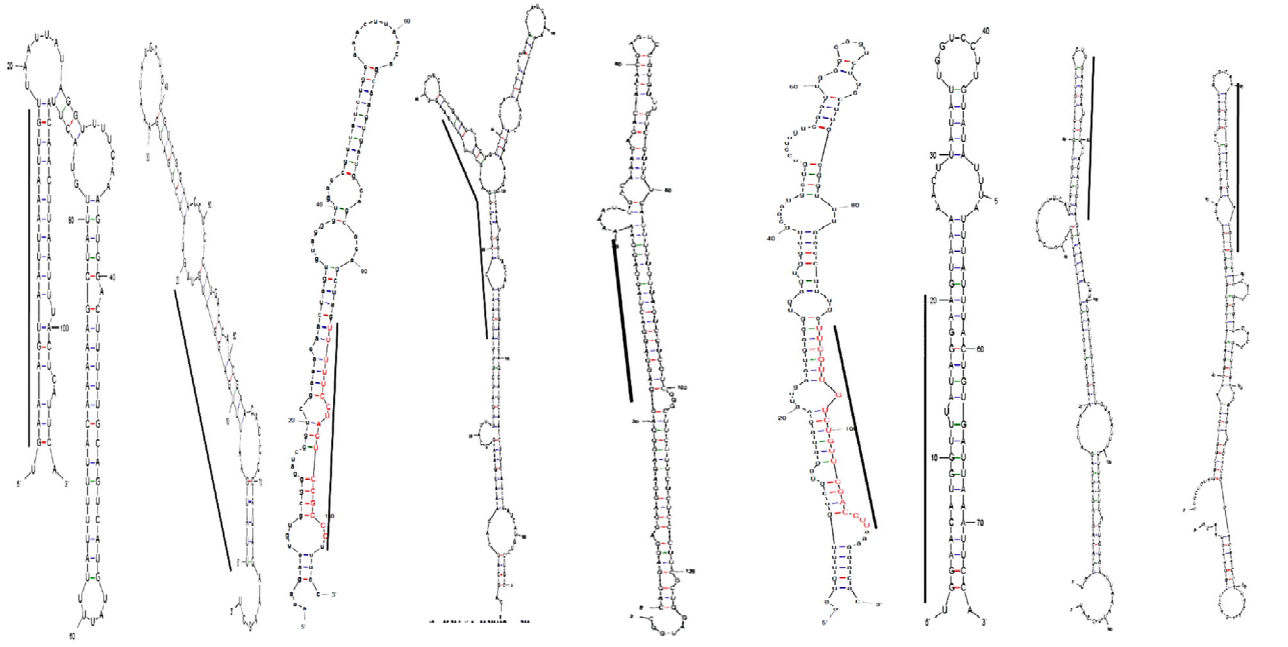
3.2. *Mimulus* miRNAs Characterization

According to MFOLD [26], the minimum folding free energies (MFE) of the newly identified *Mimulus* pre-miRNAs have a range from –12.3 to –81.2 with an average –34.5 Kcal·mol^{-1}. The pre-miRNAs length ranges from 75 - 435 nt with an average of 132 nt. The mature miRNA sequences length ranges from 20 - 23 nt. Majority (79.4%) of the *Mimulus* miRNAs have 21 nt length, followed by 20 nt (11.8%), 22 nt (5.9%) and 23 nt (3.0%). The maximum (50.0%) *Mimulus* miRNAs are observed having 2 mismatches with their homologs, followed by 1 (20.6%), 3 (14.7%), 4 (8.8%) and 0 (5.9%) mismatches. Majority (61.8%) of *Mimulus* miRNAs is located on the 5' and remaining (38.2%) are on the opposite 3' arms of the pre-miRNAs as illustrated in **Figures 1(a)**, **1(b)**. The predicted miRNA stem-loop structures show that there are at least 12 - 21 nucleotides engaged in Watson-crick or G/U base pairings between the mature miRNA and the opposite arms (miRNAs*) in the stem region and the hairpin precursors do not contain large internal loops or bulges. The *Mimulus* miRNAs characterization such as source miRNAs, pre-miRNAs

