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Computational identification of mi RNA and their predicted target genes from *Ocimum tenuiflorum* draft genome

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Abstract

Micro RNAs (mi RNA) are ~22nt long newly identified class of small non coding RNAs that are post transcriptional regulatory RNA in both plants and animals. The use of computational genomics homology search approach for the identification of conserved mi RNA from *O. tenuiflorum* drafted genome scaffold sequences with the available plant mi RNA database and screened the conserved mi RNA based on the stringent filtering criteria. In the present study, identification of 80 potential mi RNAs were done using previously known plant mi RNAs. Using the potentially identified mi RNA sequences, a total of 112 potential target genes were identified and it belongs to 12 mi RNA families; 12 of these predicted target genes encode auxin transcription factors, 16 target genes appear to play roles in lipid binding and 12 target genes have hypothetical or unknown functions. These findings significantly enlarge the scope of understanding the functions of mi RNA in *O. tenuiflorum*.

Keywords: computational identification, *Ocimum tenuiflorum*, genome

Introduction

MicroRNA are the group of ~22 nucleotide long small non coding RNAs which show an enormous role in various biological and metabolic processes in both plants and animals (Zhang *et al* 2006; Wahid *et al* 2010) [3]. A highly important medicinal plant known for its anticancer, antiviral, antibacterial, anti-inflammatory activity and still its mi RNA are not well studied (Cohen 2014) [2]. Micro RNA plays an important role in many biological processes including leaf development, stem development, root development, signal transduction and responds to various environmental stress (Li *et al.* 2016) [4]. Identification of mi RNA from plant species has attention towards its evolution of mi RNA and mi RNA target gene regulation.

Identification of mi RNA from the *O. tenuiflorum* genome can be used by computational genomics approach. Mi RNA having a 1-2% constitute of entire genome and played a significant role in plant metabolism at different level. In-silico biology, approach played a major role in identification of ortholog and paralogs of mi RNAs in entire plant genome against the publically available database. Several mi RNAs were reported in the mi Rbase database but there has been no report on mi RNA in *O. tenuiflorum*. In case of the availability of the draft genome of *O. tenuiflorum* (Upadhyay *et al* 2015) [5], this study introduce the computational approach for the discovery of new mi RNA.

In the present study, identifications of the conserved *O. tenuiflorum* mi RNA in the draft genome available in NCBI is carried out (Upadhyay *et al* 2015) [5]. The identified mi RNA of *O. tenuiflorum* would be supported in the various processed like biological processes, molecular functions and associated protein class. Most of the potential mRNAs were found to be involved in several functions including transcription factor, metabolic process and plant development (Gupta *et al* 2015) [10].

Material and Methods

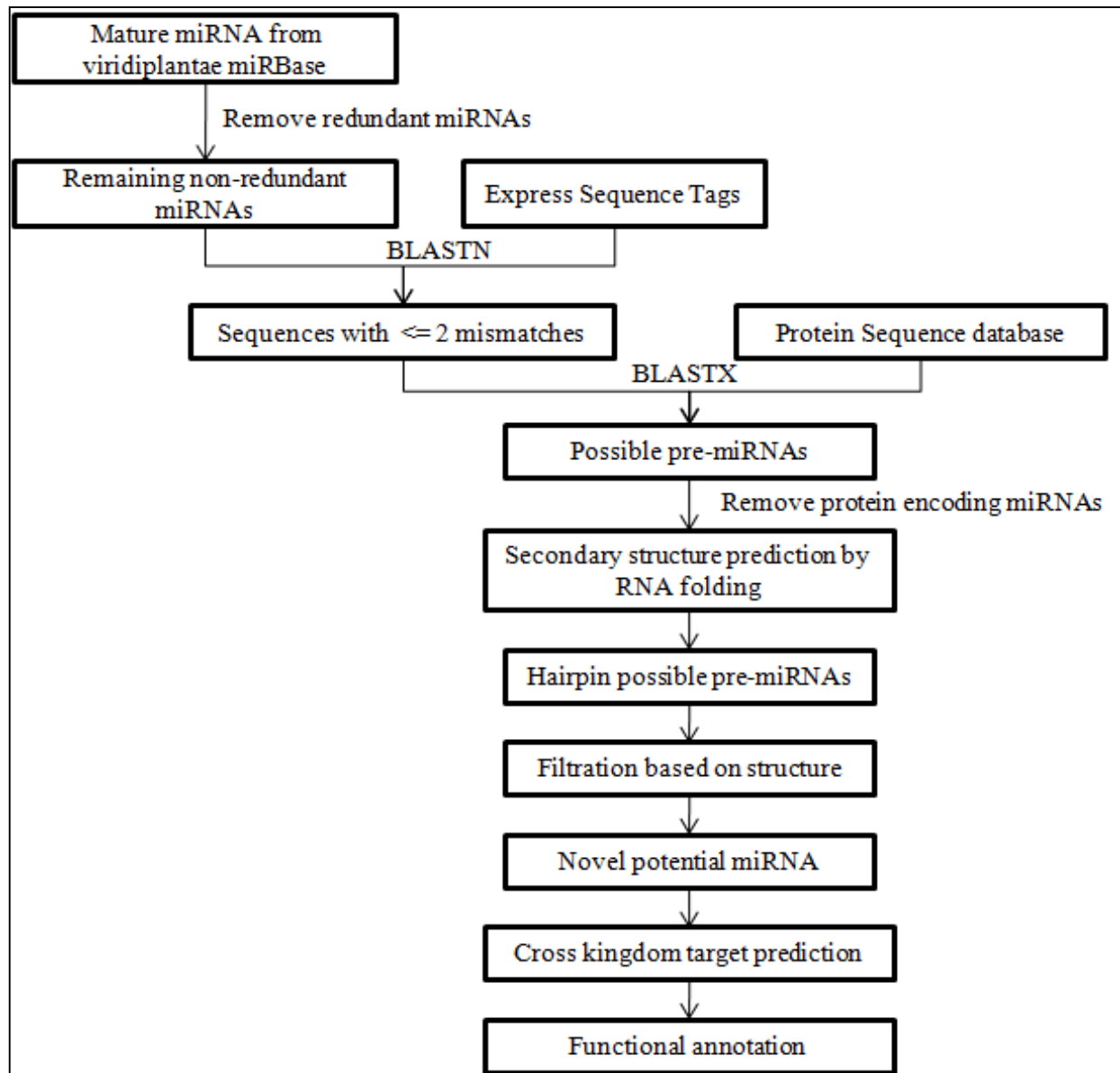


Fig 1: Schematic representation of the *O. tenuiflorum* mi RNA identification procedure.

Reference set of mi RNA and Scaffolds

To identify mi RNAs, a total of 8496 known mi RNA sequences were obtained from mi Rbase database (version 21) (<https://www.mirbase.org>). The redundant mi RNA with 100% identical mi RNA sequences were removed using in house perl script. Out of 8496 mi RNAs, only 4803 unique mi RNA were remained for the identification of conserved mi RNA search in *O. basilicum*. A total of 1, 21, 993 scaffold sequences were obtained from the NCBI (<http://www.ncbi.nlm.nih.gov/>).

Prediction of potential mi RNA

The scaffold sequences of *O. tenuiflorum* were blast against the known viridiplantae plant mi RNA using blastn with an E-value threshold 10 and word size 7 (Akter *et al* 2014; Prabu *et al* 2010; Altschul *et al* 1990) [7, 8, 9]. The mi RNA mature sequences were 18-24nt long sequences. A total of 3168 scaffold sequences were having blast hits against the miRNA database. The blast sequences with flanking region of 100bp upstream and downstream nucleotide of scaffolds were blastx against the nr database for protein coding gene removal. The

remaining scaffold sequences were used for the evaluation of mi RNA structure using mi REval (Gao *et al* 2013) [10]. The evaluated structures were plotted using the mfold web server (Zuker *et al* 2003) [11]. The following parameters were used for the secondary structure prediction in MFOLD. (1) The precursor mi RNA should be folded into the appropriate stem-loop hairpin secondary structure. (2) The minimum length of precursor mi RNA is to be 60 nucleotides. (3) The mature sequence should be located in one arm of the hairpin structure. (4) The mature miRNA sequence and its opposite mi RNA strand (mi RNA*) should not have more than 6 mismatches. (5) No loops or breaks should be allowed between the mature miRNA sequence and its opposite mi RNA strand (mi RNA*). (6) The predicted secondary structure should have higher minimum folding energy index (MFEI) and negative minimum folding energy (MFE) below -18.

