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Mining of miRNAs from EST data in *Dendrobium nobile*

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Abstract:

Dendrobium nobile is an orchid species highly popular for its therapeutic properties and is often used as a medicinal herb. Documenting miRNA-target associations in *D. nobile* is an important step to facilitate functional genomics studies in this species. Therefore, it is of interest to identify miRNA sequences from EST data available in public databases using known techniques and tools. We report 14 potential miRNAs from three ESTs of *D. nobile*. They belong to 3 miRNA families (miR390, miR528 and miR14) linking to transcription factor regulation, signal transduction, DNA and protein binding, and various cellular processes covering 34 different metabolic networks in KEGG. These results help in the understanding of miRNA-mRNAs functional networks in *Dendrobium nobile*.

Keywords: Expressed Sequence Tags, miRNA, in silico, Dendrobium nobile

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Background:

Dendrobium nobile is ornamentally and medicinally one of the most important species of flowering plants. It belongs to the Orchidaceae family, which is one of the largest families of the angiosperms and has been used as a first-rate herb in India and China since ancient times [1]. The pattern of flowering of the violet coloured flowers of D. nobile make them more fascinating and attractive [2]. The presence of various active compounds likes Dendrobine, Moscatilin, Gigantol, Nobiline and Dendrophenol in the stems and leaves of D. nobile are known to be responsible for the greatly increased medicinal property of this plant [3, 4]. These compounds are known to have strong antimutagenic properties and are anti-carcinogenic against lung carcinoma, ovary adenocarcinoma and promyelocytic leukemia [5]. Moreover genetic diversity studies indicate that *D. nobile* from Northeast India has a comparatively higher rate of genetic diversity [6, 7]. The orchid, being prized for its immense commercial importance, is often subjected to unrestrained anthropogenic pressures, thereby threatening its natural population [8]. In addition to its health benefits and economic value, D. nobile is also a wonderful source of experimental material to expound gene expression and regulation because of its versatile characteristics; the availability of decent numbers of expressed sequence tags of this species also augmented this study.

MicroRNAs are a class of endogenous small, non-coding, single stranded RNAs that act as post-transcriptional regulators in eukaryotic organisms [9]. Each miRNA is capable of regulating the expression of many genes - either by translational repression or mRNA cleavage- allowing them to simultaneously regulate multiple cellular signalling and biosynthetic pathways [10]. Plant microRNAs play important roles in plant growth and development including leaf morphology and polarity, organ development, cell differentiation and proliferation, programmed cell death, signal transduction, stress responses, hormone signalling, floral organ identity and maturity, phase transition and reproduction [11-13]. For miRNAs to be reliably distinguishable from other RNAs, Ambros et al. (2003) developed a set of criteria for miRNA identification and annotation and their guidelines for experimental verification [14]. However, those criteria for miRNA annotation have been revised by Axtell and Meyers (2018), which has been followed in this study [15]. The first miRNA to be discovered was lin-4, predicted to be of 22 nucleotides in length and found in the larval form of Caenorhabditis elegans [16]. It is responsible for regulation of the pathway that triggers the transitions of first larval stage cell division to the second [17]. In plants, RNA polymerase II is responsible to transcribe majority of primary miRNA transcripts (pri-miRNAs) from miRNA genes. Processing of pri-miRNAs to precursor miRNAs and then further to mature miRNA-miRNA* duplex is brought about by the DCL1 (Dicer-like 1) enzyme [18]. The duplex is methylated by HUA ENHANCER 1 (HEN1) and transported to the cytoplasm by HASTY, after which the guide miRNA strand is then incorporated into ARGONAUTE (AGO) protein [19]. Once a suitable pairing event between a miRNA and

target mRNA occurs, the RISC (RNA-induced silencing complex) then triggers almost complete inhibition of protein expression by either cleavage of mRNA targets or by inhibiting protein translation **[20]**. The repressional activity of miRNA is mainly based on the property of regulation of gene expression at the post-transcriptional level either by cleavage mediated mRNA degradation or inhibition of translation **[21]**. Discovery of genetic modulators in various plants has helped to comprehend their specific regulatory modules involved in complex biological processes. Understanding the biological functions of miRNAs, identification of miRNAs and their target genes is an important step in interpreting the roles of miRNAs in regulation of specific characters. Documentation of miRNAs and their targets have been very effective in a number of plants such as *Arabidopsis*, rice, maize, wheat, soybean, cotton and tea **[22, 23]**.



Figure 1: Computational pipeline for identification of putative miRNAs of *D. nobile* and their target genes

Methods:

Reference set of miRNAs and Sequence data:

A total of 38,589 previously identified mature micro-RNAs from different plants were retrieved from the miRBase database (http://www.mirbase.org/) (release 22.1). These sequences were defined as the query sequence set and used for identifying miRNAs in D. nobile Expressed Sequence Tags (ESTs). Publicly available 15,383 ESTs of the species were downloaded from National for Biotechnology Centre Information (NCBI)(https://www.ncbi.nlm.nih.gov/). Local database for BLAST was constructed for D. nobile ESTs by using the locally installed NCBI-Blast+ application (ftp://ftp.ncbi.nlm.nih.gov /blast/ executables/blast+/). Non-redundant protein sequences were used from the NR protein database of NCBI (ftp://ftp.ncbi.nlm.nih.gov/blast/db/).

