In silico microRNA identification of paprika (*Capsicum annuum*) ESTs

Authors*

Affiliations

ABSTRACT

A total of 33,311 Expressed Sequence Tags (EST) of paprika were mined from the GenBank EST database. The redundancy of the EST sequences was removed using CAP3 and it resulted 4,541 contigs 8,539 singletons as nonredundant data sets. The microRNAs were predicted using the program miRNAfinder. This program predicted a total of 85 miRNAs miRNAs. Another study was carried out to find the homologes miRNA using blast based tool named "miRseeK' A total of 13 miRNAs with significant similarity with the plant miRNAs were obtained from paprika ESTs. The targets of the predicted miRNAs were found using the program miRU2. Findings suggest that most miRNAs play an important role in plant development, signal transduction, protein degradation and in response to environmental stress. The miRNAs were found to be perfectly or nearly complementary to its target mRNAs.

INTRODUCTION

MicroRNAs (miRNAs) are a large family of about 21-22 nucleotide non-coding RNAs. miRNAs play very important roles in post transcriptional gene regulation by degradation of target mRNAs or by repression of targeted gene translation in animals, plants, and fungi [Bartel and Bartel, 2003]. According to the miRBase::Sequences (Release 12.0, Sept 2008) 8273 miRNAs have been discovered in various organisms. Out of these, 1638 genes have been predicted in plants of which 187 are from *Arabidopsis thaliana*, 353 from *Oryza sativa*, 37 from *Pinus taeda*, 220 from *Physcomitrella patens*, 58 from *Selaginella moellendorffii*, 45 from *Brassica napus*, 1 from each *Brassica oleracea*, *Brassica rapa*, *Gossypium herbecium* and *Carica papaya*, 69 from *Glycine max*, 30 from *Medicago truncatula*, 13 from *Gossypium hirsutum*, 2 from *Gossypium rammindii*, 234 from *Populus trichocarpa*, 30 from *Solanum lycopersicum*, 140 from *Vitis vinifera*, 16 from *Saccharum officinarum*, 72 from *Sorghum bicolor*, 32 from *Triticum aestivum* and 96 from *Zea mays*.

MicroRNAs (miRNAs) have been found in a wide range of eukaryotes, such as *Arabidopsis thaliana*, *Caenorhabditis elegans*, mice and human beings [Bartel, 2004]. However, the first miRNA (lin-4) was discovered *C. elegans* by the Ambros laboratory in 1993 [Lee et al., 1993]. A second miRNA, let-7, was discovered by Gary Ruvkun's group to direct the later stages of *C. elegans* development [Reinhart et al., 2000]. In plants, miRNAs are implicated in diverse aspects of plant growth and development, including leaf morphology and polarity, lateral root formation, transition from juvenile to adult vegetative phase and vegetative to flowering phase, flowering time, floral organ identity and reproduction [Sunkar et al., 2007; Mallory and Vaucheret, 2006]. miRNAs regulate the expression of many important genes; a majority of these genes are transcriptional factors. They are found to be involved in response to pathogen invasion and regulate their own biogenesis [Zhang et al., 2006b]. miRNA genes are also involved in hormone signaling [Eckardt, 2005; Guo et al., 2005; Inukai et al., 2005; Mallory et al., 2005] and environmental stress [Jones-Rhoades and Bartel, 2004; Sunkar and Zhu, 2004; Zhang et al., 2005]. miRNAs are also found to promote plant anti-virus defence. miRNAs can be applied in plant anti-virus defense in the following areas (1) Analyzing the function of viral suppressor in the process of gene silencing. (2) Designing and developing novel miRNA-mediated gene therapy. (3) Modifying plant physiological properties to enhance their anti-virus capacity. (4) Developing loss of- function transgenic plants [Lu et al., 2008].

EST analysis provides a cost-effective and rapid route towards the discovery and isolation of conserved miRNAs. One of the first uses of EST collections was in identifying genes involved in specific plant metabolic pathways [Ohlrogge and Benning, 2000]. Recently *in silico* identification of microRNAs in plants have been done by EST analysis [Zhang et al., 2005; Qiang et al., 2007; Nasaruddin et al., 2007; Jin et al., 2008]. As of Novebber 28, 2008, GenBank (National Centre for Biotechnology Information, http://www.ncbi.nlm.nih.gov/) contained 58,876,651 entries in the EST database. Among that there are 33,311 ESTs of *C. annuum*.

---

* corresponding author: manila…………..

The number of miRNAs obtained by EST analysis depends on three factors: number of previously known miRNAs, conservation of miRNA sequence and structure, and the number of ESTs in the database. EST analysis makes it possible to rapidly study miRNAs and their functions in species for which the genome sequences are not well known, which would be impossible using traditional computational approaches [Zhang et al., 2006a].

MATERIALS AND METHODS

EST mining and preprocessing and miRNA prediction

ESTs of *Capsicum annuum* were obtained from dbEST (http://www.ncbi.nlm.nih.gov/dbEST/). The squence redundancy was removed using the sequence assembly program CAP3 (http://pbil.univlyon1.fr/cap3.php). The program clustered the ESTs containing overlapping sequences as contigs and non-overlapping sequences as singletons. The processed ESTs were submitted to miRNAfinder(http://bioinfo3.noble.org/mirna/) for predicting the miRNAs present paprika. The inputted EST sequences were compared with the EST library of *Arabidopsis thaliana*. To reduce the false positives the reference genome was selected as *Oryza sativa* genomes EST library.

Development of miRseeK

A tool named “miRseeK” was created using the standalone BLAST-2.2.19 downloaded from the website (ftp://ftp.ncbi.nih.gov/blast/executables/LATEST) on 01.02.09. A web interface was made using PHP language. The clustered EST contigs of paprika was submitted as the query sequence. A database containing all plant miRNAs was created and maintained as the reference database. All available plant miRNAs were obtained from the miRBase::Sequence database (http://microrna.sanger.ac.-uk/sequences). A mismatch of 2 was allowed. The ESTs of the corresponding miRNAs predicted by “miRseek” were submitted to the mfold web server to obtain the precursor sequences. The corresponding precursor sequences were then submitted to the RNAfold Web Server. It calculates the Partition function and base pair probability matrix in addition to the minimum free energy (MFE) structure. The structures were predicted using a loop-based energy model and the dynamic programming algorithm introduced by Zuker et al. [1981].

The targets of the obtained miRNAs were predicted using the tool miRU2 (http://bioinfo3.noble.org/miRU2/). The tool also provided with a brief description of the target mRNA.

RESULTS AND DISCUSSION

Identification of novel miRNAs

A total number of 85 miRNAs were predicted from the paprika EST contigs **[Table 1].** The microRNA containing contigs namely contig3616, contig3739, contig2992, contig3629, contig3477, contig626, contig3555, contig3755, contig141, BM064546, CA516666, BM067194, BM067986, BM065268 and CA516902 were found to show significant sequence similarity with chr2 of *A. thaliana* and 64 miRNAs represented these sequences. Similarly contig4132, contig3184, EC911955 and CO912258 showed significant similarity with chr1 and 11 miRNAs were found to represent these sequences. Similarly contig3237, contig201, contig3501, contig3840 and contig1722 showed sequence similarity with chr3 and 15 miRNAs represented these contigs. Similarly Contig2482 and BM064235 were found to have significant similarity with chr4. Two miRNAs represented these sequences. Also Contig2851 and CA518435 showed sequence similarity with chr5 and a total of 4 miRNAs were found to represent these sequences.