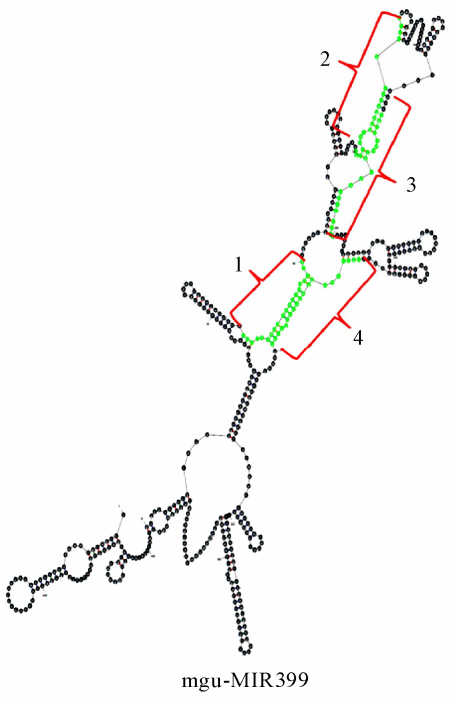








(a)



(b)

Figure 1. (a) *Mimulus* pre-miRNA secondary structures predicted with the help of Mfold. These structures are showing the mature miRNAs in stem portion, highlighted by a parallel line; (b) secondary structure of *Mimulus* pre-miRNA cluster (399). It is showing 4 mature miRNAs (highlighted by braces) in stem portion of a single pre-miRNA.

length (PL), minimum free folding energies (MFE), mature miRNA sequences (MS), number of mismatches (NM),mature sequence length (ML), source ESTs (SE), mature sequence arm (MSA) and GC percentage are summarized in **Table 1**. These findings are similar to the reported works of various groups working on miRNAs

Table 1. *Mimulus* miRNAs' characterization. The novel identified *Mimulus* miRNAs are characterized in terms of PL = Precursor miRNA Length, MFE = Minimum Free Energy, MS = Mature Sequence, MSA = Mature Sequence Arm, ML = Mature sequence Length, NM = Number of Mismatches (represented in bold & enlarged font size) and SE = Source EST.