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Identification of putative miRNAs:

Sequence and structural homologies are used for computer based predictions of miRNAs. Computational strategies provide less time consuming, valuable and efficient means for prediction and identification of miRNA genes and their targets (Figure 1). NCBI-BLAST+ program was used to screen the ESTs against the reference miRNAs obtained from miRBase by searching for homologous hits [24]. A maximum of two mismatches, threshold e-value of <0.001 and word-size value of 7 was set for the blast+ analysis. After removing redundancy the ESTs with matched hits were subjected to Blastx analysis with NR protein database, and the non-protein coding sequences were retained for further analysis of RNA secondary structure using Zuker folding algorithm by Mfoldv3.5 (http://unafold.rna.albany.edu/ ?q=mfold/ RNA-Folding-Form) [25]. The following parameters were used in defining the sequences as miRNA homologs: (1) The sequence should fold into an appropriate stem-loop secondary structure. (2) The miRNA should be present in one arm of the hairpin structure. (3) The mature miRNA and its complementary miRNA* sequence should not have more than 5 mismatches. (4) The value of Minimal Folding free Energy Index (MFEI) of precursor miRNA structures should not be less than 0.5 and should have a high Minimal Folding free Energy (MFE) value. MFE is the negative equivalent of the ΔG value [26]. The MFEI value has been calculated by using the following formula proposed by Zhang et al. [27].

AMFE = (MFE × 100)/Length of precursor MFEI = AMFE / (GC) % MFEI = [(MFE/length of the RNA sequence) × 100]/(GC)%

Prediction of putative target genes:

A plant small RNA Target Analysis Server viz. psRNATarget was used for predicting the targets of the newly identified miRNA by using Schema V2 (2017 Release) with the maximum expectation value threshold as 3 and rest of the values set as default **[28].** A maximum of two mismatches were allowed in the complementary region of target genes with the miRNAs, whereas mismatch inhibition was maintained at 10th and 11th nucleotide position along the aligned region. Target genes were identified against *Arabidopsis thaliana* transcript, TAIR V10 as genome or

Table 1: Identified	putative	miRNAs	of D.	nobile from	ESTs
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Accession no.	miRNA	Mature miRNA sequence	PL*	(C+G)%	MFE	AMFE	MFEI
HO190899.1	zma-miR528a-5p	TGGAAGGGGCATGCAGAGGAG	79	51.3	35.5	46.71	0.91
	zma-miR528b-5p	TGGAAGGGGCATGCAGAGGAG	79	51.3	35.5	46.71	0.91
HO191179.1	>cca-miR390	AAGCTCAGGAGGGATAGCG	107	43	42.55	39.76	0.92
	>lus-miR390a	AAGCTCAGGAGGGATAGCGCC	106	43.4	42.95	40.51	0.93
	>lus-miR390b	AAGCTCAGGAGGGATAGCGCC	106	43.4	42.95	40.51	0.93
	>csi-miR390b-5p	AGCTCAGGAGGGATAGCGCC	105	43.81	41.65	39.66	0.9
	>lus-miR390c	AAGCTCAGGAGGGATAGCGCC	106	43.4	42.95	40.51	0.93
	>ppt-miR390c-5p	AGCTCAGGAGGGATAGCGCC	105	43.81	41.65	39.66	0.9
	>lus-miR390d	AAGCTCAGGAGGGATAGCGCC	106	43.4	42.95	40.51	0.93
	>gma-miR390e	AGCTCAGGAGGGATAGCGCC	105	43.81	41.65	39.66	0.9
	>gma-miR390f	AAGCTCAGGAGGGATAGCGCC	106	43.4	42.95	40.51	0.93
	>gma-miR390g	AAGCTCAGGAGGGATAGCGCC	106	43.4	42.95	40.51	0.93
	>atr-miR390.1	TAAAGCTCAGGAGGGATAGCG	111	41.44	45.25	40.76	0.98
HO194934.1	>ath-miR414	GACGATGATGATGAAGATGA	169	47.93	47.8	28.28	0.59

*Precursor Length

transcriptome sequences of *D. nobile* are not available in public domain.

Gene Ontology, KEGG pathway and Phylogenetic analysis:

Annotations of the target genes were carried out using a Blastx analysis with an e-value of 10-3 against the NCBI non-redundant protein database. Blast2go version 5.2 (https://www.blast2go.com/blast2go-pro/) was used for the gene ontology and KEGG (Kyoto Encyclopaedia of Genes and Genomes) pathway analysis of the annotated target genes in order to assess the phenotypic traits which may get affected by expression of the identified miRNAs of D. nobile [29]. The phylogenetic trees were constructed using MEGA7 - a Windows OS based software. The precursor sequences of family members of the identified miRNAs, belonging to other plant species were downloaded from miRBase and collated with the D.nobile miRNA precursors. Multiple sequence alignment was carried out using MUSCLE algorithm and phylogenetic trees developed using the Maximum likelihood approach.



Figure 2: Secondary hairpin structure of precursor sequences of three identified miRNA families.



Results:

miRNA identification and characterization:

From a total of 15,383 published ESTs of *D. nobile*, 306 of them showed homology with previously deposited miRNAs in miRBase 22.1. Following the criteria given by Axtell and Meyers (2018) for plant miRNA annotation, these were further filtered to retain only the miRNAs \geq 19 nucleotides in length **[15]**. As a result only 249 miRNAs were taken from which further removal of redundancies in miRNAs and ESTs yielded 247 potential miRNA sequences. Blastx analysis of these ESTs against the NCBI non-redundant database resulted in identification of 89 sequences as non-coding sequences.

miRNA secondary structure:

The potential miRNAs were subjected to structural validation analysis in Mfold v3.5 for prediction of miRNA secondary structure. The miRNAs which showed valid stem-loop hairpin precursor, presence of complementary miRNA* sequence in the precursor with less than 6 mismatches, and an MFEI value greater than 0.5 were considered for further analysis of their target genes. Fourteen such conserved miRNAs were identified belonging to three miRNA families (Figure 2). miR528 is represented by two members, miR390 represented by 11 members and miR414 by one member. The ΔG values ranged from -47.8 to -35.5 kcal/mol. It is often considered that, lower the value of ΔG , higher is the thermodynamic stability of the miRNA precursor [30]. A lower value of ΔG corresponds to a higher MFEI value as MFE is equivalent to (- Δ G). miRNA characterization indicates that the precursor length of miRNAs varied between 79-169 bases and the mature miRNA length ranged from 19 to 21 nucleotides (Table 1).