Computational prediction of mi RNA targets

The potential mi RNA targets prediction was carried out by psNA target web server (Dai and Zhao 2011) [12]. The mi RNA

sequences were analyzed against the Arabidopsis thaliana genome with the parameters including maximum expected threshold: 2.5, Length of complementarity scoring (hsp Size): 20, maximum energy to unpair target site (UPE): 25, translation inhibition was set between 9 and 11 nucleotides. The predicted potential mi RNA targets were analyzed using the PANTHER classification system and categorized into three categories including molecular function, biological process and cellular component (Mi *et al* 2016) [13].

Results

Identification of mi RNA of *O. tenuiflorum*

To predict new mi RNA from genome using the computational approach, in this study use of different methods and structural properties to screen the mi RNA from the genome of *O. tenuiflorum* was done. Figure 1 depicts the search and filtering criteria for the identification of potential mi RNAs in *O. tenuiflorum*. The plant known mature mi RNAs is much conserved in nature within the different plant species; it is possible to perform a computational search for new mi RNAs.

A total of 1,21,993 scaffold sequences of *O. tenuiflorum* were

identified by BLAST search using all known plant mature mi RNA sequences with an E-value threshold and word size 7. The blast hit identified scaffold sequences with flanking regions were blastx against the nr database and screened the 3,168 scaffold sequences. The remaining filtered scaffold sequences were further analyzed for the presence of mi RNA using mi RE val software. The mature mi RNA predicted from the scaffolds sequences were 18-24nt long and 0-2 mismatches with known plant mi RNA. The validation process of the predicted mi RNA was initiated using MFOLD web server which confirmed the criteria mentioned in materials and methods. A total of 80 mi RNA sequences were screened from the scaffold sequences based on the target prediction from *O. tenuiflorum*. The majority of the mi RNA were belongs to highly conserved plant mi RNA family like miR160, miR166, miR845 and miR165 reported in to the *Arabidopsis thaliana*, *Glycine max* and *Oryza sativa* (Rajagopalan *et al* 2006; Subramanian *et al* 2008; Jones-Rhoades *et al* 2004) [14, 15, 16]. However, some of the mi RNA were reported in to the very less species like miR7972, miR2673b and miR398 (Li *et al* 2013; Lelandais-Brière *et al* 2009) [17, 18].

Table 1: List of mi RNA of *O. tenuiflorum*

Mi RNA ID	Scaffold ID	Mi RNA Family	Start	End	Mi RNA Seq
miR_1	AYJT01010701.1	ath-miR8174	848	867	UUAGCUUCCCUAUACACAU
miR_2	AYJT01054829.1	ptc-miR6478	2101	2120	CCAACUGAGCUAAGGUCGG
miR_3	AYJT01068083.1	hbr-miR6173	5715	5734	GUAUCCAUCGUUUACGGCU
miR_4	AYJT01093552.1	mtr-miR2673b	1143	1162	GAAGAGGAAGAGGAAGAGG
miR_5	AYJT01094031.1	ppe-miR2111b	3459	3478	AACCUCAGGAUGCAGAUUA
miR_6	AYJT01094557.1	bdi-miR172d	376	396	GAAUCCUGAUGAUGCUGCAG
miR_7	AYJT01027176.1	bdi-miR166e-3p	4244	4264	UCGGACCAGGCUUCAUUGCC
miR_8	AYJT01036162.1	lus-miR398f	4384	4403	UGUUCUCAGGUCGCCCCUG
miR_9	AYJT01036738.1	bdi-miR160f	4617	4637	GCCUGGCUCCCUGUAUGCCA
miR_10	AYJT01043994.1	bdi-miR160f	2043	2062	CCUGGCUCCCUGUAUGCCA
miR_11	AYJT01049816.1	ath-miR156g	3656	3675	UGACAGAAGAGAGUGAGCA
miR_12	AYJT01065984.1	bdi-miR160f	5180	5199	CCUGGCUCCCUGUAUGCCA
miR_13	AYJT01072104.1	bdi-miR160f	9733	9753	GCCUGGCUCCCUGUAUGCCA
miR_14	AYJT01004861.1	bdi-miR166e-3p	8349	8368	CGGACCAGGCUUCAUUGCC
miR_15	AYJT01014747.1	bdi-miR166e-3p	1950	1969	CGGACCAGGCUUCAUUGCC
miR_16	AYJT01027176.1	bdi-miR166e-3p	4244	4264	UCGGACCAGGCUUCAUUGCC
miR_17	AYJT01036738.1	bdi-miR160f	4617	4637	GCCUGGCUCCCUGUAUGCCA
miR_18	AYJT01049816.1	ath-miR156g	3656	3675	UGACAGAAGAGAGUGAGCA
miR_19	AYJT01072104.1	bdi-miR160f	9733	9753	GCCUGGCUCCCUGUAUGCCA
miR_20	AYJT01078093.1	bdi-miR169m	7644	7663	AGCCAAGGAGUCUUGCCG
miR_21	AYJT01092913.1	bdi-miR166e-3p	3981	4000	CGGACCAGGCUUCAUUGCC
miR_22	AYJT01102783.1	ata-miR393-5p	241	260	CCAAAGGGAUCGCAUUGAU
miR_23	AYJT01004861.1	bdi-miR166e-3p	8349	8368	CGGACCAGGCUUCAUUGCC
miR_24	AYJT01006281.1	bdi-miR172d	8086	8105	UGCAGCAUCAUCAGGAUUC
miR_25	AYJT01014747.1	bdi-miR166e-3p	1950	1969	CGGACCAGGCUUCAUUGCC
miR_26	AYJT01032131.1	ama-miR156	2357	2376	GACAGAAGAGAGUGAGCAC
miR_27	AYJT01032772.1	ath-miR156g	902	922	UGCUCACUCUCUCUGUCAG
miR_28	AYJT01035538.1	bdi-miR7782-3p	285	305	CUGCUCUGAUACCAUGUUGU
miR_29	AYJT01036738.1	bdi-miR160f	4617	4637	GCCUGGCUCCCUGUAUGCCA
miR_30	AYJT01072104.1	bdi-miR160f	9733	9753	GCCUGGCUCCCUGUAUGCCA
miR_31	AYJT01091626.1	cme-miR1863	1657	1676	CUCUGAUACCAUGUUAGAU
miR_32	AYJT01092913.1	bdi-miR166e-3p	3981	4000	CGGACCAGGCUUCAUUGCC
miR_33	AYJT01108810.1	gma-miR171a	2104	2123	UUGAGCCGUGCCAAUAUCA
miR_34	AYJT01112881.1	ppe-miR2111b	925	944	AAUCUGCAUCCUGAGGUUU
miR_35	AYJT01114649.1	ath-miR156g	102	121	GACAGAAGAGAGUGAGCAC
miR_36	AYJT01006281.1	bdi-miR172d	8086	8105	UGCAGCAUCAUCAGGAUUC
miR_37	AYJT01010655.1	bdi-miR845	425	444	CAACAAUUGGUAUCAGAGC
miR_38	AYJT01020309.1	bdi-miR845	243	262	CAACAAUUGGUAUCAGAGC
miR_39	AYJT01023565.1	bdi-miR845	563	582	CAACAAUUGGUAUCAGAGC
miR_40	AYJT01027186.1	bdi-miR845	491	510	CAACAAUUGGUAUCAGAGC
miR_41	AYJT01032772.1	ath-miR156g	902	922	UGCUCACUCUCUCUGUCAG