Mimulus miRNAs	Ref. miRNAs	PL	MFE	MS	MSA	ML	NM	SE
mle-MIR156a	ath-MIR156a	90	–50.50	**UGACAGAAGAGAGAGAGCAC**	5	20	1	GR192132.1
mle-MIR156b	ath-MIR156a	151	–71.00	**UGACAGAAGAUAGAGAGCAC**	5	20	1	GR194964.1
mle-MIR156c	ath-MIR156a	147	–40.80	**UGACAGAAGAGAGAGAGCAC**	5	20	1	GR196312.1
mgu-MIR157b	ath-MIR157b	124	–26.50	**-UGACAGAAGAUAGAGAGCA-**	5	21	2	GR180707.1
mgu-MIR157c	ath-MIR157b	140	–33.00	**UUGACAGAAGAGAGAGAGCAC**	5	21	1	GR086652.1
mgu-MIR159b	ath-MIR159b	154	–45.40	**UUUGGAGUGAAGGGAGCUC--**	3	21	2	GO989625.1
mgu-MIR159c	ath-MIR159b	100	–36.70	**UUUGGAGUGAAGGGAGCUC--**	5	21	2	GO953516.1
mgu-MIR160a	ath-MIR160a	92	–27.80	**UGCCUGGCUCCCUGUAUGCC-**	5	21	1	GR127528.1
mle-MIR164a	ath-MIR164a	94	–27.60	**UGGAGAAGCAGUGCACGUG--**	5	21	3	GR192152.1
mle-MIR164b	ath-MIR164a	145	–33.80	**-GGAGAAGAAGGGCACGUGCU**	5	21	2	GR163353.1
mgu-MIR164c	ath-MIR164c	90	–21.70	**-GGAGAAGAAGGGCACGUGCU**	5	21	3	GR156361.1
mgu-MIR172a	ath-MIR172a	80	–15.60	**AGAAUCUUGAUGAUGUUGCAU**	3	21	1	GR008048.1
mgu-MIR172e	ath-MIR172e	95	–20.00	**---AUCUUGAUGAUGCUGUAU**	5	21	4	GR093896.1
mgu-MIR172f	ath-MIR172e	180	–42.90	**GGAAUCCUGAUGAUGCUGCAG**	3	21	2	DV208903.1
mgu-MIR319a	ath-MIR319a	221	–78.70	**UUGGAGUGAAGGGAGCUCCA**	3	20	2	GO989626.1
mgu-MIR393a	ath-MIR393a	95	–19.10	**AUCCAAAGGGAUCGCAUUG----**	5	23	4	GR132380.1
mgu-MIR395a	ath-MIR395a	116	–54.70	**CUGAAGUGUUUGGGGGAACUC**	3	21	0	GR140768.1
mgu-MIR395b	ath-MIR395a	125	–26.5	**-UGAAGUGUUUGGGUGAACUC**	3	21	2	GR127885.1
mgu-MIR397a	ath-MIR397a	75	–30.50	**UCAUUGAGUGCAGCGUUGAUG**	5	21	0	GR112787.1
mgu-MIR399f	ath-MIR399f	435	–81.16	**UGCCAAAGGAGAUUUGCCCAA** **UGCCAAAGGAGAUUUGCCCAA** **UGCCAAAGGAGAUUUGCCCAA** **UGCCAAAGGAGAUUUGCCUGA**	5 5 3 3	21 21 21 21	2 2 2 3	GR190746.1
mgu-MIR400	ath-MIR400	140	–20.20	**-AUGAGCGUAUUAUAAGUCAC**	5	21	2	GR132977.1
mgu-MIR403b	ath-MIR403b	147	–25.90	**AUAGAUUGA-GCCCAAACUCG**	3	21	2	GO970218.1
mle-MIR417	ath-MIR417	108	–14.90	**GAAAGUAAUUAAAUUGUU--A**	5	21	4	GR203461.1
mgu-MIR419	ath-MIR419	80	–12.30	**UUAUGAAUU-UGAGGAUGUUG**	5	21	2	GR038066.1
mgu-MIR472	ath-MIR472	107	–28.30	**UUUUUCCUACUCCGCCCUUUCC**	3	22	2	GO976090.1
mgu-MIR782	ath-MIR782	167	–26.70	**-CAAACACCUUGGAAGCUUCUU**	5	22	3	GR065602.1
mgu-MIR854	ath-MIR854	130	–51.3	**GAGGAGGAGAGGGAGGAGGAG**	5	21	2	GR036365.1
mgu-MIR858	ath-MIR858	119	–27.20	**CUUCGUUGUCUGUUCGACCUU**	3	21	1	GR102844.1
mgu-MIR867a	ath-MIR867	75	–13.90	**UGGAACAUGGUUUA-UAGGAA**	5	21	2	GR189481.1
mgu-MIR867B	ath-MIR867	145	–26.10	**UGGAACAUGGUUUA-UAGGAA**	3	21	2	GR148892.1
mgu-MIR2112	ath-MIR2112	140	–37.70	**CGCAAAUCUCGAUAUCAAUGU**	3	21	3	GR150629.1

[21,22,33-35].

Sometimes the miRNAs are expressed in clusters. These miRNAs are expressed either as pre-miRNAs clusters or non-precursor miRNAs clusters. The miRNA clusters are rarely observed in plants. In the current study we also identified the *Mimulus* miRNA 399 as pre-miRNA cluster (**Figure 1(b)**. The *Mimulus* 399 pre-miRNA cluster was observed with four mature miRNA sequences. The same family is reported as cluster miRNA in plants [34].

To validate these novel miRNAs as strong candidates of miRNAs the relationship between them and known protein is very significant. The *Mimulus* pre-miRNAs were subjected through Blastx against the protein database at National Center for Biotechnology Information (NCBI) and found no homology with known proteins. This result is confirmed our identified pre-miRNAs as strong candidates in *Mimulus*.

3.3. Sequence and Structural Features Filter

The sequence and structural features filter is introduced by Li *et al.*, (2006) in animals' miRNA validation and by Barozai *et al.*, (2008) in plant [21,27]. It is useful to filter the false positive and validate the candidates. The filter is composed of four indices, namely GC content, core minimum free energy (mfe), hairpin mfe and the ratio of core mfe to hairpin mfe (ch_ratio).