Target gene prediction and annotation:

It has been demonstrated in several studies that most plant miRNAs bind to their target mRNA sequences with perfect or nearperfect sequence complementarity **[31, 32]**. This provides an effective approach for discovering probable miRNA targets by comparing and aligning miRNAs with mRNA sequences. In order to identify genes plausibly recognised by the potential miRNAs, psRNA Target - a web-based server was used for searching target genes against *A. thaliana* transcriptome acquired from TAIR10. A total of 138 genes were identified as target genes of 14 identified miRNAs, where 4 genes having unknown functions were discarded. Out of the 134 retained targets, only 3 genes exhibit translational repression by corresponding miRNAs whereas all the rest of the genes show cleavage mode of regulation (**Table 2**).



Figure 3: GO reports of the identified target genes showing percentage of sequences representing each class in three different categories viz. Biological processes, Cellular component and Molecular Function



Table 2: Predicted target genes of *D. nobile* miRNAs.

miRNIA Acc	Target Acc	Export	Description	Inhibition
dao miR200	AT2C1718E 1	2	Description	Cleanage
dno-miR390	AT5G17105.1	3	predicted protein	Translation
dno-miK390	AT3G03640.1	3	server the owner protein kinase	Classication
dno-miK390.1	ATIG05500.1	3	synaptotagmin-5	Cleavage
dno-miR390.1	ATIG/8930.1	3	Deta-aniyrinisynnase	Cleavage
dno-miR390.1	AT2G41000.3	3	wheeholdering give protein failing	Cleavage
dno-mik390.1	A14G12980.1	3	cytochrome bob1 and DOMON domain-containing protein At4g12980	Cleavage
dno-mik390.1	AT5G11700.1	3	epirin type-b receptor	Cleavage
dno-miR390.1	A15G39862.1	3	putative non-LTR retroelement reverse transcriptase	Cleavage
dno-miR390b	A15G48480.1	3	Lactoyigiutathionelyase / giyoxalase I family protein	Cleavage
dno-miR390b-5p	A13G52890.2	3	serine/threonine-protein kinase	Cleavage
dno-miR390c-5p	ATIG4/890.1	3	receptor-like protein 12	Cleavage
dno-miR390e	A15G05570.1	2.5	transducin family protein / WD-40 repeat family protein	Cleavage
dno-miR390e	A15G05570.2	2.5	transducin family protein / WD-40 repeat family protein	Cleavage
dno-miR390e	AT3G11050.1	3	putative ferritin subunit precursor	Cleavage
dno-miR390f	A14G32820.1	3	Tetratricopeptide repeat (TPR)-like superfamily protein	Cleavage
dno-miR390f	AT4G32820.2	3	Tetratricopeptide repeat (TPR)-like superfamily protein	Cleavage
dno-miR414	AT1G74890.1	0.5	two-component response regulator ARR15-like	Cleavage
dno-miR414	AT1G80960.2	1	F-box and Leucine Rich Repeat domains containing protein	Cleavage
dno-miR414	A13G59220.1	1	PRN1_ARATHRecName: Full=Pirin-1; AltName: Full=AtPirin1	Cleavage
dno-miR414	AT5G20370.1	1	serine-rich protein-like protein	Cleavage
dno-miR414	AT5G23240.1	1	DNAJ heat shock N-terminal domain-containing protein	Cleavage
dno-miR414	AT5G61510.1	1	GroES-like zinc-binding alcohol dehydrogenase family protein	Cleavage
dno-miR414	AT1G05490.1	1.5	SNF2 domain-containing protein CLASSY 3-like	Cleavage
dno-miR414	AT1G45160.1	1.5	Protein kinasesuperfamily protein	Cleavage
dno-miR414	AT1G48970.1	1.5	translation initiation factor eIF-2B subunit delta	Cleavage
dno-miR414	AT1G53770.2	1.5	O-fucosyltransferase family protein	Cleavage
dno-miR414	AT1G78270.1	1.5	UDP-glycosyltransferase 85A4	Cleavage
dno-miR414	AT2G15345.1	1.5	Plant invertase/pectin methylesterase inhibitor superfamily protein	Cleavage