miR_42	AYJT01035538.1	bdi-miR7782-3p	285	305	CUGCUCUGAUACCAUGUUGU
miR_43	AYJT01040828.1	gma-miR396a-3p	15868	15887	UCAAUAAAGCUGUGGAAG
miR_44	AYJT01043994.1	bdi-miR160f	2043	2062	CCUGGCUCCUGUAUGCCA
miR_45	AYJT01048710.1	cre-miR1171	16624	16645	GGGGUGGAGUGGAGUGGAGUG
miR_46	AYJT01065984.1	bdi-miR160f	5180	5199	CCUGGCUCCUGUAUGCCA
miR_47	AYJT01072772.1	gma-miR396e	3923	3942	UCCACAGCUUUCUUGAACU
miR_48	AYJT01078093.1	bdi-miR169m	7644	7663	AGCCAAGGAUGACUUGCCG
miR_49	AYJT01091626.1	cme-miR1863	1657	1676	CUCUGAUACCAUUGUAGAU
miR_50	AYJT01096882.1	ath-miR156i	288	308	AUGACAGAAGCAUAGAGAGC
miR_51	AYJT01102783.1	ata-miR393-5p	241	260	CCAAAGGGAUCGCAUUGAU
miR_52	AYJT01121550.1	cre-miR1171	739	760	GGAGUGGAGUGGAGUGGAGUG
miR_53	AYJT01006281.1	bdi-miR172d	8086	8105	UGCAGCAUCAUCAGGAUUC
miR_54	AYJT01009661.1	bdi-miR845	1304	1323	CUCUGAUACCAUUGUUGG
miR_55	AYJT01009958.1	bdi-miR160f	663	682	GCAUACAGGGAGCCAGGCA
miR_56	AYJT01012471.1	rgl-miR7972	2139	2159	UGUCAGGCUUGUUAUUCUCC
miR_57	AYJT01013340.1	bdi-miR845	172	191	CUCUGAUACCAUUGUUGG
miR_58	AYJT01014396.1	bdi-miR845	695	714	CUCUGAUACCAUUGUUGG
miR_59	AYJT01035538.1	bdi-miR7782-3p	285	305	CUGCUCUGAUACCAUGUUGU
miR_60	AYJT01040828.1	gma-miR396a-3p	15868	15887	UCAAUAAAGCUGUGGAAG
miR_61	AYJT01043994.1	bdi-miR160f	2043	2062	CCUGGCUCCUGUAUGCCA
miR_62	AYJT01046914.1	cme-miR168	4209	4228	CCCACCUGCACCAAGCGA
miR_63	AYJT01048710.1	cre-miR1171	16624	16645	GGGGUGGAGUGGAGUGGAGUG
miR_64	AYJT01054264.1	bgm-miR156	2576	2596	UGCUCUCUAUCUUCUGUCA
miR_65	AYJT01054984.1	csi-miR393	798	818	AUCAUUGCGAUCCCUUUGGA
miR_66	AYJT01065984.1	bdi-miR160f	5180	5199	CCUGGCUCCUGUAUGCCA
miR_67	AYJT01070772.1	mdm-miR164a	3406	3426	GCAUGUGCCCUGCUUCUCCA
miR_68	AYJT01072772.1	gma-miR396e	3923	3942	UCCACAGCUUUCUUGAACU
miR_69	AYJT01076765.1	bra-miR164c-5p	1977	1996	CACGUGCCUUGAUUCUCCA
miR_70	AYJT01080473.1	bdi-miR845	379	398	CUCUGAUACCAUUGUUGG
miR_71	AYJT01080922.1	cme-miR168	146	165	CCCACCUGCACCAAGCGA
miR_72	AYJT01083838.1	bdi-miR845	795	814	CUCUGAUACCAUUGUUGG
miR_73	AYJT01092544.1	bdi-miR845	1080	1099	CUCUGAUACCAUUGUUGG
miR_74	AYJT01096882.1	ath-miR156i	288	308	AUGACAGAAGCAUAGAGAGC
miR_75	AYJT01104936.1	bnm-miR171g	211	231	AUAUUGGCGCGGCUCAAUCA
miR_76	AYJT01106367.1	ptc-miR6478	7055	7074	CGACCUUAGCUCAGUUGGU
miR_77	AYJT01108111.1	ptc-miR6478	1611	1630	CGACCUUAGCUCAGUUGGU
miR_78	AYJT01110558.1	csi-miR393	1339	1360	AUCAUUGCGAUCCCUUUGGAA
miR_79	AYJT01112881.1	ppe-miR2111b	925	944	AAUCUGCAUCCUGAGGUUU
miR_80	AYJT01121550.1	cre-miR1171	739	760	GGAGUGGAGUGGAGUGGAGUG

Target Identification and Functional annotation

To identify the potential target of mi RNA, in this study use of ps RNA target server with the parameter mentioned in material and method were done. In these studies, 112 unique target genes for 28miRNA families were identified. These key target genes of the predicted mi RNAs were belonging to several biological function including auxin response transcription factor, GRAS family transcription factor,

debranching enzyme, transposable element gene, developmental regulator, homeobox-lucine zipper family protein, squamous promoter binding protein and unknown or hypothetical protein. The predicted target genes were involved in different categories including biological process, molecular function and cellular component. The different potential target genes were categorized in to the protein class.

Table 2: List of mi RNA and their target genes of *O. tenuiflorum*

Mi RNA ID	Target ID	Score	Binding Position	Mi RNA Sequence	Target Sequence
miR_1	AT5G49743.1	0	84-102	UUAGCUUCCCUAUACACAU	AUGUGUAUAGGGAAGCUAA
	AT2G22350.1	0	806-824	UUAGCUUCCCUAUACACAU	AUGUGUAUAGGGAAGCUAA
	AT4G12275.1	0	1031-1049	UUAGCUUCCCUAUACACAU	AUGUGUAUAGGGAAGCUAA
	AT5G35416.1	0	4578-4596	UUAGCUUCCCUAUACACAU	AUGUGUAUAGGGAAGCUAA
	AT1G43250.1	0	5158-5176	UUAGCUUCCCUAUACACAU	AUGUGUAUAGGGAAGCUAA
miR_2	AT1G57200.1	0	1-19	CCAACUGAGCUAAGGUCGG	CCGACCUUAGCUCAGUUGG
	AT5G07315.1	0	1-19	CCAACUGAGCUAAGGUCGG	CCGACCUUAGCUCAGUUGG
	AT1G57320.1	0	1-19	CCAACUGAGCUAAGGUCGG	CCGACCUUAGCUCAGUUGG
	AT5G03445.1	0	1-19	CCAACUGAGCUAAGGUCGG	CCGACCUUAGCUCAGUUGG
	AT1G57010.1	0	1-19	CCAACUGAGCUAAGGUCGG	CCGACCUUAGCUCAGUUGG
miR_3	ATCG01210.1	0	756-774	GUAUCCAUCGUUACGGCU	AGCCGUAACGAUGGAUAC
	ATCG00920.1	0	756-774	GUAUCCAUCGUUACGGCU	AGCCGUAACGAUGGAUAC
miR_4	AT2G06040.1	0	49-67	GAAGAGGAAGAGGAAGAGG	CCUCUCCUCUCCUCUUC
	AT4G28190.2	0	496-514	GAAGAGGAAGAGGAAGAGG	CCUCUCCUCUCCUCUUC
	AT3G60300.2	0	84-102	GAAGAGGAAGAGGAAGAGG	CCUCUCCUCUCCUCUUC
	AT3G60300.1	0	36-54	GAAGAGGAAGAGGAAGAGG	CCUCUCCUCUCCUCUUC