As presented in (**Table 2**), identified miRNAs of *Mimulus* have a range of GC content (33.8 to 55.3), core mfe (–56.10 to –19.80 kcal·mol^{-1}, 72%), hairpin mfe (–81 to –25 kcal·mol^{-1}, 75%) and ch_ratio (40.0 to 95.0, 80%). The GC content and ch_ratio are within the range given by Li *et al.* (2006) and Barozai *et al.* (2008) [21, 27].

3.4. Conservation and Phylogenetic Studies of *Mimulus* miRNAs

The novel *Mimulus* miRNA (mir-156) is studied for conservation and phylogeny. The *Mimulus* miRNA (mir-156) has showed conservation with *Arabidopsis thaliana*, *Vitis vinifera*, *Oryza sativa* and *Populus trichocarpa* miRNAs as shown in **Figure 2**. The Phylogenetic analysis of the same miRNA (mir-156) sequences suggested that the *Mimulus* is more closed to *Vitis vinifera* (vvi) than the *Populus trichocarpa* (ptc) *Arabidopsis thaliana* (ath) and *Oryza sativa* (osa) as shown in **Figure 3**. The results are in agreement with the reported works [21, 34].

3.5. *Mimulus* miRNA Targets

The prediction of novel *Mimulus* miRNAs targets is a crucial step for validation of miRNAs identified on homology basis. Total 22 targets (**Table 3**) were annotated for the novel identified *Mimulus* miRNAs. Almost all of the predicted targets are reported as miRNA targeted proteins in various organisms [21,22,34].

The transcription factors are the famous and well known class of proteins targeted by miRNAs in almost all plant and animal species [4,20-22]. The novel identified *Mimulus* miRNAs also target this class of proteins. The predicted putative *Mimulus* targets for miRNAs; 156, 157, 164, 319, 397, 399, 417, 472, 782 and 854 are Squamosa-promoter binding protein, MYB transcriptional regulator, MYB transcription factor MIXTA-like 5 protein (MYBML5), MYB transcription factor MIXTA-like 6 protein (MYBML6), MYB transcription factor MIXTA-like 9 protein (MYBML9), MYB transcription factor MIXTA-like 5 protein (MYBML5), MYB transcriptional regulator, MYB transcription factor MIXTA- like 2 protein (MYBML2) and MYB transcription factor MIXTA-like 1 (MYBML1) respectively. The other *Mimulus* miRNA families' putative targets are Dihydrofla-vonol-4-reductase, Cycloidea-like protein A (CYCA), DNA-directed RNA polymerase II, NADH dehydrogenase, External transcribed spacer, Maturase (matR), Leafy-like protein and Maturase K (matK). Our findings are in agreement with the earlier reported works of many researchers group in the same field [10,20-22].

4. CONCLUSIONS

We have identified novel thirty four miRNAs belonging to twenty one (21) families in *Mimulus* from ESTs sequences. All twenty one (21) families and their thirty four members are repotted for the first time. These findings will be helpful in understanding the gene regulation

Table 2. Comparison of *Mimulus*, *Arabidopsis thaliana* and Li *et al.*, Reference values of GC-content, core mfe, hairpin mfe and ch-ratio.

Reference	GC content	core mfe	Hairpin mfe	ch_ratio
Li *et al.*	30 - 60 (93%)	–42 ~ –17 (99%)	–50.2 ~ –24.2 (99%)	50 - 96 (99%)
***Arabidopsis* Homologs**	36.4 - 51.1	–54.2 ~ –23.5	–79.4 ~ –48.3	42 - 93
Mimulus	33.8 - 55.3	–56.1 ~ –19.8	–81 ~ –25	40 - 95

Table 3. Putative *Mimulus* miRNA targets. The *Mimulus* miRNA families and their putative targeted proteins function, Genbank Acc. and RNA-hybrid results are provided.