dno-miR414	AT2G17525.1	1.5	pentatricopeptide repeat-containing protein At2g17525, mitochondrial	Cleavage
dno-miR414	AT2G22000.1	1.5	elicitor peptide 6 precursor	Cleavage
dno-miR414	AT2G30790.1	1.5	oxygen-evolving enhancer protein 2-1, chloroplastic	Cleavage
dno-miR414	AT2G35960.1	1.5	NDR1/HIN1-like protein 12	Cleavage
dno-miR414	AT2G35970.1	1.5	NDR1/HIN1-like protein 12	Cleavage
dno-miR414	AT2G36460.2	1.5	Aldolasesuperfamily protein	Cleavage
dno-miR414	AT3G13730.1	1.5	3-epi-6-deoxocathasterone 23-monooxygenase	Cleavage
dno-miR414	AT3G17100.1	1.5	transcription factor bHLH147-like	Cleavage
dno-miR414	AT3G27640.1	1.5	denticless protein homolog	Cleavage
dno-miR414	AT3G43590.1	1.5	protein AIR1	Cleavage
dno-miR414	AT4G16790.1	1.5	glycoprotein homolog	Cleavage
dno-miR414	AT1G22850.1	2	SNARE associated Golgi protein family	Cleavage
dno-miR414	AT1G26780.2	2	transcription factor MVB177	Cleavage
dno-miR414	AT1G75180.2	2	Erythronate-4-phosphate dehydrogenase family protein	Cleavage
dno-miR414	AT2G36320.1	2	zinc finger A20 and AN1 domain-containing stress-associated protein 6-like	Cleavage
dno-miR414	AT3G13930.1	2	dihydrolipovllysine-residue acetyltransferase component 2 of pyruvatedebydrogenase complex	Cleavage
dno-miR414	AT3G21380.1	2	iaralin-related lectin 36	Cleavage
dno-miR414	AT4G323001	2	G-type lectin S-recentor-like serine/threonine-protein kinase SD2-5	Cleavage
dno-miR414	AT4G37630.1	2	cyclin d5	Cleavage
dno-miR414	AT4G39410.1	2	probable WRKY transcription factor 13	Cleavage
dno-miR414	AT5G11720.1	2	alpha-durosidase	Cleavage
dno-miR414	AT1C05310.1	25	probable pectinesterase 8	Cleavage
dno miP414	AT1C12420.1	2.5	Ploane permiser nucleoside tripheenbatebudreleseesuperfamily protein	Cleavage
dno-miR414	AT1C1/020.1	2.5	DELLA protein GAL	Cleavage
dno-miR414	AT1C15710.1	2.5	Arogenatedebydrogenase 2. chloroplastic	Cleavage
dno-miP414	AT1C21226 1	2.5	Nuclear epockle RNA-hinding protain R	Cleavage
dno-miR414	AT1C263001	2.5	harbarina bridge anzuma lika /	Cleavage
dno-miR414	AT1G20390.1	2.0	agamous-like MADS-box protein ACI 29	Cleavage
dno miR414	AT1C44920.1	2.0	agamous-met where-bux protein AGL27	Cleavage
dno-miK414	ATIG44030.1	2.5	entylene-responsive transcription factor EKr014	Cleavage
dno-miR414	ATIC51640.1	2.5	exocyst complex component EAO/OA1	Cleavage
dno-m1K414	ATIG52160.1	2.5	tkivase Z. 1 KZS, mitocnonariai	Cleavage
dno-miK414	ATIG54160.1	2.5	nuclear transcription factor 1 subtint A-5	Cleavage
dno-m1K414	ATIG60940.1	2.5	serine/inreonine-protein kinase SKKZA	Cleavage
dno miR414	AT1G00090.1	∠.⊃ 2 ⊑	transition of the second	Cleavage
dno miR414	AT1G08/20.1	∠.⊃ 2 ⊑	transariation factor TCD15 like	Cleavage
dno miR414	AT1G09090.1	∠.⊃ 2 ⊑	uanscription factor fCr 10-fike	Cleavage
dno-miK414	ATIG/1220.2	2.5	UDF-glucose:glycoproteinglucosyltransferase	Cleavage
dno-miK414	A12G01530.1	2.5	MLP-like protein 328	Cleavage
ano-miK414	A12G04620.1	2.5	zinc transporter-like protein	Cleavage
ano-miK414	A12G06850.1	2.5	xyloglucanendotransglucosylase/hydrolase	Cleavage
ano-miR414	A12G21530.1	2.5	SMAD/FHA domain-containing protein	Cleavage
dno-miK414	A12G23530.1	2.5	cell division cycle-associated protein 7	Cleavage
ano-miR414	A12G25110.1	2.5	stromal cell-derived factor 2-like protein	Cleavage
dno-miR414	A12G28610.1	2.5	WUSCHEL-related homeobox 3	Cleavage
dno-miR414	AT2G32310.1	2.5	CCT motif family protein	Cleavage
dno-miR414	AT2G35110.2	2.5	protein NAP1 isoform X1	Cleavage