Since the predicted miRNAs did not contain any homologs in any other plant miRNAs these can be considered as novel miRNAs and grouped to new miRNA families.

Plant miRNAs are conserved

The results obtained from miRseeK gave a list of miRNAs with similarity ranging from 17 to 22. A similarity between 19 to 22 was taken into account. A mismatch of 2 was allowed. The e value was set to 2e-3 to obtain sequences with significant similarity. The result displayed the miRNAs homologous to the plant miRNA. The search reported a total of 13 miRNAs **[Table 2]** from *Capsicum annuum.* A keen observation of the results also reveals the fact that most of the predicted miRNAs were homologous to the miR156 family.

In paprika out of the 13 identified ESTs which contain miRNAs, most of them were obtained from tissues under stress. 8 ESTs were found to be obtained from tissues under hypersensitive response against pathogen, 3 each from leaves inoculated with *Xanthomonas campestris* and the fruit pericarp.. In this study, majority of the obtained microRNAs in paprika were expressed in response to stress. Such kind of observations were also reported in the study conducted by Zhang et al., [2005] in the crops *Aegilops speltoides, Citrus sinensis, Lactuca sativa, Persea americana, Triticum turgidum,* etc. Though the tool was unable to predict the novel miRNAs present it was reliable in the prediction of the homologous miRNAs present in plants.

Most miRNAs target genes related to plant growth and development

From the results obtained from miRU2, it was observed that one single miRNA may have many targets. The result shows that majority of the genes’ functions were related to plant growth and development. Many miRNAs have been found to target transcription factors which show its role in inhibiting transcription. The obtained result complements with the earlier studies done by Zhang et al [2006b].

Table 3 shows the list of targets predicted by miRU2 for miRNAs obtained from *miRNAfinder*. According to the results pap-miR1 is found to target GRF2 (GENERAL REGULATORY FACTOR 2) which functions in protein binding, protein phosphorylated amino acid binding and is expressed during seedling growth. pap-miR15 is found to target CUC3 gene which is involved in multicellular organismal development, meristem initiation, organ boundary specification between lateral organs and the meristem and has transcription factor activity. pap-miR25 is found to target PTAC2 (PLASTID TRANSCRIPTIONALLY ACTIVE2) gene which is involved in positive regulation of transcription, transcription from plastid promoter and expressed in seed, embryo, cotyledon, hypocotyl, root, shoot, etc.

Table 4 shows the list of targets predicted by miRU2 for miRNAs obtained from *miRseeK*. According to the results pap-miR4 was found to target the gene CYP71B21 which is found in plasma membrane, vacuole and has carbohydrate transmembrane transporter activity, nucleoside transmembrane transporter activity, sugar:hydrogen symporter activity, etc. pap-miR8 was found to target the gene PHB involved in regulation of transcription, DNA-dependent, determination of bilateral symmetry, adaxial/abaxial pattern formation, polarity specification of adaxial/abaxial axis, meristem initiation, primary shoot apical meristem specification and has transcription factor activity. pap-miR12 was found to target the gene RPT5B involved in glucose mediated signaling and has ATPase activity.

From the results [Table 3,4] miRNAs were found to target the genes which all are very essential for the plant growth and development and so it the miENAs does not function properly or does not bind properly to their targets, it can cause abnormalities in the development of plant organs. These relates with the findings done by Chen [2005]. Dysfunction of individual miRNAs and/or their ability to properly regulate their targets has been shown to cause floral and leaf-patterning defects (miR159 and miR319; Palatnik et al., 2003; Achard et al., 2004; Millar and Gubler, 2005), floral development and timing defects (miR172; Aukerman and Sakai, 2003; Chen, 2004), loss of organ polarity and altered vascular development (miR165/166; McConnell et al., 2001; Emery et al., 2003; Juarez et al., 2004; Mallory et al., 2004b; McHale and Koning, 2004; Zhong and Ye, 2004; Kim et al., 2005), defective organ separations and aberrant numbers of floral organs (miR164; Laufs et al., 2004; Mallory et al., 2004a; Baker et al., 2005), aberrant phyllotaxis, reduced fertility, and abortion of the shoot apical meristem (miR168; Vaucheret et al., 2004), and cotyledon and rosette leaf shape and symmetry defects, reduced fertility, and misexpression of early auxin response genes (miR160; Mallory et al., 2005).

CONCLUSION

The work supports and proves that miRNAs are conserved in plants. The work also identifies the presence of many novel miRNAs in paprika. Most of the miRNAs were found to target transcription factors and genes involved in plant growth and development. This work also puts a special emphasis on the fact that miRNAs are involved in plant stress responses esp. in response to pathogenic attack. This can be further considered as one of the causes of diseases affecting paprika like fruit rot.

ACKNOWLEDGEMENT

This work was supported by a grant from Department of Biotechnology (BTISnet), Government of India, New Delhi, India.

REFERENCES

Achard P, Herr A, Baulcombe D C and Harberd N P (2004). Modulation of floral development by a gibberellinregulated microRNA. *Development* 131 : 3357–3365.

Aukerman M J and Sakai H (2003). Regulation of flowering time and floral organ identity by a MicroRNA and its APETALA2-like target genes. *Plant Cell* 15 : 2730– 2741.

Baker C C, Sieber P, Wellmer F and Meyerowitz E M (2005). The early extra petals1 mutant uncovers a role for microRNA miR164c in regulating petal number in Arabidopsis. *Curr. Biol.* 15 : 303–315.

Bartel B, Bartel DP (2003). MicroRNAs: At the Root of Plant Development. *Plant Physiology* 132:709–717.

Bartel DP (2004) MicroRNAs: Genomics, biogenesis, mechanism, and function. *Cell* 116:281–297.

Chen X (2004). A microRNA as a translational repressor of *APETALA2* in *Arabidopsis* flower development. *Science* 303**:** 2022–2025.

Chen X (2005). microRNA biogenesis and function in plants. *FEBS Letters* 579:5923–5931.

Eckardt NA (2005). MicroRNAs regulate auxin homeostasis and plant development. *Plant Cell* 17:1335–1338

Emery J F, Floyd S K, Alvarez J, Eshed Y, Hawker N P, Izhaki A, Baum S F and Bowman J L (2003). Radial patterning of Arabidopsis shoots by class III HD-ZIP and KANADI genes. *Curr. Biol.* 13 : 1768–1774.

Guo HS, Xie Q, Fei JF, and Chua NH(2005). MicroRNA directs mRNA cleavage of the transcription factor *NAC1* to downregulate auxin signals for *Arabidopsis* lateral root development. *Plant Cell* 17:1376–1386.

Inukai Y, Sakamoto T, Ueguchi-Tanaka M, Shibata Y, Gomi K, Umemura I, Hasegawa Y, Ashikari M, Kitano H, Matsuoka M (2005). *Crown rootless1*, Which Is Essential for Crown Root Formation in Rice, Is a Target of an AUXIN RESPONSE FACTOR in Auxin Signaling. *Plant Cell* 17(5): 1387–1396.

Jin W, Li N, Zhang B, Wu F, Li W, Guo A, Deng Z (2008). Identification and verification of microRNA in wheat (Triticum aestivum). *Plant Res* 121: 351–355.

Jones-Rhoades M W, Bartel D P (2004). Computtional Identification of Plant MicroRNAs and Their Targets, Including a Stress-Induced miRNA. *Molecular Cell* 14: 787–799.

Juarez M T, Kui J S, Thomas J, Heller B A and Timmermans M C (2004). microRNA-mediated repression of rolled leaf1 specifies maize leaf polarity. *Nature* 428 : 84–88.