Mimulus **miRNA families**	**Targets**		
	Function	**Genbank Acc.**	**RNA-Hybrid Result**
156	Squamosa promoter binding protein	HM011588	target 5' A A U 3' UGCUCUCU UCUUCUGUCA ACGAGAGA AGAAGACAGU miRNA 3' C G 5'
156	Dihydroflavonol-4-reductase	EU305680	target 5' A C AAUC A 3' UGUU CUCUCUCUUU UCA ACGA GAGAGAGAAG AGU miRNA 3' C AC 5'
157	MYB transcriptional regulator	EU305682	target 5' A A A A U 3' GCU UUUUUC UUC GUCGA CGA AGAGAG AAG CAGUU miRNA 3' CA G A 5'
160	Palmate-like pentafoliata 1 transcription factor	HM453337	target 5' U CCGC AUC U 3' GCA CGGG GAGCCAGGC CGU GUCC CUCGGUCCG miRNA 3' C AU U 5'
164	MYB transcription factor (MYBML5)	HQ202277	target 5' A CAAUA UCG A 3' AGC GCUUU UCUUCUUC UCG CGGGA AGAAGAGG miRNA 3' UGCA 5'
172	Cycloidea-like protein A	EU363011	target 5' G UUAUCCGACGAGU UA A G 3' GUGCA AC AUUAUCAAGA UCU UACGU UG UAGUAGUUCU AGA miRNA 3' A 5'
172	DNA-directed RNA polymerase II	AJ558241	target 5' C UGUUUCGGAAUAA C C 3' GUGUA GCAUCAUC GGGUUU UACGU UGUAGUAG UCUAAG miRNA 3' U A 5'
319	MYB transcription factor (MYBML6)	HQ202279	target 5' G CCAA C 3' GGAGCUCCC UAUUCCG CCUCGAGGG GUGAGGU miRNA 3' A AA U 5'
393	NADH dehydrogenase	EU551661	target 5' A GAU C 3' CAA UGAUCCCUUUGGAU GUU GCUAGGGAAACCUA miRNA 3' AC 5'
395	External transcribed spacer	AY943103	target 5' G AAUUAAGUGUUU U 3' CCCCCA GACGCUUU GGGGGU UUGUGAAG miRNA 3' CUCAA UC 5'
397	MYB transcription factor (MYBML9)	HQ204201	target 5' C CACA G 3' UCAGC UG GCUCAAUGG AGUUG AC UGAGUUACU miRNA 3' GU CG G 5'
399	MYB transcription factor (MYBML5)	HQ202275	target 5' A UCGU G C 3' GGC UCUCC UGGCG CCG AGAGG ACCGU miRNA 3' AAC UUU AA 5'
400	Maturase-R	HQ593753	target 5' U CCACC G 3' GCUUG UACGCUCG UGAAU AUGCGAGU miRNA 3' CAC AUU A 5'

403	Leafy-like protein	AF381769	target 5' A AAAUUU G U 3' GAG UUGG UU AAUUUAU CUC AACC GA UUAGAUA miRNA 3' G A C G 5'
417	MYB transcriptional regulator	EU305681	target 5' U A 3' GCGGU GUUAUUUUC UGUUA UAAUGAAAG miRNA AAU 5'
419	Dihydroflavonol-4-reductase	EU305679	target 5' U UCUGACUGC C 3' UAACAUCUUCAA GUUUAU GUUGUAGGAGUU UAAGUA miRNA 3' UU 5'
472	MYB transcription factor (MYBML2)	HQ202269	target 5' U UAGU AGUUUUU G 3' GG GGGGCGGAG GGAGGAG CC UCCCGCCUC CCUUUUU miRNA 3' UU AU 5'
782	MYB transcription factor (MYBML2)	HQ202267	target 5' A CA A G 3' AAG GGGCUUC AAGGUGU UUC UUCGAAG UUCCACA miRNA 3' G AAC 5'
854	MYB transcription factor (MYBML1)	HQ197364	target 5' C GC A 3' CUCC UCCUUCUUCUCCUCC GAGG AGGAGGGAGAGGAGG miRNA 3' AG 5'
858	Cycloidea-like protein A	AY319629	target 5' C GCCCG U 3' GGG CGAGCAGAUGAUGG UCC GCUUGUCUGUUGCU miRNA 3' U A UC 5'
867	Maturase K	AY849605	target 5' U AA G 3' UCC GAGCCA GUUCUA AGG UUUGGU CAAGGU miRNA 3' AUA A 5'
2112	DNA-directed RNA polymerase II	AJ558242	target 5' C GAC C 3' UGAUG UGAGGUUUGU ACUAU GCUCUAAACG miRNA 3' UGUA A C 5'