	AT4G28190.1	0	213-231	GAAGAGGAAGAGGAAGAGG	CCUCUCCUCUCCUCUCCUCUCC
miR_5	AT5G02035.1	0	20-38	AACCUCAGGAUGCAGAUUA	UAAUCUGCAUCCUGAGGUU
	AT3G09285.1	0	32-50	AACCUCAGGAUGCAGAUUA	UAAUCUGCAUCCUGAGGUU
miR_6	AT4G36920.1	0	1329-1348	GAAUCCUGAUGAUGCUGCAG	CUGCAGCAUCAUCAGGAUUC
	AT4G36920.2	0	1293-1312	GAAUCCUGAUGAUGCUGCAG	CUGCAGCAUCAUCAGGAUUC
	AT3G54990.1	0	965-984	GAAUCCUGAUGAUGCUGCAG	UUGCAGCAUCAUCAGGAUUC
	AT5G60120.1	0	1647-1666	GAAUCCUGAUGAUGCUGCAG	AUGCAGCAUCAUCAGGAUUC
	AT5G60120.2	0	1810-1829	GAAUCCUGAUGAUGCUGCAG	AUGCAGCAUCAUCAGGAUUC
miR_7	AT1G52150.1	0.5	1269-1288	UCGGACCAGGCUUCAUUC	UGGAAUGAAGCCUGGUCCGG
	AT1G52150.2	0.5	1269-1288	UCGGACCAGGCUUCAUUC	UGGAAUGAAGCCUGGUCCGG
	AT1G52150.3	0.5	1269-1288	UCGGACCAGGCUUCAUUC	UGGAAUGAAGCCUGGUCCGG
miR_8	AT5G14550.1	0.5	1651-1669	UGUUCUCAGGUCGCCCCUG	CAGGGGUGACCUGAGAACA
miR_9	AT1G77850.1	0.5	1409-1428	GCCUGGCUCCUGUAUGCCA	UGGCAUGCAGGGAGCCAGGC
miR_10	AT1G77850.1	0.5	1409-1427	CCUGGCUCCUGUAUGCCA	UGGCAUGCAGGGAGCCAGGC
miR_11	AT2G33810.1	0.5	788-806	UGACAGAAGAGAGUGAGCA	UGCUCUCUCUCUUCUGUCA
miR_12	AT1G77850.1	0.5	1409-1427	CCUGGCUCCUGUAUGCCA	UGGCAUGCAGGGAGCCAGGC
miR_13	AT1G77850.1	0.5	1409-1428	GCCUGGCUCCUGUAUGCCA	UGGCAUGCAGGGAGCCAGGC
miR_14	AT1G52150.1	1	1269-1287	CGGACCAGGCUUCAUUC	UGGAAUGAAGCCUGGUCCGG
	AT1G52150.2	1	1269-1287	CGGACCAGGCUUCAUUC	UGGAAUGAAGCCUGGUCCGG
	AT1G52150.3	1	1269-1287	CGGACCAGGCUUCAUUC	UGGAAUGAAGCCUGGUCCGG
miR_15	AT1G52150.1	1	1269-1287	CGGACCAGGCUUCAUUC	UGGAAUGAAGCCUGGUCCGG
	AT1G52150.2	1	1269-1287	CGGACCAGGCUUCAUUC	UGGAAUGAAGCCUGGUCCGG
	AT1G52150.3	1	1269-1287	CGGACCAGGCUUCAUUC	UGGAAUGAAGCCUGGUCCGG
miR_7	AT1G30490.1	1	796-815	UCGGACCAGGCUUCAUUC	UGGGAUGAAGCCUGGUCCGG
	AT4G32880.1	1	935-954	UCGGACCAGGCUUCAUUC	UGGGAUGAAGCCUGGUCCGG
miR_9	AT2G28350.1	1	1329-1348	GCCUGGCUCCUGUAUGCCA	AGGAAUACAGGGAGCCAGGC
miR_11	AT1G53160.1	1	593-611	UGACAGAAGAGAGUGAGCA	UGCUCUCUCUCUUCUGUCA
	AT3G57920.1	1	846-864	UGACAGAAGAGAGUGAGCA	UGCUCUCUCUCUUCUGUCA
	AT1G27360.2	1	1214-1232	UGACAGAAGAGAGUGAGCA	UGCUCUCUCUCUUCUGUCA
	AT1G27360.3	1	1241-1259	UGACAGAAGAGAGUGAGCA	UGCUCUCUCUCUUCUGUCA
miR_13	AT2G28350.1	1	1329-1348	GCCUGGCUCCUGUAUGCCA	AGGAAUACAGGGAGCCAGGC
miR_20	AT1G54160.1	1	1285-1303	AGCCAAGGAUGACUUGCCG	CGGGAAGUCAUCCUUGGCU
miR_21	AT1G52150.1	1	1269-1287	CGGACCAGGCUUCAUUC	UGGAAUGAAGCCUGGUCCGG
	AT1G52150.2	1	1269-1287	CGGACCAGGCUUCAUUC	UGGAAUGAAGCCUGGUCCGG
	AT1G52150.3	1	1269-1287	CGGACCAGGCUUCAUUC	UGGAAUGAAGCCUGGUCCGG
miR_22	AT1G12820.1	1	1885-1903	CCAAAGGGAUCGCAUUGAU	AACAAUGCGAUCCCUUUGG
	AT3G26810.1	1	2000-2018	CCAAAGGGAUCGCAUUGAU	AACAAUGCGAUCCCUUUGG
miR_14	AT1G30490.1	1.5	796-814	CGGACCAGGCUUCAUUC	UGGGAUGAAGCCUGGUCCGG
	AT4G32880.1	1.5	935-953	CGGACCAGGCUUCAUUC	UGGGAUGAAGCCUGGUCCGG
miR_24	AT4G24580.1	1.5	995-1013	UGCAGCAUCAUCAGGAUUC	GAAUCCUGAUGAUGAUGCA
miR_15	AT1G30490.1	1.5	796-814	CGGACCAGGCUUCAUUC	UGGGAUGAAGCCUGGUCCGG
	AT4G32880.1	1.5	935-953	CGGACCAGGCUUCAUUC	UGGGAUGAAGCCUGGUCCGG
miR_26	AT2G33810.1	1.5	787-805	GACAGAAGAGAGUGAGCAC	UUGCUCUCUCUCUUCUGUC
	AT3G57920.1	1.5	845-863	GACAGAAGAGAGUGAGCAC	GUGCUCUCUCUCUUCUGUC
	AT1G27360.2	1.5	1213-1231	GACAGAAGAGAGUGAGCAC	GUGCUCUCUCUCUUCUGUC
	AT1G27360.3	1.5	1240-1258	GACAGAAGAGAGUGAGCAC	GUGCUCUCUCUCUUCUGUC
	AT5G50670.1	1.5	1102-1120	GACAGAAGAGAGUGAGCAC	GUGCUCUCUCUCUUCUGUC
miR_27	AT2G33815.1	1.5	156-175	UGCACACUCUCUUCUGUCAG	CUGACAGAAGAGAGUAAGCA
miR_28	AT3G43955.1	1.5	38-57	CUGCUCUGAUACCAUGUUGU	GUAACAUGGUAUCAGAGCAU
miR_9	AT4G30080.1	1.5	1508-1527	GCCUGGCUCCUGUAUGCCA	GGGUUUACAGGGAGCCAGGC
miR_13	AT4G30080.1	1.5	1508-1527	GCCUGGCUCCUGUAUGCCA	GGGUUUACAGGGAGCCAGGC
miR_31	AT2G11140.1	1.5	44-62	CUCUGAUACCAUGUUAGAU	UUCUAAUAUGGUAUCAGAG
	AT1G26990.1	1.5	37-55	CUCUGAUACCAUGUUAGAU	UUCUAAUAUGGUAUCAGAG
miR_21	AT1G30490.1	1.5	796-814	CGGACCAGGCUUCAUUC	UGGGAUGAAGCCUGGUCCGG
	AT4G32880.1	1.5	935-953	CGGACCAGGCUUCAUUC	UGGGAUGAAGCCUGGUCCGG
miR_33	AT4G00150.1	1.5	855-873	UUGAGCCGUGCCAAUAUCA	GGAAUUGGCGCGGCUCAA
	AT3G60630.1	1.5	1044-1062	UUGAGCCGUGCCAAUAUCA	GGAAUUGGCGCGGCUCAA
	AT2G45160.1	1.5	1002-1020	UUGAGCCGUGCCAAUAUCA	GGAAUUGGCGCGGCUCAA
	AT3G47170.1	1.5	531-549	UUGAGCCGUGCCAAUAUCA	UGGAAUUGGGAUGGCUCAG
miR_34	AT3G27150.1	1.5	291-309	AAUCUGCAUCCUGAGGUUU	AAACCUAAGGAUGCAGAUU
miR_35	AT2G33810.1	1.5	787-805	GACAGAAGAGAGUGAGCAC	UUGCUCUCUCUCUUCUGUC
	AT3G57920.1	1.5	845-863	GACAGAAGAGAGUGAGCAC	GUGCUCUCUCUCUUCUGUC
	AT1G27360.2	1.5	1213-1231	GACAGAAGAGAGUGAGCAC	GUGCUCUCUCUCUUCUGUC
	AT1G27360.3	1.5	1240-1258	GACAGAAGAGAGUGAGCAC	GUGCUCUCUCUCUUCUGUC
	AT5G50670.1	1.5	1102-1120	GACAGAAGAGAGUGAGCAC	GUGCUCUCUCUCUUCUGUC
miR_24	AT4G04350.1	2	2256-2274	UGCAGCAUCAUCAGGAUUC	AAAUCCUGAUGAUGUUGUA
	AT1G73910.1	2	135-153	UGCAGCAUCAUCAGGAUUC	GGAAUUGAUGAUGCUGCA
miR_37	AT5G29032.1	2	1735-1753	CAACAAUUGGUAUCAGAGC	GCUCUGAAACCAGUUGUUG