Kim J, Jung J-H, Reyes JL, Kim Y-S, Kim S-Y, Chung K-S, Kim JA, Lee M, Lee Y, Narr Kim V, Chua NH and Park C-

M (2005). microRNA-directed cleavage of ATHB15 mRNA regulates vascular development in Arabidopsis inflorescence stems.*Plant J*. 42, 84–94.

Laufs P, Peaucelle A, Morin H and Traas J (2004). Micro-RNA regulation of the CUC genes is required for boundary size control in Arabidopsis meristems. *Development* 131 : 4311–4322.

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.

Lu Y, Gan Q, Chi X, Qin S(2008). Roles of microRNA in plant defense and virus offense interaction. *Plant Cell Rep* 27:1571–1579.

Mallory AC , Bartel DP, Bartel B(2005). MicroRNA-directed regulation of *Arabidopsis* AUXIN RESPONSE FACTOR17 is essential for proper development and modulates expression of early auxin response genes. *Plant Cell* 17:1360–1375.

Mallory AC, Dugas, DV, Bartel DP and Bartel B (2004b). MicroRNA regulation of NAC-domain targets is required for proper formation and separation of adjacent embryonic, vegetative, and floral organs. *Curr. Biol.* 14 :1035–1046.

Mallory AC, Reinhart B J, Jones-Rhoades MW, Tang G, Zamore PD, Barton MK and Bartel DP (2004a). MicroRNA control of PHABULOSA in leaf development: importance of pairing to the microRNA 50 region. *EMBO J.* 23 : 3356–3364.

Mallory AC, Vaucheret H (2006). Functions of microRNAs and related small RNAs in plants. *Nat Genet* 38(suppl):S31-36.

McConnell JR, Emery J, Eshed Y, Bao N, Bowman J and Barton MK (2001). Role of PHABULOSA and PHAVOLUTA in determining radial patterning in shoots. Nature 411 : 709–713.

McHale N A and Koning R E (2004). MicroRNA-directed cleavage of Nicotiana sylvestris PHAVOLUTA mRNA regulates the vascular cambium and structure of apical meristems. *Plant Cell* 16 : 1730–1740.

Millar AA and Gubler F (2005). The Arabidopsis GAMYB-like genes, MYB33 and MYB65, are microRNA-regulated genes that redundantly facilitate anther development. *Plant Cell* 17 : 705–721.

Nasaruddin N M, Harikrishna K, Othman R Y, Hoon L S, Harikrishna J A (*2007*). Computational prediction of microRNAs from Oil Palm (Elaeis guineensis Jacq.)expressed sequence tags. *AsPac J. Mol. Biol. Biotechnol.* 15(3): 107-113.

Ohlrogge J, Benning C (2000). Unraveling plant metabolism by EST analysis. *Current Opinion in Plant Biology* 3:224–228.

Palatnik J F, Allen E, Wu X, Schommer C, Schwab R, Carrington J C and Weigel D (2003). Control of leaf morphogenesis by microRNAs. *Nature* 425 : 257–263.

Qiang G, AnLing X, Qing Y, ZhiMin Y (2007). Bioinformatic identification of microRNAs and their target genes from *Solanum tuberosum* expressed sequence tags. *Chinese Science Bulletin* 52(17):2380-2389.

Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, Horvitz HR, Ruvkun G.(2000). The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans. *Nature* 403(6772):901-6.

Sunkar R, Chinnusamy V, Zhu J, Zhu J K (2007). Small RNAs as big players in plant abiotic stress responses and nutrient deprivation. Trends in Plant Sci 12(7):301-309.

Sunkar R, Zhu JK (2004). Novel and stress-regulated micro-RNAs and other small RNAs from Arabidopsis. *Plant Cell* 16: 2001-2019.

Vaucheret H, Vazquez F, Crete P and Bartel D P (2004). The action of ARGONAUTE1 in the miRNA pathway and its regulation by the miRNA pathway are crucial for plant development. *Genes Dev.* 18 : 1187–1197.

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.

Zhang B, Pan X, Cannon CH, Cobb GP, Anderson TA (2006a). Conservation and divergence of plant microRNA genes. *The Plant Journal* 46:243–259.

Zhang B, Pan X, Cobb G P, Anderson T A (2006b). Plant microRNA: A small regulatory molecule with big impact. *Developmental Biology* 289:3– 16.

Zhong R and Ye Z-H (2004). amphivasal vascular bundle 1, a Gain-of-Function Mutation of the IFL1/REV Gene, Is Associated with Alterations in the Polarity of Leaves, Stems and Carpels. *Plant Cell Physiol.* 45 : 369-385.

Zuker M, Stiegler P (1981). Optimal computer folding of large RNA sequences using thermodynamics and auxiliary information. *Nucleic Acid Res.* 9(1): 133-148.

Table 1. List of miRNAs predicted by miRNAfinder

SL No.	INPUTTED EST	PREDICTED miRNA
pap-miR1	Contig3616 ;Contig3739 ;Contig2992 ; Contig3629; Contig3477 ;Contig3237 ;Contig626 ; Contig3555; Contig201 ;Contig3755 BM064546;	GGAUCGCGGCGACGUGGGUG
pap-miR2	Contig3616 ;Contig3739 ;Contig2992 ;Contig3629 ; Contig3477 ;Contig3237 ;Contig626 ; Contig3555; Contig201 ;Contig3755 ; BM064546	CCGCCCGCGACGUCGCGAGA
pap-miR3	Contig3477 ;Contig2992;Contig626 ; Contig3555; Contig3616 ;Contig3739; BM067194	CUAGUAGCUGGUUCCCCCCG
pap-miR4	Contig3477 ;Contig2992;Contig626 ;Contig3555; BM067194	UCGGGUAAAGCCAAUGAUUA
pap-miR5	Contig3616 ;Contig3739;Contig3629; Contig2992; Contig626	UGUACACACCGCCCGUCGCU