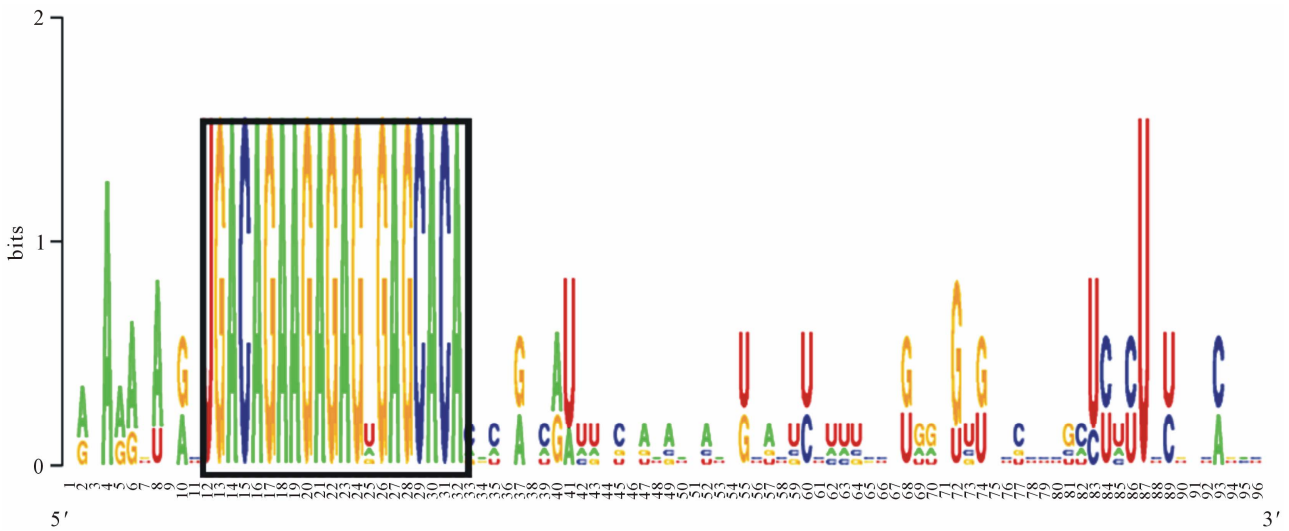


Figure 2. The *Mimulus* miRNA conservation studies. Alignment of pre-miRNA (156) of *Mimulus* with *Arabidopsis thaliana*, *Vitis vinifera*, *Oryza sativa* and *Populus trichocarpa* pre-miRNAs, using Weblogo: a sequence logo generator, showing miRNA sequences conservation, highlighted in a box.

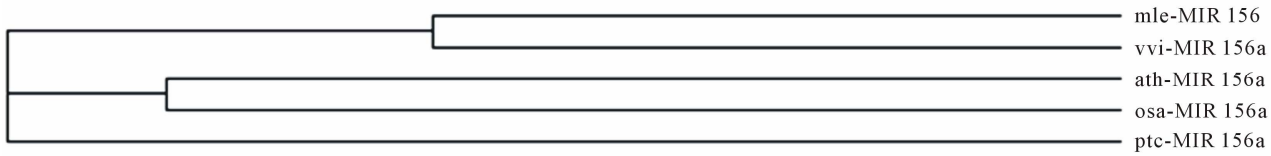


Figure 3. The *Mimulus* miRNA phylogenetic analysis. The Phylogenetic analysis of the pre-miRNA (156) of *Mimulus* (mle) with *Arabidopsis thaliana* (ath), *Vitis vinifera* (vvi), *Oryza sativa* (osa) and *Populus trichocarpa* (ptc) miRNAs, is done with the help of Clustalw and cladogram tree was generated using neighbor joining clustering method. The Phylogenetic tree showed that on the basis of pre-miRNA sequences, the *Mimulus* is more closed to *Vitis vinifera* (vvi) than the *Populus trichocarpa* (ptc) *Arabidopsis thaliana* (ath) and Oryza sativa (osa).

concept in the ecological model plant *Mimulus*. It also strengthens the bioinformatics approach for new pre-miRNAs identification from plant species whose genome is not yet sequenced. The ESTs based identification confirmed the miRNAs expression.

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