miR_38	AT5G29032.1	2	1735-1753	CAACAAUUGGUAUCAGAGC	GCUCUGAAACCAGUUGUUG
miR_39	AT5G29032.1	2	1735-1753	CAACAAUUGGUAUCAGAGC	GCUCUGAAACCAGUUGUUG
miR_40	AT5G29032.1	2	1735-1753	CAACAAUUGGUAUCAGAGC	GCUCUGAAACCAGUUGUUG
miR_27	AT3G63380.1	2	982-1001	UGCUCACUCUCUCUGUCAG	AUGACAGGAGAGAGUGACCA
	AT4G14490.1	2	659-677	UGCUCACUCUCUCUGUCAG	UUGA-AGAAGAGAGUGAGCA
miR_28	AT2G11140.1	2	46-65	CUCUCUGUAACCAUGUUGU	CUAAUUGGUAUCAGAGCAU
	AT1G26990.1	2	39-58	CUCUCUGUAACCAUGUUGU	CUAAUUGGUAUCAGAGCAU
	AT3G29156.1	2	52-71	CUCUCUGUAACCAUGUUGU	UUGACAUGGUAUCAGAGCAA
miR_43	AT2G46060.1	2	2065-2083	UCAAUAAAGCUGUGGAAG	AUUCUCACAGCUUUAUUGG
	AT2G46060.2	2	2035-2053	UCAAUAAAGCUGUGGAAG	AUUCUCACAGCUUUAUUGG
	AT3G54280.1	2	1031-1049	UCAAUAAAGCUGUGGAAG	CUUCCAUAUUUUUGUUGA
	AT3G54280.2	2	1124-1142	UCAAUAAAGCUGUGGAAG	CUUCCAUAUUUUUGUUGA
miR_10	AT2G28350.1	2	1329-1347	CCUGGCUCUUGUAUGCCA	AGGAAUACAGGGAGCCAGG
miR_45	AT2G10310.1	2	1068-1088	GGGUGGAGUGGAGUGGAGUG	UUCUCCACUCCACUCGACCUC
	AT4G06506.1	2	2981-3001	GGGUGGAGUGGAGUGGAGUG	UUCUCCACUCCACUCGACCUC
miR_12	AT2G28350.1	2	1329-1347	CCUGGCUCUUGUAUGCCA	AGGAAUACAGGGAGCCAGG
miR_47	AT2G34530.2	2	774-792	UCCACAGCUUUCUUGAACU	AGUUCAAGAAGUUAUGGA
	AT2G34530.1	2	774-792	UCCACAGCUUUCUUGAACU	AGUUCAAGAAGUUAUGGA
	AT5G01370.1	2	626-644	UCCACAGCUUUCUUGAACU	GGUUCGAGAAGGUUGUGGA
	AT5G58980.1	2	1272-1290	UCCACAGCUUUCUUGAACU	AGUUCAAGAAGGCUCGCGGA
miR_20	AT3G20910.1	2	1036-1054	AGCCAAGGAUGACUUGCCG	CGGCAAUUCAUUCUUGGCU
	AT5G12840.1	2	1038-1056	AGCCAAGGAUGACUUGCCG	CGGCAAUUCAUUCUUGGCU
	AT5G12840.4	2	1120-1138	AGCCAAGGAUGACUUGCCG	CGGCAAUUCAUUCUUGGCU
	AT5G12840.3	2	1220-1238	AGCCAAGGAUGACUUGCCG	CGGCAAUUCAUUCUUGGCU
miR_31	AT4G07810.1	2	1235-1253	CUCUGAUACCAUGUUGAU	AUCUAAAUGGUAUAGAG
	AT3G43955.1	2	36-54	CUCUGAUACCAUGUUGAU	CUGUAACAUGGUAUCAGAG
	AT3G30815.1	2	29-47	CUCUGAUACCAUGUUGAU	GUUCACAUGGUAUCAGAG
miR_50	AT3G14770.1	2	1373-1392	AUGACAGAAGCAUAGAGAGC	AUUCUCUCUGUUCUGUCAU
	AT1G41830.1	2	1971-1990	AUGACAGAAGCAUAGAGAGC	UCUUUAUAUGCUUUUGUCAU
miR_22	AT3G62980.1	2	1712-1730	CCAAAGGGAUCGCAUUGAU	GACAAUGCGAUCCCUUGG
	AT3G23690.1	2	407-425	CCAAAGGGAUCGCAUUGAU	GUCAGAGCGAUCCCUUGG
miR_52	AT4G15640.1	2	3-23	GGAGUGGAGUGGAGUGGAGUG	GACUCUACUCUACUCUACUCU
miR_24	AT1G49015.1	2.5	129-147	UGCAGCAUCAUCAGGAUUC	GAAUUUUGAUGAUGUUGUG
miR_54	AT1G03310.1	2.5	1678-1696	CUCUGAUACCAUUGUUGG	UCAGCAGUUGGUAUUGGAG
	AT1G03310.2	2.5	1686-1704	CUCUGAUACCAUUGUUGG	UCAGCAGUUGGUAUUGGAG
miR_55	AT4G39680.2	2.5	2295-2313	GCAUACAGGGAGCCAGGCA	UGUUUGGCUCUCUGUGUGU
	AT4G39680.1	2.5	2354-2372	GCAUACAGGGAGCCAGGCA	UGUUUGGCUCUCUGUGUGU
	AT3G26890.5	2.5	112-130	GCAUACAGGGAGCCAGGCA	UGUUUGGUUUCUUGUAUGC
	AT3G26890.4	2.5	134-152	GCAUACAGGGAGCCAGGCA	UGUUUGGUUUCUUGUAUGC
	AT3G26890.1	2.5	319-337	GCAUACAGGGAGCCAGGCA	UGUUUGGUUUCUUGUAUGC
miR_56	AT3G27925.1	2.5	1581-1600	UGUCAGGCUUGUUAUUCUCC	UGGGAGUGACGAGUCUGACA
	AT5G04020.1	2.5	593-612	UGUCAGGCUUGUUAUUCUCC	AGAGAAUAGCAAGCUUGAGA
miR_57	AT1G03310.1	2.5	1678-1696	CUCUGAUACCAUUGUUGG	UCAGCAGUUGGUAUUGGAG
	AT1G03310.2	2.5	1686-1704	CUCUGAUACCAUUGUUGG	UCAGCAGUUGGUAUUGGAG
miR_58	AT1G03310.1	2.5	1678-1696	CUCUGAUACCAUUGUUGG	UCAGCAGUUGGUAUUGGAG
	AT1G03310.2	2.5	1686-1704	CUCUGAUACCAUUGUUGG	UCAGCAGUUGGUAUUGGAG
miR_28	AT3G30815.1	2.5	31-50	CUCUCUGUAACCAUGUUGU	UCAACAUGGUAUCAGAGCAU
miR_43	AT1G59865.2	2.5	419-437	UCAAUAAAGCUGUGGAAG	CUUCCAAGGUAUCUUGGAG
miR_10	AT4G30080.1	2.5	1508-1526	CCUGGCUCUUGUAUGCCA	GGGUUACAGGGAGCCAGG
miR_62	AT2G46190.1	2.5	8-26	CCCACCUGCACCAAGCGA	UCGCUUGGUGCAGGUCAGC
miR_45	AT4G15640.1	2.5	3-23	GGGUGGAGUGGAGUGGAGUG	GACUCUACUCUACUCUACUCU
miR_64	AT2G16000.1	2.5	3673-3692	UGCUCUCUAUCUUCUGUCA	UUGAUAGAGGAUUAAGAGCA
	AT1G72350.1	2.5	129-148	UGCUCUCUAUCUUCUGUCA	UCGACAGAAGAUAGAGAUA
	AT2G13440.1	2.5	2137-2156	UGCUCUCUAUCUUCUGUCA	UGGGUAGAAGAUAGGGAGCA
	AT1G17310.1	2.5	144-163	UGCUCUCUAUCUUCUGUCA	AAGACAGAAGAUAGAGAUA
miR_65	AT1G04790.1	2.5	1906-1925	AUCAAUAGCAUCCCUUGGA	UCCACAAGGAUUGCAUUGAU
miR_12	AT4G30080.1	2.5	1508-1526	CCUGGCUCUUGUAUGCCA	GGGUUACAGGGAGCCAGG
miR_67	AT5G41080.1	2.5	1011-1030	GCAUGUCCCUUGUUCUCCA	CGGAGAGGCAGUGACCAUGC
	AT5G41080.2	2.5	1030-1049	GCAUGUCCCUUGUUCUCCA	CGGAGAGGCAGUGUACAUGC
miR_47	AT3G19040.1	2.5	1434-1452	UCCACAGCUUUCUUGAACU	CUUUCAAGAAGGCUGUGGA
miR_69	AT1G59860.1	2.5	336-354	CACGUGCCCUUGCUUCUCCA	AGGAGAAGCAGGACACGUG
	AT1G07400.1	2.5	486-504	CACGUGCCCUUGCUUCUCCA	AGGAGAAGCAGGACACGUG
	AT2G29654.1	2.5	25-43	CACGUGCCCUUGCUUCUCCA	UGGAGAAGUGGGCCACGUG
miR_70	AT1G03310.1	2.5	1678-1696	CUCUGAUACCAUUGUUGG	UCAGCAGUUGGUAUUGGAG
	AT1G03310.2	2.5	1686-1704	CUCUGAUACCAUUGUUGG	UCAGCAGUUGGUAUUGGAG
miR_71	AT2G46190.1	2.5	8-26	CCCACCUGCACCAAGCGA	UCGCUUGGUGCAGGUCAGC
miR_72	AT1G03310.1	2.5	1678-1696	CUCUGAUACCAUUGUUGG	UCAGCAGUUGGUAUUGGAG
	AT1G03310.2	2.5	1686-1704	CUCUGAUACCAUUGUUGG	UCAGCAGUUGGUAUUGGAG