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dno-miRi44 AT3C02150.1 25 probable calcium-binding protein CML40 Cleavage dno-miRi44 AT3C02150.2 25 transcription factor TCP13 Cleavage dno-miRi44 AT3C022151.2 25 transcription factor TCP13 Cleavage dno-miRi44 AT3C22711.2 25 transcription factor TCP13 Cleavage dno-miRi44 AT3C22711.2 25 transcription factor TCP13 Cleavage dno-miRi44 AT3C42901.2 25 reposed Cleavage dno-miRi44 AT3C42901.2 25 reposed Cleavage dno-miRi44 AT3C64901.2 25 reposed phote pathyles to a constraint opticin (RC21) Cleavage dno-miRi44 AT4C18001.2 25 reposed phote pathyles to a constraint opticin (RC21) Cleavage dno-miRi44 AT4C18001.2 25 probable phote photelyles to a constraint opticin (RC21) Cleavage dno-miRi44 AT4C18001.2 25 probable photelyles to a constraint opticin (RC21) Cleavage dno-miRi44 AT4C18001.2 25 probable photelyles to a constraint o	dno-miR414	AT3G01770.1	2.5	transcription factor GTE9 isoform X1	Cleavage
doo-miR144 AT3C02150.1 25. inscription factor TCP13 Cleavage doo-miR144 AT3C02270.1 25. publicly Explanding CP13 Cleavage doo-miR144 AT3C0270.1 25. price (horining Profile) Cleavage doo-miR144 AT3C0470.1 25. FFAscading Profile Cleavage doo-miR144 AT3C04700.1 25. FFAscading Profile Cleavage doo-miR144 AT4C01800.1 25. FFAscading Profile Cleavage doo-miR144 AT4C01800.1 25. Profile/FrAsf07070-like Cleavage doo-miR144 AT4C01800.1 25. Profile/FrAsf07070-like Cleavage doo-miR144 AT4C01800.1 25. Profile/FrAsf07070-like Cleavage doo-miR144 AT4C01800.1 25.	dno-miR414	AT3G01830.1	2.5	probable calcium-binding protein CML40	Cleavage
doom:R144AT3C2270.125puttore F-box protein AF3g2420Cleavagedoom:R144AT3C2270.125Regulator of chromosome condensation (RCC1) family with FVVE zinc finger domain-containing proteinCleavagedoom:R144AT3C2480.125Refree dromosome condensation (RCC1) family with FVVE zinc finger domain-containing proteinCleavagedoom:R144AT3C4810.125Grossen dromosome condensation (RCC1) family with FVVE zinc finger domain-containing transcription factor AB3Cleavagedoom:R144AT3C4810.125Frobse-activating protein profeCleavagedoom:R144AT3C6400.125Frobse-activating protein OR23Cleavagedoom:R144AT3C6400.125Frobse-activating protein OR23Cleavagedoom:R144AT3C6400.125Frobse-protein A269700.16Cleavagedoom:R144AT4C3780.125Frobse-protein A269700.16Cleavagedoom:R144AT4C3780.125Frobse-protein A268Cleavagedoom:R144AT4C3780.125Inf-Fide protein A28Cleavagedoom:R144AT4C3780.125Inf-Fide protein A28Cleavagedoom:R144AT4C3780.125Inf-Fide protein A28Cleavagedoom:R144AT4C3780.125Inf-Fide protein A28Cleavagedoom:R144AT4C3780.125Inf-Fide protein A28Cleavagedoom:R144AT4C3780.125Inf-Fide protein A28Cleavagedoom:R144AT4C3780.125Inf-Fide protein A28Cleavage <td>dno-miR414</td> <td>AT3G02150.1</td> <td>2.5</td> <td>transcription factor TCP13</td> <td>Cleavage</td>	dno-miR414	AT3G02150.1	2.5	transcription factor TCP13	Cleavage
dnomiki44 ATSC2270.1 2.5 putative Fhox protein Adsg2420 Clavage dnomiki44 ATSC2240.01 2.5 B3 domain-containing transcription factor AB3 Clavage dnomiki44 ATSC450.01 2.5 B3 domain-containing transcription factor AB3 Clavage dnomiki44 ATSC450.01 2.5 GTThese-activating protein gyp7 Clavage dnomiki44 ATSC4500.11 2.5 FTAss-activating protein gyp7 Clavage dnomiki44 ATSC4500.11 2.5 FTAss-activating protein Ox23 Clavage dnomiki44 ATSC4500.11 2.5 FTAss-activating protein Ox23 Clavage dnomiki44 ATSC3700.11 2.5 FTASS-activating protein Ox23 Clavage dnomiki44 ATGC3700.11 2.5 FTASSCA70.11 Clavage Clavage dnomiki44 ATGC3700.11 2.5 Protein/Activating protein Ox23 Clavage Clavage dnomiki44 ATGC3700.12 2.5 Protein/Activating protein Ox23 Clavage Clavage dnomiki44 ATGC3700.12 2.5 Prot	dno-miR414	AT3G02150.2	2.5	transcription factor TCP13	Cleavage
ab.om.101414 ATG22320.1 2.5 Regulator of chromosome condensation (RCC1) family with FVPE zinc finger domain-containing protein Cleavage dno.m18144 ATG2460.1 2.5 Bi domain-containing transcription factor ABIS Cleavage dno.m18144 ATG2460.1 2.5 serine/threonine-protein phosphatase 6 regulatory subunit 3-like isoform X1 Cleavage dno.m18144 ATG26400.1 2.5 Frobay protein gyP7 Cleavage dno.m18144 ATG26400.1 2.5 Frobay protein gyP7 Cleavage dno.m18144 ATG26400.1 2.5 Frobay kelch-repeat protein OR23 Cleavage dno.m18144 ATG16100.1 2.5 Frobay kelch-repeat protein OR23 Cleavage dno.m18144 ATG2840.1 2.5 Probay kelch-repeat protein OR23 Cleavage dno.m18144 ATG2840.1 2.5 Probay kelch-repeat protein OR23 Cleavage dno.m18144 ATG2840.1 2.5 Prophay keyraperfamily protein Cleavage dno.m18144 ATG2840.1 2.5 Prophay keyraperfamily protein Cleavage dno.m18144 ATG2840.1	dno-miR414	AT3G22770.1	2.5	putative F-box protein At3g23420	Cleavage