	;Contig3555 ;Contig201; Contig3477; Contig3237	
pap-miR6	Contig3616 ;Contig3739;Contig3629; Contig2992 ; Contig626 ;Contig3555 ;Contig201;Contig3477; Contig3237	GAAUGAUCCGGUGAAGUGUU
pap-miR7	Contig3840; Contig1722	GAAGCUAGCUGGUGAGAGUG
pap-miR8	Contig3840 ; Contig1722	UCAUAUUCAUUGAUGAGAUC
pap-miR9	Contig3616 ;Contig3739	UCGGGUAAAGCCAAUGAUUAG
pap-miR10	Contig626;Contig3555;Contig3477; Contig2992 BM067194;	UCAGCGGACUGCUCGAGCUG
pap-miR11	Contig626;Contig3555;Contig3477; Contig2992 BM067194;	GCGGCGAGAGCGGGUCGCCG
pap-miR12	Contig626 ;Contig3555;Contig3477;Contig3755; Contig3616;Contig3739 ; Contig2992;Contig3629 ; Contig201; BM064546; BM067194	ACCUCAGCCUGCUAACUAGC
pap-miR13	Contig626;Contig3555 ; Contig3477;Contig3755; Contig3616;Contig3739 ; Contig2992;Contig3629; Contig201 ; BM064546; BM067194	GGCUUCUUAGAGGGACUAUG
pap-miR14	Contig141	GGUCACAAAGGAGGCCAGGU
pap-miR15	Contig141	ACUUGAGCUCGUCGUUGAUG
pap-miR16	Contig3755 ;Contig3616 ;Contig3739 ; Contig2992; BM064546; BM067194	UAGAUGUUCUGGGCCGCACGC
pap-miR17	Contig3755 ;Contig3616 ;Contig3739 ; Contig2992; BM064546; BM067194	CGUGAUGGGGAUAGAUCAU
pap-miR18	Contig626 ;Contig3555 ;Contig201	GAUGUUCUGGGCCGCACGC
pap-miR19	Contig626 ;Contig3555 ;Contig201	CGUGAUGGGGAUAGAUCAUUG
pap-miR20	Contig3477	AGAUGUUCUGGGCCGCACGC
pap-miR21	Contig3477	CGUGAUGGGGAUAGAUCAUU
pap-miR22	Contig2851	UGGUGUCAUGGUUGGUAUGG
pap-miR23	Contig2851	UCCAAUCGAACAUGGUAUUG
pap-miR24	Contig2482 BM064235;	CGGAAAGCAGUUGGAGGAUG
pap-miR25	Contig2482; BM064235	CCACCCUUCAUCUUGUUCUC
pap-miR26	Contig2992 ; BM067194	AGACCUUCGGAAUUUGAAG
pap-miR27	Contig2992 BM067194;	GCUUUUUGAUCCUUCGAUGU
pap-miR28	Contig3477 ; Contig2992; Contig3616;Contig3739 ; BM067194	GGCUUGGCAGAAUCAGCGGG
pap-miR29	Contig3477 ; Contig2992; Contig3616;Contig3739; BM067194	CCCUGUUGAGCUUGACUCUA
pap-miR30	Contig3501	ACUUCCCCUGGGGGUAGGGU
pap-miR31	Contig3501	UGCCCGAGCGGUUAAUGGGGA
pap-miR32	Contig3616;Contig3739	UAGACCUUCGGAAUUUGAAG
pap-miR33	Contig3616;Contig3739	GCUUUUUGAUCCUUCGAUGUC
pap-miR34	Contig3477;Contig3616 ;Contig3739 ; Contig2992;Contig626 ;Contig3555; BM067194	CGUCGCGUGGUGUCCGGUGC
pap-miR35	Contig3477;Contig3616 ;Contig3739 ;Contig2992 BM067194;	CGCAUCAGGUCUCCAAGGUG
pap-miR36	Contig626 ;Contig3555 ; Contig201	UUGAUCCUUCGAUGUCGGCU
pap-miR37	Contig626 ;Contig3555 ; Contig201	GAGCUGGGUUUAGACCGUC
pap-miR38	Contig626 ;Contig3555	CGCAUCAGGUCUCCAAGGUGA
pap-miR39	Contig626; Contig3555 ;Contig3755; Contig3477 ; Contig3616 ;Contig3739;Contig3629 ;Contig2992; BM064546; BM067194	GACCUCAGCCUGCUAACUAGC
pap-miR40	Contig626;Contig3555; Contig3755 ; Contig3477; Contig3616 ;Contig3739 ;Contig3629; Contig2992;Contig201; BM064546; BM067194	GACAGACUGAGAGCUCUUUC
pap-miR41	Contig626;Contig3555; Contig3755 ; Contig3477;Contig3616 ;Contig3739 ;Contig3629; Contig2992;Contig201 BM064546; BM067194;	GGUGGAGCGAUUUGUCUGGU
pap-miR42	Contig4132	CCACGUCGAUUCUGGAAAGU
pap-miR43	Contig4132	GAGGUUCGAGAAGGAGGCUG
pap-miR44	Contig626 ;Contig3555 ; Contig3755; Contig3477; Contig3616 ;	GGGGAGUAUGGUCGCAAGGC

	Contig3739 ; Contig3629 ; Contig2992; Contig201 BM064546; BM067194;	
pap-miR45	Contig626 ;Contig3555 ;Contig3755;Contig3477; Contig3616 ; Contig3739 ; Contig3629 ; Contig2992; Contig201; BM064546; BM067194	AGCCUGCGGCUUAAUUUGAC
pap-miR46	Contig3477;Contig3616 ;Contig3739;Contig2992 ; Contig626 ; Contig3555; BM067194	GAAGCCGGGUUACGGUGCCC
pap-miR47	Contig3477;Contig3616 ;Contig3739;Contig2992 ; Contig626 ; Contig3555; BM067194	UGGUCAUGGAAGUCGAAAUC
pap-miR48	Contig3477;Contig3616; Contig3739; Contig2992;Contig626;Contig3555 BM067194;	GCUGGAAGAGCACCGCACGU
pap-miR49	Contig3477;Contig3616; Contig3739; Contig2992; Contig626;Contig3555; BM067194;	GGUGUCCGGUGCGCCCCCGG
pap-miR50	Contig4132;Contig3184; EC911955;	ACAAGCCCCUUCGUCUCCCA
pap-miR51	Contig4132;Contig3184 ; EC911955	GUGGGACGUGUUGAGACUGG
pap-miR52	Contig3755;Contig3616 ;Contig3739 ;Contig2992; BM064546; BM067194	GGUGGAGCGAUUUGUCUGG
pap-miR53	Contig4132	ACCUCCCAGGCUGAUUGUGC
pap-miR54	Contig4132 EC911955;	AGGAUGGUCAGACCCGUGAG
pap-miR55	CA516666	AAGCGCUUUUGCUACUGAGA
pap-miR56	CA516666	UUCUCAUAGCGAGGCGCUUC
pap-miR57	CA516666	CCCCGGAUGAUCGUGUUGGG
pap-miR58	CA516666	ACCUCGUACGAUCGUGUCGG
pap-miR59	BM067986;BM065268	GUAGGGUGGGUGGUACCGCU
pap-miR60	BM067986;BM065268	GAGCGAGUGAAACUCAUCAC
pap-miR61	CA516666	AUCCCCUUCGUCAGCUGUUU
pap-miR62	CA516666	CAAGCAGUUAUAUGGAUACG
pap-miR63	CA516666	UGCCCAUUCACAAGGACUCG
pap-miR64	CA516666	UCAAGAUAUUGUGUUUGGAG
pap-miR65	CA516666	UCCCCUUCGUCAGCUGUUUG
pap-miR66	CA516666	UUGAAUGGAUGGCGGAGCGU
pap-miR67	CA516666	UGGUGGUGCUUCAGGAUUCC
pap-miR68	CA516666	UGCGUCCAAGAUCAGAACGA
pap-miR69	BM067986;BM065268	CUGGAGCUACCCACUUCGAU
pap-miR70	BM067986;BM065268	GAUCUAGUGGUAUUUUUAUG
pap-miR71	BM065268;BM067986	AUUUGUUCUUCGCCGCUUUG
pap-miR72	BM065268;BM067986	UGGGAGUAGUGACUAGCGUU
pap-miR73	BM067986; BM065268	GAUCCAGUAGCCGGGAAGGG
pap-miR74	BM067986; BM065268	CCCCUGUCCAGUGCCUGGCAG
pap-miR75	CA518435	GCACAGAAAGUGCCUGAGGAU
pap-miR76	CA518435	CAUCCUGGCAUGAUUCAGG
pap-miR77	EC911955	CCACCACUGGUGGUUUUGAG
pap-miR78	EC911955	CCUUGCUUUCACCCUUGGUG
pap-miR79	CA516666	GGACCAAUUUACGAUGUCUU
pap-miR80	CA516666	AGGCCACGUGGAUUGGCCAG
pap-miR81	CO912258	GCAGUUAGUGGUGCAACUAG
pap-miR82	CO912258	GCUCGUUGACACCAACAACGA
pap-miR83	EC911955	UACCUCCCAGGCUGAUUGUGC
pap-miR84	CA516902	GCAGCGCGCUUGGGGAUGCA
pap-miR85	CA516902	CUAUUAUCGCCCAGCCCCCU