miR_73	AT1G03310.1	2.5	1678-1696	CUCUGAUACCAUUGUUGG	UCAGCAGUUGGUAUUGGAG
	AT1G03310.2	2.5	1686-1704	CUCUGAUACCAUUGUUGG	UCAGCAGUUGGUAUUGGAG
miR_50	AT1G57600.1	2.5	204-223	AUGACAGAAGCAUAGAGAGC	GCUUUCUAUGCUUAUGUCGU
	AT5G62630.1	2.5	2103-2122	AUGACAGAAGCAUAGAGAGC	UUUCUCUGUGUUUUUGUUAU
miR_75	AT1G53060.1	2.5	67-86	AUAUUGGCGCGGCUCAAUCA	GGAUUGAGCCGUGACAAUGU
miR_76	AT2G47030.1	2.5	1777-1795	CGACCUUAGCUCAGUUGGU	GUUAACUGGGCUAAGGUUG
miR_77	AT2G47030.1	2.5	1777-1795	CGACCUUAGCUCAGUUGGU	GUUAACUGGGCUAAGGUUG
miR_78	AT1G04790.1	2.5	1905-1925	AUCAUUGCGAUCCCUUGGAA	UUCCACAAGGAUUGCAUUGAU
miR_34	AT2G23370.1	2.5	822-840	AAUCUGCAUCCUGAGGUUU	GGACCUUAGGAUGCAGAU
	AT5G52010.1	2.5	890-908	AAUCUGCAUCCUGAGGUUU	AAGCCUCAGGCUGCGGAUU
	AT1G07010.1	2.5	1206-1224	AAUCUGCAUCCUGAGGUUU	GAACUUCAGGUUGCAGAU
	AT1G07010.3	2.5	1246-1264	AAUCUGCAUCCUGAGGUUU	GAACUUCAGGUUGCAGAU
miR_52	AT5G11550.1	2.5	50-70	GGAGUGGAGUGGAGUGGAGUG	CACUUCACUCCACUUCACUUG
	AT3G30300.1	2.5	223-243	GGAGUGGAGUGGAGUGGAGUG	CACUUCACUUCACUUCACUU

Table 3: Functional annotation of target genes

Mi RNA ID	Target Acc.	Target Description
miR_1	AT5G49743.1	transposable element gene
	AT2G22350.1	transposable element gene
	AT4G12275.1	transposable element gene
	AT5G35416.1	transposable element gene
	AT1G43250.1	transposable element gene
miR_2	AT1G57200.1	pre-tRNA
	AT5G07315.1	pre-tRNA
	AT1G57320.1	pre-tRNA
	AT5G03445.1	pre-tRNA
	AT1G57010.1	pre-tRNA
miR_3	ATCG01210.1	chloroplast-encoded 16S ribosomal RNA
	ATCG00920.1	chloroplast-encoded 16S ribosomal RNA
miR_4	AT2G06040.1	Leucine-rich repeat,
	AT4G28190.2	Developmental regulator, ULTRAPETALA
	AT3G60300.2	RWD domain-containing protein
	AT3G60300.1	RWD domain-containing protein
	AT4G28190.1	Developmental regulator, ULTRAPETALA
miR_5	AT5G02035.1	Protien coding gene
	AT3G09285.1	Protien coding gene
miR_6	AT4G36920.1	Integrase-type DNA-binding superfamily protein
	AT4G36920.2	Integrase-type DNA-binding superfamily protein
	AT3G54990.1	Integrase-type DNA-binding superfamily protein
	AT5G60120.1	target of early activation tagged (EAT) 2
	AT5G60120.2	target of early activation tagged (EAT) 2
miR_7	AT1G52150.1	Homeobox-leucine zipper family protein / lipid-binding START domain-containing protein
	AT1G52150.2	Homeobox-leucine zipper family protein / lipid-binding START domain-containing protein
	AT1G52150.3	Homeobox-leucine zipper family protein / lipid-binding START domain-containing protein
miR_8	AT5G14550.1	Core-2/I-branching beta-1,6-N-acetylglucosaminyltransferase family protein
miR_9	AT1G77850.1	auxin response factor 17
miR_10	AT1G77850.1	auxin response factor 17
miR_11	AT2G33810.1	squamosa promoter binding protein-like 3
miR_12	AT1G77850.1	auxin response factor 17
miR_13	AT1G77850.1	auxin response factor 17
miR_14	AT1G52150.1	Homeobox-leucine zipper family protein / lipid-binding START domain-containing protein
	AT1G52150.2	Homeobox-leucine zipper family protein / lipid-binding START domain-containing protein
	AT1G52150.3	Homeobox-leucine zipper family protein / lipid-binding START domain-containing protein
miR_15	AT1G52150.1	Homeobox-leucine zipper family protein / lipid-binding START domain-containing protein
	AT1G52150.2	Homeobox-leucine zipper family protein / lipid-binding START domain-containing protein
	AT1G52150.3	Homeobox-leucine zipper family protein / lipid-binding START domain-containing protein
miR_7	AT1G30490.1	Homeobox-leucine zipper family protein / lipid-binding START domain-containing protein
	AT4G32880.1	homeobox gene 8
miR_9	AT2G28350.1	auxin response factor 10
miR_11	AT1G53160.1	squamosa promoter binding protein-like 4
	AT3G57920.1	squamosa promoter binding protein-like 15
	AT1G27360.2	squamosa promoter-like 11
	AT1G27360.3	squamosa promoter-like 11
miR_13	AT2G28350.1	auxin response factor 10
miR_20	AT1G54160.1	nuclear factor Y, subunit A5
miR_21	AT1G52150.1	Homeobox-leucine zipper family protein / lipid-binding START domain-containing protein
	AT1G52150.2	Homeobox-leucine zipper family protein / lipid-binding START domain-containing protein