ano-mR414 ATSC345901 2.5 B3 domain-containing transcription factor AB3 Cleavage dno-mR414 ATSC345901 2.5 GTRase-activating protein gyp7 Cleavage dno-mR414 ATSC345901 2.5 GTRase-activating protein gyp7 Cleavage dno-mR414 ATSC349001 2.5 F-box protein AfSg40790-like Cleavage dno-mR414 ATGC36001 2.5 F-box protein AfSg40790-like Cleavage dno-mR414 ATGC36001 2.5 F-box protein AfSg40790-like Cleavage dno-mR414 ATGC37001 2.5 r-box protein AfSg40790-like Cleavage dno-mR414 ATGC37001 2.5 r-box protein TC/2 Cleavage dno-mR414 ATGC37001 2.5 r-box protein 3726 Cleavage dno-mR414 ATGC37001 2.5 rotebalt protein 328 Cleavage dno-mR414 ATGC37001 2.5 rotebalt protein 328 Cleavage dno-mR414 ATGC37001 2.5 rotebalt protein 328 Cleavage dno-mR414 ATGC37001	dno-miR414	AT3G232701	2.5	Regulator of chromosome condensation (RCC1) family with FYVE zinc finger domain-containing protein	Cleavage
dos.mi814 ATSG45001 2.5 oreing/threating-prior properties for regulatory subunit 3-like isoform X1 Cleavage dos.mi8144 ATSG45001 2.5 GTPasa-activating profetin py7 Cleavage dos.mi8144 ATSG47001 2.5 Frobable properties py7 Cleavage dos.mi8144 ATSG47001 2.5 Frobable properties profetin py7 Cleavage dos.mi8144 ATSG17001 2.5 Frobable properties profetin py7 Cleavage dos.mi8144 ATSG17001 2.5 Frobable properties profetin py7 Cleavage dos.mi8144 ATSG17001 2.5 Frobable properties profetin py7 Cleavage dos.mi8144 ATSG178001 2.5 transcription factor TC72 Cleavage Cleavage dos.mi8144 ATG23801 2.5 pptidy1-prolyCis-trans isomerase FKB17-1, chloroplastic Cleavage Cleavage dos.mi8144 ATG23801 2.5 Plosphorylassesperfamily protein Cleavage Cleavage Cleavage dos.mi8144 ATG23801 2.5 ATC transporter B family member 2.3, mitochondrial Cleavage	dno-miR414	AT3C24650.1	2.5	B3 domain containing transcription factor ABI3	Cleavage
uborninitian A 1502020. 2.5 General protein protein protein protein able of subort A1 Clearage downinitian A 1505020. 2.5 probability prot 13 Clearage downinitian A 1505020. 2.5 probability prot 13 Clearage downinitian A 1505020. 2.5 probability prot 13 Clearage downinitian A 1505020. 2.5 probability protein 1023 Clearage downinitian A 1505020. 2.5 probability protein 1023 Clearage downinitian A 1505020. 2.5 probability protein 1023 Clearage downinitian A 1505020. 2.5 Prophory protein Clearage downinitian A 1505020. 2.5 MLP-like protein 328 Clearage downinitian A 1505020. 2.5 MLP-like protein 328 Clearage downinitian A 15050200. 2.5 MLP-like protein 328 Clearage downinitian A 15050500. 2.5 Stell association protein Clearage downinitian A 15050500.<	dno miR414	AT2C45100.1	2.5	avria (threating protein photos 6 radiulatory subunit 2 like isoform Y1	Cleavage
ubornmittiATSC200212.5optication optical by 21Closs optication optical by 21ubornmittiATSC200212.5F-kox / helds. Age0730-likeCloss optication optication OR23ubornmittiATAC1100012.5F-kox / helds. Age0730-likeCloss optication	dno miR414	AT2C40250.1	2.5	CTDea activities protain gup?	Cleavage
UncertaintsA 13502920.1 2.5 Froome product hydrae isClear agedno mikitiA 13500200.1 2.5 F-boxy footan A260780-likeClear agedno mikitiA 145010001 2.5 F-boxy footan A260780-likeClear agedno mikitiA 146018001 2.5 F-boxy footan A260780-likeClear agedno mikitiA 146018001 2.5 F-boxy footan A260780-likeClear agedno mikitiA 146018001 2.5 Franker fragment and the clear ageClear agedno mikitiA 146028001 2.5 F-boxy footan A260780-likeClear agedno mikitiA 146028001 2.5 MLP-kite fragment and the clear ageClear agedno mikitiA 146028001 2.5 MLP-kite fragment and the clear ageClear agedno mikitiA 146028001 2.5 Froot hair specific 16Clear ageClear agedno mikitiA 146028001 2.5 root hair specific 16Clear ageClear agedno mikitiA 146028001 2.5 reot hair specific 16Clear ageClear agedno mikitiA 146028001 2.5 reot hair specific 16Clear ageClear agedno mikitiA 146028001 2.5 reot hair specific 16Clear ageClear agedno mikitiA 146028001 2.5 reot hair specific 16Clear ageClear agedno mikitiA 146028001 2.5 reot hair specific 16Clear ageClear agedno mikitiA 146028001 2.5 reot hair specifi	da a miD414	AT2CE4020.1	2.5	archella materializza 12	Cleavage
uno-miki+i A1.5.00/94.1 2.5 F-box protein A1.500/94-142 Cleavage uno-miki+i A14(20100) 2.5 F-box kich-repeat protein OR23 Cleavage uno-miki+i A14(21160) 2.5 probable phospholipidily droperoxide glutathione peroxidase 6, mitochondrial Cleavage uno-miki+i A14(21870) 2.5 cellulose synthase A catalytic subuni 8 [UDP-forming] Cleavage uno-miki+i A14(21870) 2.5 cellulose synthase A catalytic subuni 8 [UDP-forming] Cleavage uno-miki+i A14(22880) 2.5 MLP-like protein 325 Cleavage uno-miki+i A14(22730) 2.5 universal stress protein P140554 Cleavage uno-miki+i A14(22780) 2.5 rot hair specific 16 Cleavage uno-miki+i A14(23910) 2.5 signal recognition particle receptor subunit alpha-like Cleavage uno-miki+i A14(23900) 2.5 bZIP transcription factor Cleavage uno-miki+i A14(23900) 2.5 bZIP transcription factor Cleavage uno-miki+i A15(20480) 2.5	dno-miK414	AT3G34920.1	2.5		Cleavage