Table 2. List of miRNAs predicted by miRseeK

Micro ID	Query Sequence	Homolog miRNA ID	Homolog miRNA Sequence	Similarity	Strand	Query Region	E value
pap-1	Contig638	>vvi-miR171e MIMAT0005695 >osa-miR171a MIMAT0000645 >bna-miR171f MIMAT0005610 >bna-miR171g MIMAT0004446 >ath-miR171a MIMAT0000202	gatattggcgcggctcaatca	21/21	+/-	123-143	4e-006
pap-2	Contig1767; Contig2790	>ath-miR414 MIMAT0001322	tgacgaagatgatgaagatga	20/21	+/-	347-367;926-946	5e-004; 0.001
pap-3	Contig2235	>vvi-miR156f MIMAT0005645 >vvi-miR156g MIMAT0005646 >sly-miR156a MIMAT0009138 >sly-miR156c MIMAT0009140 >sly-miR156b MIMAT0009139 >ptc-miR156j MIMAT0001899 >ptc-miR156h MIMAT0001897 >ptc-miR156g MIMAT0001896 >ptc-miR156i MIMAT0001898 >gra-miR157b MIMAT0005811 >gra-miR157a MIMAT0005810 >gma-miR156d MIMAT0001672 >gma-miR156e MIMAT0001673 >gma-miR156c MIMAT0001674 >bna-miR156b MIMAT0005636 >bna-miR156c MIMAT0005639 >ath-miR157c MIMAT0000174 >ath-miR157a MIMAT0000172 >ath-miR157b MIMAT0000173	tgctctctatcttctgtcaa	20/21	+/-	536-555	9e-006
pap-4	Contig3998	>vvi-miR403e MIMAT0006573 >vvi-miR403d MIMAT0006572 >vvi-miR403b MIMAT0006570 >vvi-miR403f	ttagattcacgcacaaactc	20/21	+/+	299-318	9e-006

		MIMAT0006574 >vvi-miR403c MIMAT0006571 >vvi-miR403a MIMAT0006569 >ptc-miR403b MIMAT0002057 >ptc-miR403a MIMAT0002056 >ptc-miR403c MIMAT0003944 >ath-miR403 MIMAT0001004					
pap-5	gil51300065	>vvi-miR171e MIMAT0005695 >osa-miR171a MIMAT0000645 >bna-miR171f MIMAT0005610 >bna-miR171g MIMAT0004446 >ath-miR171a MIMAT0000202	gatattggcgcggctcaatca	21/21	+/-	438-458	2e-006
pap-6	gil25036197	>ppt-miR1046-3p MIMAT0005160	tggtgaaaaatctgaaaaat	19/21	+/+	64-83	0.002
pap-7	gil25035721	>osa-miR396d MIMAT0001600 >osa-miR396e MIMAT0001601	gttcaagaaagcctgtgga	19/21	+/-	137-155	3e-005
pap-8	gil25033610	>ppt-miR166j MIMAT0005046 >ppt-miR166l MIMAT0005048 >ppt-miR166k MIMAT0005047	ggaatgaagcctggtccgga	20/21	+/-	221-240	7e-006
pap-9	gil25016881	>vvi-miR156f MIMAT0005645 >vvi-miR156g MIMAT0005646 >sly-miR156a MIMAT0009138 >sly-miR156c MIMAT0009140 >sly-miR156b MIMAT0009139 >smo-miR156c MIMAT0005215 >ptc-miR156j MIMAT0001899 >ptc-miR156h MIMAT0001897 >ptc-miR156g MIMAT0001896 >ptc-miR156i MIMAT0001898 >gra-miR157b MIMAT0005811 >gra-miR157a MIMAT0005810 >gma-miR156d MIMAT0001672 >gma-miR156e	gtgctctctctcttctgtcaa	20/21	+/-	417-437	4e-004

		MIMAT0001673 >gma-miR156c MIMAT0001674 >bna-miR156b MIMAT0005636 >bna-miR156c MIMAT0005639 >ath-miR157c MIMAT0000174 >ath-miR157a MIMAT0000172 >ath-miR157b MIMAT0000173					
pap-10	gil25015879	>osa-miR395o MIMAT0003872	gagttcctccaaactcttcat	20/21	+/-	305-325	4e-004
pap-11	gil22785044	>zma-miR156j MIMAT0001710 >sbi-miR156d MIMAT0001444 >smo-miR156d MIMAT0005216 >osa-miR156k MIMAT0001020 >mtr-miR156 MIMAT0001654 >gma-miR156b MIMAT0001692 >bna-miR156a MIMAT0004445	tgtgctccctctcttctgtca	20/21	+/-	35-55	4e-004
pap-12	gil51300065	>zma-miR171a MIMAT0001385	atattggcgcggctcaatca	20/20	+/-	439-458	7e-006
pap-13	gil22785044	>vvi-miR156a MIMAT0005640 >ptc-miR156k MIMAT0001900	gtgctccctctcttctgtca	20/20	+/-	36-55	7e-006

Table 3. List of targets predicted by miRU2 for miRNAs obtained from miRNAfinder

SL No.	Target Acc.	Target Sequence (reverse)	Target Description
pap-miR1	AT1G78300.1; AT1G13260.1	UGACCACGUCACGGCGAUCC; UGCCUACGACGUCGCGGUUC	GRF2 (GENERAL REGULATORY FACTOR 2); protein phosphorylated amino acid binding;;RAV1 (Related to ABI3/VP1 1); DNA binding / transcription factor
pap-miR2	AT5G07150.1; AT1G59640.1	UCUC-CGGCGUGGUGGGCGG; UCACGCGUUGUCUCAGGCGG	leucine-rich repeat family protein;;ZCW32 (BIGPETAL, BIGPETALUB); DNA binding / transcription factor
pap-miR3	AT1G70070.1; AT5G51220.1	CUGGGAGAAACAGCUGCUGC ;CGGGGUUAACUUGCUGCUAG	PDE317 (PIGMENT DEFECTIVE 317); ATP-dependent helicase;ubiquinol-cytochrome C chaperone family protein
pap-miR4	AT1G49950.2; AT1G50840.1	UGAUCAUUCUCUUUACUUGG; AAUUGAUUGGCAUUACCCAA	ATTRB1/TRB1 (TELOMERE REPEAT BINDING FACTOR 1); DNA binding / transcription factor;;POLGAMMA2 (polymerase gamma 2); DNA binding / DNA-directed DNA polymerase
pap-miR5	AT2G28900.1; AT2G06210.1	AGCUGCUGGUGGAGUGUACA; GGCAAUGGCGAGUGUGUACA	OEP16 (OUTER ENVELOPE PROTEIN 16); protein translocase;;ELF8 (EARLY FLOWERING 8); binding
pap-miR6	AT1G19200.1; AT1G32180.1	ACUAUUUCGCCGGAUUAUUU; AAACCCUCACUGGAUCAUUC	senescence-associated protein-related;;ATCSLD6 (Cellulose synthase-like D6); cellulose synthase/ transferase, transferring glycosyl groups
pap-miR7	AT2G34680.1; AT2G43330.1	CUCUCUCUCCAGCUAUUUUC; CACUCAAACCAGCUCGCUUU	AIR9 (Auxin-Induced in Root cultures 9); protein binding;;ATINT1 (INOSITOL TRANSPORTER 1); carbohydrate transporter/ sugar porter
pap-miR8	AT3G18660.2; AT4G31530.1	CAUCUUCUCGAUGGAUAUGG; GGUUUCAUCAGUAGGUGUGA	PGSIP1 (PLANT GLYCOGENIN-LIKE STARCH INITIATION PROTEIN 1); transferase, transferring gly-