	AT1G52150.3	Homeobox-leucine zipper family protein / lipid-binding START domain-containing protein
miR_22	AT1G12820.1	auxin signaling F-box 3
	AT3G26810.1	auxin signaling F-box 2
miR_14	AT1G30490.1	Homeobox-leucine zipper family protein / lipid-binding START domain-containing protein
	AT4G32880.1	homeobox gene 8
miR_24	AT4G24580.1	Rho GTPase activation protein (RhoGAP) with PH domain
miR_15	AT1G30490.1	Homeobox-leucine zipper family protein / lipid-binding START domain-containing protein
	AT4G32880.1	homeobox gene 8
miR_26	AT2G33810.1	squamosa promoter binding protein-like 3
	AT3G57920.1	squamosa promoter binding protein-like 15
	AT1G27360.2	squamosa promoter-like 11
	AT1G27360.3	squamosa promoter-like 11
	AT5G50670.1	Squamosa promoter-binding protein-like (SBP domain) transcription factor family protein
miR_27	AT2G33815.1	other RNA
miR_28	AT3G43955.1	transposable element gene
miR_9	AT4G30080.1	auxin response factor 16
miR_13	AT4G30080.1	auxin response factor 16
miR_31	AT2G11140.1	transposable element gene
	AT1G26990.1	transposable element gene
miR_21	AT1G30490.1	Homeobox-leucine zipper family protein / lipid-binding START domain-containing protein
	AT4G32880.1	homeobox gene 8
miR_33	AT4G00150.1	GRAS family transcription factor
	AT3G60630.1	GRAS family transcription factor
	AT2G45160.1	GRAS family transcription factor
	AT3G47170.1	HXXXD-type acyl-transferase family protein
miR_34	AT3G27150.1	Galactose oxidase/kelch repeat superfamily protein
miR_35	AT2G33810.1	squamosa promoter binding protein-like 3
	AT3G57920.1	squamosa promoter binding protein-like 15
	AT1G27360.2	squamosa promoter-like 11
	AT1G27360.3	squamosa promoter-like 11
	AT5G50670.1	Squamosa promoter-binding protein-like (SBP domain) transcription factor family protein
miR_24	AT4G04350.1	tRNA synthetase class I (I, L, M and V) family protein
	AT1G73910.1	actin-related proteins 4A
miR_37	AT5G29032.1	transposable element gene
miR_38	AT5G29032.1	transposable element gene
miR_39	AT5G29032.1	transposable element gene
miR_40	AT5G29032.1	transposable element gene
miR_27	AT3G63380.1	ATPase E1-E2 type family protein / haloacid dehalogenase-like hydrolase family protein
	AT4G14490.1	SMAD/FHA domain-containing protein
miR_28	AT2G11140.1	transposable element gene
	AT1G26990.1	transposable element gene
	AT3G29156.1	transposable element gene
miR_43	AT2G46060.1	transmembrane protein-related
	AT2G46060.2	transmembrane protein-related
	AT3G54280.1	DNA binding;ATP binding;nucleic acid binding;binding;helicases;ATP binding;DNA binding;helicases
	AT3G54280.2	DNA binding;ATP binding;nucleic acid binding;binding;helicases;ATP binding;DNA binding;helicases
miR_10	AT2G28350.1	auxin response factor 10
miR_45	AT2G10310.1	transposable element gene
	AT4G06506.1	transposable element gene
miR_12	AT2G28350.1	auxin response factor 10
miR_47	AT2G34530.2	unknown protein;
	AT2G34530.1	unknown protein;
	AT5G01370.1	ALC-interacting protein 1
	AT5G58980.1	Neutral/alkaline non-lysosomal ceramidase
miR_20	AT3G20910.1	nuclear factor Y, subunit A9
	AT5G12840.1	nuclear factor Y, subunit A1
	AT5G12840.4	nuclear factor Y, subunit A1
	AT5G12840.3	nuclear factor Y, subunit A1
miR_31	AT4G07810.1	transposable element gene
	AT3G43955.1	transposable element gene
	AT3G30815.1	transposable element gene
miR_50	AT3G14770.1	Nodulin MtN3 family protein
	AT1G41830.1	SKU5-similar 6
miR_22	AT3G62980.1	F-box/RNI-like superfamily protein
	AT3G23690.1	basic helix-loop-helix (bHLH) DNA-binding superfamily protein
miR_52	AT4G15640.1	Unknown protein

miR_24	AT1G49015.1	DPP6 N-terminal domain-like protein
miR_54	AT1G03310.1	debranching enzyme 1
	AT1G03310.2	debranching enzyme 1
miR_55	AT4G39680.2	SAP domain-containing protein
	AT4G39680.1	SAP domain-containing protein
	AT3G26890.5	Unknown protein
	AT3G26890.4	Unknown protein
	AT3G26890.1	Unknown protein
miR_56	AT3G27925.1	DegP protease 1
	AT5G04020.1	calmodulin binding
miR_57	AT1G03310.1	debranching enzyme 1
	AT1G03310.2	debranching enzyme 1
miR_58	AT1G03310.1	debranching enzyme 1
	AT1G03310.2	debranching enzyme 1
miR_28	AT3G30815.1	transposable element gene
miR_43	AT1G59865.2	Unknown protein
miR_10	AT4G30080.1	auxin response factor 16
miR_62	AT2G46190.1	Mitochondrial glycoprotein family protein
miR_45	AT4G15640.1	Unknown protein
miR_64	AT2G16000.1	transposable element gene
	AT1G72350.1	MADS-box transcription factor family protein
	AT2G13440.1	glucose-inhibited division family A protein
	AT1G17310.1	MADS-box transcription factor family protein
miR_65	AT1G04790.1	RING/U-box superfamily protein
miR_12	AT4G30080.1	auxin response factor 16
miR_67	AT5G41080.1	PLC-like phosphodiesterases superfamily protein
	AT5G41080.2	PLC-like phosphodiesterases superfamily protein
miR_47	AT3G19040.1	histone acetyltransferase of the TAFII250 family 2
miR_69	AT1G59860.1	HSP20-like chaperones superfamily protein
	AT1G07400.1	HSP20-like chaperones superfamily protein
	AT2G29654.1	Unknown protein
miR_70	AT1G03310.1	debranching enzyme 1
	AT1G03310.2	debranching enzyme 1
miR_71	AT2G46190.1	Mitochondrial glycoprotein family protein
miR_72	AT1G03310.1	debranching enzyme 1
	AT1G03310.2	debranching enzyme 1
miR_73	AT1G03310.1	debranching enzyme 1
	AT1G03310.2	debranching enzyme 1
miR_50	AT1G57600.1	MBOAT (membrane bound O-acyl transferase) family protein
	AT5G62630.1	hipl2 protein precursor
miR_75	AT1G53060.1	Legume lectin family protein
miR_76	AT2G47030.1	Plant invertase/pectin methylesterase inhibitor superfamily
miR_77	AT2G47030.1	Plant invertase/pectin methylesterase inhibitor superfamily
miR_78	AT1G04790.1	RING/U-box superfamily protein
miR_34	AT2G23370.1	Unknown protein
	AT5G52010.1	C2H2-like zinc finger protein
	AT1G07010.1	Calcineurin-like metallo-phosphoesterase superfamily protein
	AT1G07010.3	Calcineurin-like metallo-phosphoesterase superfamily protein
miR_52	AT5G11550.1	ARM repeat superfamily protein
	AT3G30300.1	O-fucosyltransferase family protein