dno-mikiii Aid.00001 2.5 i-box/kelch-repeat protein OK25 Cleavage dno-mikiii Aid.01001 2.5 probable phospholinghidydroproside glutathione peroxidase 6, mitochondrial Cleavage dno-mikiii Aid.018901 2.5 probable phospholinghidydroprosoide glutathione peroxidase 6, mitochondrial Cleavage dno-mikiii Aid.018901 2.5 peptidyl-prolylcis-trans isomerase FKBPI7-1, chloroplastic Cleavage dno-mikiii Aid.028001 2.5 MLPilke protein 328 Cleavage dno-mikiii Aid.028001 2.5 Phosphorylasesuperfamily protein Cleavage dno-mikiii Aid.028001 2.5 root hair specific 16 Cleavage dno-mikiii Aid.028001 2.5 root hair specific 16 Cleavage dno-mikiii Aid.03001 2.5 signal recognitro particle receptor subunit alpha-like Cleavage dno-mikiii Aid.03001 2.5 signal recognitro particle receptor subunit alpha-like Cleavage dno-mikiii Aid.03001 2.5 sepresed in response to phosphade starvation protein Cleavage <	dno-mik414	A13G60/90.1	2.5	F-box protein Atsg60/90-like	Cleavage
dno-miki14 Al 4(1160) 2.5 probable phospholipditydroperoside glutathione peroxidase 6, mitochondrial Cleavage dno-miki14 Al 4(1180) 2.5 transcription factor TC2 Cleavage dno-miki14 Al 4(1180) 2.5 cellulose synthase A catalytic subunit 8 [UDP-forming] Cleavage dno-miki14 Al 4(2380) 2.5 Phosphoryfases FKBPT7-1, chicroplastic Cleavage dno-miki14 Al 4(23280) 2.5 MLP-like protein 328 Cleavage dno-miki14 Al 4(22730) 2.5 MLP-like protein 328 Cleavage dno-miki14 Al 4(22780) 2.5 rot hair specific 16 Cleavage dno-miki14 Al 4(23780) 2.5 rot hair specific 16 Cleavage dno-miki14 Al 4(2390) 2.5 signal recognition particle receptor subunit alpha-like Cleavage dno-miki14 Al 4(2390) 2.5 bZP transcription factor Cleavage dno-miki14 Al 5(2390) 2.5 bZP transcription factor Cleavage dno-miki14 Al 5(2390) 2.5 bZP transcription factor	dno-miR414	A14G03030.1	2.5	F-box/ kelch-repeat protein OR23	Cleavage
dno-mikl41 AT4G1890.1 2.5 transcription factor 1CP Cleavage dno-mikl41 AT4G1898.01 2.5 petidyl-prolyticis-trans isomerase FKBPI7-1, chloroplastic Cleavage dno-mikl41 AT4G2880.1 2.5 MLP-like protein 328 Cleavage dno-mikl41 AT4G2880.1 2.5 MLP-like protein 328 Cleavage dno-mikl41 AT4G2880.1 2.5 MLP-like protein 328 Cleavage dno-mikl41 AT4G2880.1 2.5 Not transporter 8 family member 23, mitochondrial Cleavage dno-mikl41 AT4G29180.2 2.5 root hair specific 16 Cleavage dno-mikl41 AT4G29180.2 2.5 root hair specific 16 Cleavage dno-mikl41 AT4G390.1 2.5 extra-arge GTP-binding protein 2 Cleavage dno-mikl41 AT5G0330.1 2.5 extra-arge GTP-binding protein 2 Cleavage dno-mikl41 AT5G0330.1 2.5 extra-arge GTP-binding protein 3 Cleavage dno-mikl41 AT5G3630.1 2.5 phospholipiddacylgyterolacyltarsferase 1 Cleavage	dno-miR414	AT4G11600.1	2.5	probable phospholipidhydroperoxide glutathione peroxidase 6, mitochondrial	Cleavage
dno-miR414 AT4G18780.1 2.5 cellulose synthase A catalytic subunit 8 [UDP-forming] Cleavage dno-miR414 AT4G2380.1 2.5 MLP-like protein 328 Cleavage dno-miR414 AT4G2380.1 2.5 MLP-like protein 328 Cleavage dno-miR414 AT4G2370.1 2.5 miversal stress protein PHOS34 Cleavage dno-miR414 AT4G2370.1 2.5 vortexal stress protein PHOS34 Cleavage dno-miR414 AT4G2370.1 2.5 vortexal stress protein PHOS34 Cleavage dno-miR414 AT4G2390.1 2.5 vortexal stress protein 210 momber 23, mitochondrial Cleavage dno-miR414 AT4G3990.1 2.5 vortexal stress protein 210 momber 23, mitochondrial Cleavage dno-miR414 AT4G3990.1 2.5 vortexal stress protein 20 Cleavage dno-miR414 AT5G03545.1 2.5 vortexal stress protein 210 momber 23, mitochondria Cleavage dno-miR414 AT5G1360.1 2.5 vortexal stress protein 43 homolog E Cleavage dno-miR414 AT5G1360.1 2.5 vor	dno-miR414	AT4G18390.1	2.5	transcription factor TCP2	Cleavage
dno-miR414 AT4G19830.1 2.5 peptidyl-prolylcis-trans isomerase FKBPI7-1, chloroplastic Cleavage dno-miR414 AT4G2340.1 2.5 Phosphorylasesuperfamily protein Cleavage dno-miR414 AT4G2340.1 2.5 Phosphorylasesuperfamily protein Cleavage dno-miR414 AT4G2380.1 2.5 AFC transporter B family member 23, mitochondrial Cleavage dno-miR414 AT4G2380.1 2.5 roth air specific 16 Cleavage dno-miR414 AT4G3980.1 2.5 roth air specific 16 Cleavage dno-miR414 AT4G3980.1 2.5 extra-large GTT-binding protein 2 Cleavage dno-miR414 AT4G3980.1 2.5 extra-large GTT-binding protein 2 Cleavage dno-miR414 AT5G3851.1 2.5 bzlT transcription factor Cleavage dno-miR414 AT5G3651.1 2.5 syntxin-21 Cleavage dno-miR414 AT5G4380.1 2.5 syntxin-21 Cleavage dno-miR414 AT5G4780.1 2.5 syntxin-21 Cleavage dno-miR414	dno-miR414	AT4G18780.1	2.5	cellulose synthase A catalytic subunit 8 [UDP-forming]	Cleavage