			cosyl groups ;;catalytic/ coenzyme binding
pap-miR9	AT1G33970.3; AT1G49950.2	UGAUCGUUGUUUUUACCGGU ; UUGAUCAUUCUCUUUACUUGG	GTP binding;;ATTRB1/TRB1 (TELOMERE REPEAT BINDING FACTOR 1); DNA binding / transcription factor
pap-miR10	AT2G30800.1; AT3G06340.2	AGGGUCGAGCAGGCCGCUGC; CAGGUUGAGCAGAUCGCUAA	HVT1 (HELICASE IN VASCULAR TISSUE AND TAPETUM); ATP binding / helicase/ nucleic acid binding;;heat shock protein binding / unfolded protein binding
pap-miR11	AT3G46640.1; AT4G16480.1	CGGAGACGACGCUCUCGUCGC AUGCGACUUGCUCUCUCCGC	PCL1 (PHYTOCLOCK 1); DNA binding / transcription factor;;ATINT4 (INOSITOL TRANSPORTER 4); carbohydrate transporter/ myo-inositol:hydrogen symporter/ sugar porter
pap-miR12	AT5G04720.1; AT3G44530.1	ACUAGUUAUGAGGCUGGGGU; GCUGGUUUGCAGGUUGAUGU	ADR1-L2 (ADR1-LIKE 2); ATP binding / nucleoside-triphosphatase/ nucleotide binding / protein binding;;HIRA (ARABIDOPSIS HOMOLOG OF HISTONE CHAPERONE HIRA); nucleotide binding / protein binding
pap-miR13	AT2G26290.1; AT2G34860.2	UAGAGUCUAUCCAAGAAGUU; CAAAGGCCUUCUUAGAAGGC	ARSK1 (ROOT-SPECIFIC KINASE 1); kinase;;EDA3 (embryo sac development arrest 3)
pap-miR14	AT1G08520.1; AT2G36530.1	CCCUGGUUAUCUUUGUGGUU; AAGUGGUCUUCUUUGUGACG	CHLD/PDE166 (PIGMENT DEFECTIVE 166); ATP binding / magnesium chelatase/ nucleoside-triphosphatase/ nucleotide binding;;LOS2 (Low expression of osmotically responsive genes 1); phosphopyruvate hydratase
pap-miR15	AT1G76420.1; AT1G03880.1	CAACAUCGACGAGCUCAAAG; CAUAGGCGAACAGUUCGAGU	CUC3 (CUP SHAPED COTYLEDON3); transcription factor;;CRU2 (CRUCIFERIN 2); nutrient reservoir
pap-miR16	AT5G57090.1; AT5G45140.1	GCGUGACGCCGAGAACGUCAA; CGAGCGGCUCAUGAUAUCUA	EIR1 (ETHYLENE INSENSITIVE ROOT 1); auxin:hydrogen symporter/ transporter;;NRPC2 (nuclear RNA polymerase C 2); DNA binding / DNA-directed RNA polymerase
pap-miR17	AT4G33330.1; AT1G71480.1	AUGACUCCAUCUCCAUUACC; AUGAUUUAUCCUCUGUCACA	PGSIP3 (PLANT GLYCOGENIN-LIKE STARCH INITIATION PROTEIN 3); transferase, transferring glycosyl groups;;nuclear transport factor 2 (NTF2) family protein
pap-miR18	AT1G32750.1; AT3G62010.1	GCUUGUGGCACCAGAGCAUG; GUGUGCUGGUGCAGGACAUU	HAF01 (Histone acetyltransferase TAFII250 family); DNA binding ;;metal ion binding / oxidoreductase
pap-miR19	AT1G21310.1; AT5G03650.1	CUAUUAGAUAUCUCUAUCACG; CAAUGAUGUAUACUCAUCAUG	ATEXT3 (EXTENSIN 3); structural constituent of cell wall;;SBE2.2 (STARCH BRANCHING ENZYME 2.2)
pap-miR20	AT1G13260.1	GCGUGUGGCUCGGGACAUUC	RAV1 (Related to ABI3/VP1 1); DNA binding / transcription factor
pap-miR21	AT1G24625.1	AAUGUUUGGUCACCAUCACA	ZFP7 (ZINC FINGER PROTEIN 7); nucleic acid binding / transcription factor/ zinc ion binding
pap-miR22	AT1G11190.1	UUAGACUAGCCAUGAUACUA	BFN1 (BIFUNCTIONAL NUCLEASE I); nucleic acid binding
pap-miR23	AT1G31800.1	UGAUUUCAUGUUUGAUUGGA	CYP97A3/LUT5 (CYTOCHROME P450-TYPE MONOOXYGENASE 97A3); carotene beta-ring hydroxylase/ oxygen binding
pap-miR24	AT4G21750.2	UAUCCUCCAUUUGUUUUCUU	ATML1 (MERISTEM LAYER 1); DNA binding
pap-miR25	AT1G74850.1	GAGAAGAAGAUAAAUGGUGA	PTAC2 (PLASTID TRANSCRIPTIONALLY ACTIVE2); binding
pap-miR26	AT3G61850.2	UUUGAAACUUCCGAAGUUCU	DAG1 (DOF AFFECTING GERMINATION 1); DNA binding / transcription factor
pap-miR27	AT3G14940.1	CCGUCGCAGGAUCAAGAAGC	ATPPC3 (PHOSPHOENOLPYRUVATE CARBOXYLASE 3); phosphoenolpyruvate carboxylase
pap-miR28	AT2G47310.1; AT3G56100.1	UCCGCCGUUUCCGCCGAGUC ; CUUGCUGACUCUUCUAAGCU	flowering time control protein-related / FCA gamma-related;;MRLK (MERISTEMATIC RECEPTOR-LIKE KINASE); ATP binding / protein serine/threonine kinase
pap-miR29	AT5G59690.1; AT4G19490.1	UGGAGUCAAGCGUAUCAGUG; UGGAGAUAAGCUCAACAGAG	histone H4;;protein binding
pap-miR30	AT2G23380.1	AUUCCAUCGCCAGUGGAAGU	CLF (CURLY LEAF); transcription factor
pap-miR31	AT3G09650.1	UUUCAUUAACCCCUCCGGUA	HCF152 (HIGH CHLOROPHYLL FLUORESCENCE