Table 4: Gene Ontology analysis of *O. tenuiflorum*

Target Gene	Molecular Function	Biological Process	Cellular Component
AT4G28190.2			Cell part; Organelle;
AT3G60300.2		Cellular process; Metabolic process;	Cell part; Organelle;
AT3G60300.1		Cellular process;	
AT4G28190.1			Organelle;
AT1G54160.1	Binding;	Metabolic process; Response to stimulus;	
AT4G00150.1	Binding;	Cellular process; Metabolic process;	Cell part; Organelle
AT3G60630.1	Binding;	Cellular process; Metabolic process;	Cell part;
AT2G45160.1	Binding;	Cellular process; Metabolic process;	Cell part; Organelle;
AT3G47170.1	Catalytic activity;		
AT4G04350.1	Catalytic activity;	Cellular process; Metabolic process;	Cell part; Organelle;
AT1G73910.1	Structural molecular activity;	Cellular process; Localization;	Cell part;
AT3G63380.1	Catalytic activity; Transporter activity;	Metabolic process;	Cell part; Organelle
AT2G46060.1			Membrane;
AT5G58980.1	Catalytic activity;	Cellular process; Metabolic process;	
AT3G20910.1	Binding;	Metabolic process; Response to stimulus;	

AT5G12840.1	Binding;	Metabolic process; Response to stimulus;	
AT3G14770.1	Transporter activity;	Cellular process; Localization;	Membrane;
AT1G41830.1	Catalytic activity;		Cell Junction; Cell part; Macromolecular complex; Membrane
AT1G49015.1	Translation regulation activity	Cellular process; Metabolic process;	Cell part; Macromolecular complex;
AT1G03310.1	Catalytic activity;	Metabolic process;	
AT5G04020.1	Binding;		Membrane;
AT1G59865.2			
AT2G46190.1	Binding; Translation regulation activity;	Cellular process; Metabolic process;	Cell part; Organelle
AT2G16000.1			
AT1G72350.1	Binding;	Cellular process; Developmental process; Metabolic process; Multicellular organismal process;	Cell part; Organelle
AT2G13440.1	Binding;	Cellular process; Metabolic process;	Cell part;
AT1G17310.1	Binding;	Cellular process; Developmental process; Metabolic process; Multicellular organismal process;	Cell part; Organelle;
AT5G41080.1		Cellular process; Metabolic process;	
AT5G41080.2		Cellular process;	
AT3G19040.1	Binding; Catalytic activity	Biogenesis; Cellular process; Metabolic process;	Cell part; Macromolecular complex; Organelle;
AT1G59860.1		Metabolic process; Response to stimulus;	
AT1G07400.1		Metabolic process; Response to stimulus;	
AT1G57600.1	Catalytic activity;	Cellular process; Metabolic process;	
AT2G47030.1	Catalytic activity;	Biological regulation	
AT3G30300.1			Cell part; Organelle;

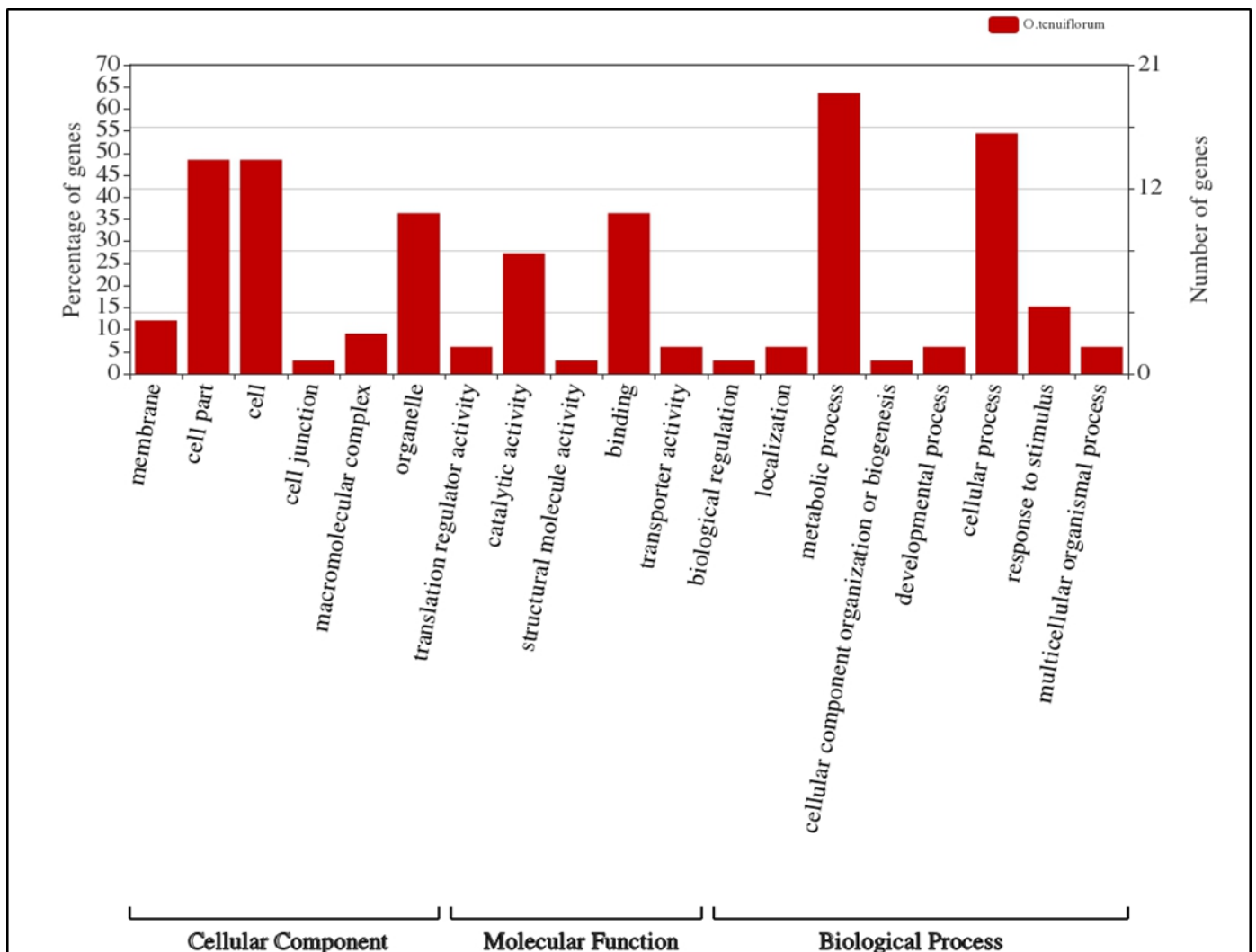


Figure 2: Gene ontology categorized in MF, BP and CC in O.tenuiflorum

Discussion

In recent years, many studies have demonstrated that plant mi RNAs are involved in various biological and metabolic processes. The mi RNA sequence is too short and conserved in nature. Therefore, the viridiplantae plant mi RNA from mi Rbase repository is used to search the homolog of mi RNA of *Ocimum tenuiflorum* in the publically available Genome database. By computational approach, identification of 80 miRNAs belonging to 12 different mi RNA families was done (Singh and Sharma 2014; Singh *et al* 2016) ^[20, 21]. To differentiate mi RNA from the other r RNA, minimum free energy criteria was considered and MFE is lower than -18.0 kcal/mol. The lower the value of MFE, the higher the stability of structure of corresponding potential mi RNA sequence.

To get the better understanding of predicted mi RNAs in plant development and other activities, the targets were predicted. The targets were predicted based on computational genomics approach and shows that the most of the target genes coding for transcription factor, lipid binding, several enzyme and regulatory development. Some of predicted mi RNA were having a complementarities with multiple target genes or group of target genes for the regulation of various physiological process as well as plant development. The present research has successfully finding of 12 mi RNAs with their different gene ontologies categories including plant development, cellular process, metabolic process and response to stimulus.

Conclusion

This study identifies 80 novel candidate mi RNA belonging to 12 different mi RNA families in *Ocimum tenuiflorum* genomics sequence based on bioinformatics approaches. This is steps towards to understanding the function and processing of mi RNAs in *O. tenuiflorum*. It is a bioinformatics approach for new mi RNA identification from the draft genome. The identified function of the predicted mi RNA target genes were involved in cell growth, development and several transcription factors. Our study indicates the identified mi RNA were conserved nature in plants and regulate the several biological process and metabolic process.

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