			152)
pap-miR32	AT4G29060.1; AT4G32730.2	CAUCAAAGUCAGAAGGUUUG; CUUCAAGUUCUGAGGGCCU	EMB2726 (EMBRYO DEFECTIVE 2726); translation elongation factor ;;PC-MYB1 (myb domain protein 3R1); DNA binding / transcription factor
pap-miR33	AT3G18780.1; AT3G56510.2	AGAUCAAAGGCUUAAAAAGC; ACGAUGAAGAGUCAAAAGGC	ACT2 (ACTIN 2); structural constituent of cytoskeleton ;;TATA-binding protein binding
pap-miR34	AT2G15130.1; AT4G16480.1	GCAACGGACUUCACGUGGCG; ACACCUUACAUCAUGCGACU	plant basic secretory protein (BSP) family protein;;ATINT4 (INOSITOL TRANSPORTER 4); carbohydrate transporter/ myo-inositol:hydrogen symporter/ sugar porter
pap-miR35	AT2G34430.1; AT3G04260.1	CAUCUUGGAAAUGUGAUGCA; ACCUUGGAGAACUUGAUGCA	LHB1B1 (Photosystem II light harvesting complex gene 1.4); chlorophyll binding;;PTAC3 (PLASTID TRANSCRIPTIONALLY ACTIVE3); DNA binding
pap-miR36	AT5G65700.1; AT1G61140.1	UGCCACCGUCGAAGGAUCAG; ACCCAACAACCGAGGAUCAA	BAM1 (big apical meristem 1); ATP binding / kinase/ protein serine/threonine kinase ;;EDA16 (embryo sac development arrest 16); ATP binding / DNA binding / helicase/ protein binding / zinc ion binding
pap-miR37	AT4G24390.1; AT3G09710.1	GGCGGAUUUGAGCUUAGCUC; GAUGGUUUAAAGUUCAGUUU	F-box family protein (FBX14) ;;IQD1 (IQ-DOMAIN 1); calmodulin binding
pap-miR38	AT1G10130.1; AT1G23935.1	UUAUUUUCGGGAACUGAUGUG ; CCCCUAUAAGGUCUGAUGCG	ECA3 (ENDOPLASMIC RETICULUM-TYPE CALCIUM-TRANSPORTING ATPASE 3); calcium-transporting ATPase/ calmodulin binding;;apoptosis inhibitory protein 5 (API5)-related
pap-miR39	AT2G47860.1; AT3G28380.1	GAUAGUUAUCUCGCUGAGGUG GCAAGUGAAUUGGUUGAGGUC	phototropic-responsive NPH3 family protein;;PGP17 (P-GLYCOPROTEIN 17); ATP binding / ATPase/ ATPase, coupled to transmembrane movement of substances / nucleoside-triphosphatase/ nucleotide binding
pap-miR40	AT1G77090.1; AT2G28170.1	GGGAGAGUUCCUGGUCUGUC; AAAGAGCGUCUUGCUCUGUU	thylakoid lumenal 29.8 kDa protein;;ATCHX7 (cation/hydrogen exchanger 7); monovalent cation:proton antiporter
pap-miR41	AT1G69250.1; AT2G29890.1	GCAAGACAAAUGGCUCCGCA; ACCAGACAAAGCUAUUCACC	nuclear transport factor 2 (NTF2) family protein / RNA recognition motif (RRM)-containing protein;;VLN1 (VILLIN 1); actin binding
pap-miR42	AT5G24590.2	GCUUUUCAGAGUCGGUGUGG	TIP (TCV-INTERACTING PROTEIN); transcription factor
pap-miR43	AT1G08135.1	UAUUUUCCUUCUAGAGCCUC	ATCHX6B/CHX6B (CATION/H+ EXCHANGER 6B); monovalent cation:proton antiporter
pap-miR44	AT3G58810.1; AT4G22010.1	GUCUUGCGUCCUUAUUUCUC; GCCGUACGAUCAUACUCGCC	MTPA2; efflux permease/ zinc ion transporter;;SKS4 (SKU5 Similar 4); copper ion binding / oxidoreductase ;;protease inhibitor/seed storage/lipid transfer protein (LTP) family protein
pap-miR45	AT5G62320.1; AT4G03430.1	GUUUAAUUAGGCCGUAGGUU; GUCAAAUUAUGUAGCGGGUU	MYB99 (myb domain protein 99); DNA binding / transcription factor;;STA1 (STABILIZED1); RNA splicing factor, transesterification mechanism
pap-miR46	AT5G07390.1; AT2G13650.2	GGGAGGAGUAACUCGGUUUU; GGGCUUUGUUAUCCGGUUUA	ATRBOHA (RESPIRATORY BURST OXIDASE HOMOLOG A); FAD binding / calcium ion binding / iron ion binding / oxidoreductase ;;GONST1 (GOLGI NUCLEOTIDE SUGAR TRANSPORTER 1)
pap-miR47	AT1G70300.1; AT1G70300.1	GAUUGGGACUUGUAUGGUUA; GAUUGGGACUUGUAUGGUUA	KUP6 (K+ uptake permease 6); potassium ion transporter;;KUP6 (K+ uptake permease 6); potassium ion transporter
pap-miR48	AT5G04770.1; AT4G21280.2	ACAUUCGGUGAAUUUCCAGC; CCUUCCGGUGGCCUUCCAGC	ATCAT6/CAT6 (CATIONIC AMINO ACID TRANSPORTER 6); amino acid transporter/ cationic amino acid transporter;;PSBQ/PSBQ-1/PSBQA; calcium ion binding
pap-miR49	AT3G07300.2; AT2G25940.1	CUGGAGGUGGACAGGACAUC; CCGGGAGUGCUCGGGAUGCC	GTP binding / translation initiation factor ;;ALPHA-VPE (ALPHA-VACUOLAR PROCESSING ENZYME); cysteine-type endopeptidase
pap-miR50	AT4G21390.1; AT5G03790.1	UGGGAGAUCAGAGGAGCUUGU ; UGGAAGGCGAAGCAGCUUGA	B120; protein kinase/ sugar binding;;ATHB51/LMI1 (LATE MERISTEM IDENTITY1); DNA binding / transcription factor

pap-miR51	AT2G29840.1; AT2G33835.1	UCAGCCUCAAGAAGUUCUAU; CCAGUUUCAUCAGGUGCCAA	protein binding / zinc ion binding;;FES1 (FRIGIDA-ESSENTIAL 1); nucleic acid binding
pap-miR52	AT5G20700.1; AT3G49950.1	CCGGCCAAAUUUUCUCCACC; CUCAACAACAUCGCUCCACC	senescence-associated protein-related;;scarecrow transcription factor family protein
pap-miR53	AT2G14720.1	GUACAAUCAACAAUGGAGGU	VSR-2 (Vacuolar sorting receptor 2); calcium ion binding / peptidase
pap-miR54	AT1G25540.1	UGUAUGGGUCUGACCAUUUA	PFT1 (PHYTOCHROME AND FLOWERING TIME 1)
pap-miR55	AT2G13370.1	UGUCACUAGCAAAAGUGUUG	CHR5 (chromatin remodeling 5); ATP binding / DNA binding / chromatin binding / helicase
pap-miR56	AT1G19950.1	GGAGCACCGCGCUAAGAGAA	abscisic acid-responsive HVA22 family protein
pap-miR57	AT1G26880.2	UCCGACAGGAUCAUUCGGGC	60S ribosomal protein L34 (RPL34A)
pap-miR58	AT1G65660.1; AT1G79820.1	GUUAGAACAACUGGUGGUGG; CUUACACCAUUAUGCGAGGU	SMP1 (swellmap 1); nucleic acid binding ;;SGB1; carbohydrate transporter/ sugar porter
pap-miR59	AT1G20260.1; AT3G17690.1	GACGAUAUCACUCACCCUAC; AGUGGUCCUAUUCACUCUAC	(VACUOLAR ATP SYNTHASE SUBUNIT B3); ATP binding / hydrogen ion transporting ATP synthase, rotational mechanism / hydrogen ion transporting ATPase, rotational mechanism;;ATCNGC19 (cyclic nucleotide gated channel 19); calmodulin binding / cyclic nucleotide binding / ion channel
pap-miR60	AT2G18790.1; AT5G60450.1	UGGAUGCGAUUCACUCGCUC; UUGAUGGGUUUUACUUGUUU	PHYB (PHYTOCHROME B); G-protein coupled photoreceptor/ signal transducer;;ARF4 (AUXIN RESPONSE FACTOR 4); transcription factor
pap-miR61	AT4G35610.1	AAACCGAUGACGAAGGGGAU	zinc finger (C2H2 type) family protein
pap-miR62	AT2G24180.1	CGUAUUCAUGUCAAUGCUUG	CYP71B6 (CYTOCHROME P450 71B6); oxygen binding
pap-miR63	AT3G20660.1	CGAGUUCUUGGGAAUGGACG	carbohydrate transporter/ sugar porter
pap-miR64	AT3G22600.1	CUUCAAACACAACAUCUUCA	protease inhibitor/seed storage/lipid transfer protein (LTP) family protein
pap-miR65	AT1G14530.2; AT3G46790.1	GAAACAGCUGAAAAAGGGGU; GAAUCAUCUGAUGAAAGGGA	(TOM THREE HOMOLOG); virion binding;;CRR2 (CHLORORESPIRATORY REDUCTION 2); binding
pap-miR66	AT1G63020.1; AT4G02080.1	ACGUUCCUCAGUCCAUUCAA; ACGUUUCGUAAUUUAUUCAA	NRPD1a (nuclear RNA polymerase D 1a); DNA binding / DNA-directed RNA polymerase ;;ASAR1 (Arabidopsis thaliana secretion-associated RAS super family 2); GTP binding
pap-miR67	AT2G36010.1; AT2G29130.1	GGAGUGGUGCAGCACCACCA; GGAAUCACGCGGCACUACCA	E2F3 (E2F TRANSCRIPTION FACTOR-3); transcription factor;;LAC2 (laccase 2); copper ion binding / oxidoreductase
pap-miR68	AT1G79840.1	UCGUGCAGCUCUUGGACGCA	GL2 (GLABRA 2); DNA binding / transcription factor
pap-miR69	AT1G19690.1; AT2G22690.2	AUCAGACUGGGUGGUUUCAG ; UUUGAAAUGGGUAGCUUCAA	catalytic/ coenzyme binding ;;protein binding / zinc ion binding
pap-miR70	AT2G31450.2; AT3G25750.1	UACAAAAAGACUAUUGGAUC; CGAAAAGAUACCAUUAGAUU	DNA binding / endonuclease/ iron ion binding;;F-box family protein
pap-miR71	AT5G02240.1; AT2G42240.1	CGAGGAGGCCAAGAACAAAG; CUAAGCGUUCAAGAACAGAU	catalytic/ coenzyme binding ;;nucleic acid binding
pap-miR72	AT1G72660.2	AGCGCUAGGCACUACCCUCA	GTP binding
pap-miR73	AT1G06220.2; AT1G77850.1	CCCCUCCCGGUUCCUGGGUU; UUUUAUCCGGCUGCUGGAUG	MEE5 (maternal effect embryo arrest 5); translation elongation factor/ translation factor, nucleic acid binding;;ARF17 (AUXIN RESPONSE FACTOR 17); transcription factor
pap-miR74	AT4G39990.1	UCCCAGGUUCUGGACAGGAG	ATGB3 (GTP-BINDING PROTEIN 3); GTP binding
pap-miR75	AT5G02740.1	UCCUGCGGCAGUCUUUGUGC	Nucleotide binding
pap-miR76	AT2G34680.1	CCGGAAUCGUCGCCGGGAUU	AIR9 (Auxin-Induced in Root cultures 9); protein binding
pap-miR77	AT1G53500.1	CUCAGAAUCCUGAGUGGUGG	MUM4 (MUCILAGE-MODIFIED 4); catalytic
pap-miR78	AT1G28560.1	CAGCGAGGGAGAGAGCAGGG	SRD2 (SHOOT REDIFFERENTIATION DEFECTIVE 2); DNA binding
pap-miR79	AT2G23740.1	AUGACAUUGCAAAAUGGUUC	nucleic acid binding / transcription factor/ zinc ion binding
pap-miR80	AT5G49030.1	UUGCUAAUCCACCUGAGCCU	OVA2 (OVULE ABORTION 2); ATP binding / aminoacyl-tRNA ligase

pap-miR81	AT2G47310.1	CCAGUAACAACACUAGCUGU	flowering time control protein-related / FCA gamma-related
pap-miR82	AT2G46440.1	CGUCGCUGGUGUCAGAGAGA	TCNGC11 (cyclic nucleotide gated channel 11); calmodulin binding / cyclic nucleotide binding / ion channel
pap-miR83	AT4G16250.1	UGCAGUUAGCCUUGCAGGUG	PHYD (PHYTOCHROME DEFECTIVE D); G-protein coupled photoreceptor/ signal transducer
pap-miR84	AT1G18840.1	UUCAUCGCCAAACGUGCUGC	IQD30; calmodulin binding
pap-miR85	AT5G45160.1	GGGGGACUUGCUGGUGAUAG	root hair defective 3 GTP-binding (RHD3) family protein

Table 4. List of targets predicted by miRU2 for miRNAs obtained from miRseeK

Micro ID	Target Acc.	Target Sequence	Target Description
pap-1	AT3G60630.1	GAUAUUGGCGCGGCUCAAUCA	scarecrow transcription factor family protein
pap-2	AT1G06950.1	GAAGAAGAUGAUGAAGAUGA	ATTIC110/TIC110 (TRANSLOCON AT THE INNER ENVELOPE MEMBRANE OF CHLOROPLASTS 110)
pap-3	AT4G28660.1	GGUUCUUUAACUUCUGUCAA	photosystem II reaction centre W (PsbW) family protein
pap-4	AT3G26190.1	GAGCUUGUGCUUGAGUCUGA	CYP71B21 (cytochrome P450, family 71, subfamily B, polypeptide 21); oxygen binding
pap-5	AT1G20840.1	AUCUCGCCGUGGCUCGAUCA	TMT1 (TONOPLAST MONOSACCHARIDE TRANSPORTER1); carbohydrate transporter/ nucleoside transporter/ sugar porter
pap-6	AT3G51770.2	UUUAUUUAUUUUUUUCAUCA	ETO1 (ETHYLENE OVERPRODUCER 1)
pap-7	AT2G22840.1	CGUUCAAGAAAGCCUGUGGA	AtGRF1 (GROWTH-REGULATING FACTOR 1)
pap-8	AT2G34710.1	GGGAUGAAGCCUGGUCCGGA	PHB (PHABULOSA); DNA binding / transcription factor
pap-9	AT4G28660.1	GGUUCUUUAACUUCUGUCAA	photosystem II reaction centre W (PsbW) family protein
pap-10	AT3G56100.1	AUUUCCUUCAAACGCUUGAU	MRLK (MERISTEMATIC RECEPTOR-LIKE KINASE); ATP binding / protein serine/threonine kinase
pap-11	AT3G15270.1	CCGCUCUCUCUCUUCUGUCA	SPL5 (SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 5); DNA binding / transcription factor
pap-12	AT1G09100.1	AUGUUGGAGCUGCUCAACCA	RPT5B (26S PROTEASOME AAA-ATPASE SUBUNIT RPT5B); ATPase/ calmodulin binding
pap-13	AT1G30220.1	UGGCUCAAUCUUUUUUGUCA	ATINT2 (INOSITOL TRANSPORTER 2); carbohydrate transporter/ sugar porter