dno-miR414AT4G236012.5MLP-like protein 328Cleavagedno-miR414AT4G272012.5Phosphorylasesuperfamily proteinCleavagedno-miR414AT4G272012.5universal stress protein PT0S34Cleavagedno-miR414AT4G278012.5ABC transporter 8 family member 23, mitochondrialCleavagedno-miR414AT4G29180.12.5root hair specific 16Cleavagedno-miR414AT4G300012.5signal recognition particle receptor subunit alpha-likeCleavagedno-miR414AT4G390012.5signal recognition particle receptor subunit alpha-likeCleavagedno-miR414AT4G390012.5extra-large GTP-binding protein 2Cleavagedno-miR414AT6G390012.5extra-large GTP-binding protein 2Cleavagedno-miR414AT5G0354512.5expressed in response to phosphate starvation proteinCleavagedno-miR414AT5G136012.5phospholipid.diavjkg)cerolacyltransferase 1Cleavagedno-miR414AT5G48012.5phospholipid.diavjkg)cerolacyltransferase 1Cleavagedno-miR414AT5G48012.5zin-finger homeodomain protein 3Cleavagedno-miR414AT5G427012.5zin-finger homeodomain protein 13Cleavagedno-miR414AT5G427012.5zin-finger homeodomain protein 13Cleavagedno-miR414AT5G427012.5protably inactive leucine-rich repeat receptor-like protein kinase At5g48300Cleavagedno-miR414AT5G547012.5 <td>dno-miR414</td> <td>AT4G19830.1</td> <td>2.5</td> <td>peptidyl-prolylcis-trans isomerase FKBP17-1, chloroplastic</td> <td>Cleavage</td>	dno-miR414	AT4G19830.1	2.5	peptidyl-prolylcis-trans isomerase FKBP17-1, chloroplastic	Cleavage
dnomiR414AT4C2440.12.5Phosphorylasesuperfamily proteinCleavagednomiR414AT4C28620.12.5ABC transporter B family member 23, mitochondrialCleavagednomiR414AT4C29180.22.5root hair specific 16CleavagednomiR414AT4C39180.22.5root hair specific 16CleavagednomiR414AT4C39180.22.5signal recognition particle receptor subunit alpha-likeCleavagednomiR414AT4C39180.22.5signal recognition particle receptor subunit alpha-likeCleavagednomiR414AT4C39180.12.5bZP transcription factorCleavagednomiR414AT3C03540.12.5bZP transcription factorCleavagednomiR414AT3C03540.12.5expressed in response to phosphate starvation proteinCleavagednomiR414AT3C03540.12.5sphosphotipid-dav/glycrensrase1CleavagednomiR414AT3C16480.12.5shnoebox protein BEL1 homologCleavagednomiR414AT3C4720.12.5ethylene responsive element binding factor 2 (ATERF2)CleavagednomiR414AT3C4720.12.5ethylene responsive element binding factor 2 (ATERF2)CleavagednomiR414AT3C64790.12.5ethylene responsive element binding factor 2 (ATERF2)CleavagednomiR414AT3C64790.12.5ethylene responsive element binding factor 2 (ATERF2)CleavagednomiR414AT3C64790.12.5ethylene responsive element binding factor 2 (ATERF2)CleavagednomiR414	dno-miR414	AT4G23680.1	2.5	MLP-like protein 328	Cleavage
innomikilitik AT4C27320.1 2.5 unvitexal stress protein PHOS34 Cleavage innomikilitik AT4C29180.1 2.5 ABC transporter B family member 23, mitochondrial Cleavage innomikilitik AT4C29180.1 2.5 root hair specific 16 Cleavage innomikilitik AT4C34300.1 2.5 root hair specific 16 Cleavage innomikilitik AT4C34300.1 2.5 signal recognition particle receptor subunit alpha-like Cleavage innomikilitik AT4C3300.1 2.5 extra-large GTP-binding protein 2 Cleavage innomikilitik AT5C0330.01 2.5 extra-large of phosphate stravation protein Cleavage innomikilitik AT5C0400.1 2.5 expressed in response to phosphate stravation protein Cleavage innomikilitik AT5C4400.1 2.5 syntaxin-21 Cleavage innomikilitik AT5C44030.1 2.5 stra-large phosphate stravation protein film Cleavage innomikilitik AT5C4720.1 2.5 zinc-finger homodomain protein 13 Cleavage innomikilitik AT5C4720.1	dno-miR414	AT4G24340.1	2.5	Phosphorylasesuperfamily protein	Cleavage
inno-miRi414 AT4C28620.1 2.5 ARC transporter B family member 23, mitochondrial Cleavage inno-miRi414 AT4C29180.2 2.5 root hair specific 16 Cleavage inno-miRi414 AT4C30100.2 2.5 root hair specific 16 Cleavage inno-miRi414 AT4C30300.1 2.5 signal recognition particle receptor subunit alpha-like Cleavage inno-miRi414 AT4C30300.1 2.5 centra-large GTP-binding protein 2 Cleavage inno-miRi414 AT5C03451.1 2.5 centra-sign protein 48 homolog E Cleavage inno-miRi414 AT5C03451.1 2.5 centra-sign protein 48 homolog E Cleavage inno-miRi414 AT5C0480.1 2.5 syntaxin-21 Cleavage inno-miRi414 AT5C480.1 2.5 syntaxin-21 Cleavage inno-miRi414 AT5C4410.1 2.5 syntaxin-21 Cleavage inno-miRi414 AT5C4470.1 2.5 ethylene responsive element binding factor 2 (ATERF2) Cleavage inno-miRi414 AT5C4780.1 2.5 ethylene responsive element binding factor 2 (dno-miR414	AT4G27320.1	2.5	universal stress protein PHOS34	Cleavage
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dno-miR414AT4G29180.22.5root hair specific 16Cleavagedno-miR414AT4G3600.12.5signal recognition particle receptor shunit alpha-likeCleavagedno-miR414AT4G36390.12.5bzIIP transcription factorCleavagedno-miR414AT4G34390.12.5celtariage GTP-binding protein 2Cleavagedno-miR414AT5G0345.12.5cell division control protein 48 homolog ECleavagedno-miR414AT5G0345.12.5expressed in response to phosphate starvation proteinCleavagedno-miR414AT5G1480.12.5phospholipid.diacylg/ycerolacyltransferase 1Cleavagedno-miR414AT5G4830.12.5BAG family molecular chaperone regulator 2Cleavagedno-miR414AT5G41410.12.5homeobox protein BEL1 homologCleavagedno-miR414AT5G41410.12.5ethylene responsive lement binding factor 2 (ATERF2)Cleavagedno-miR414AT5G41410.12.5probably inactive leucine-rich repeat receptor-like protein kinase At5g48380Cleavagedno-miR414AT5G4740.12.5probable LRR receptor-like serine/threnine-protein kinase At4g26540Cleavagedno-miR414AT5G4780.12.5probable LRR receptor-like serine/threnine-protein kinase At4g26540Cleavagedno-miR414AT5G4780.12.5copper transcription factor 21-likeCleavagedno-miR414AT5G4780.12.5copper transcription factor ERF118-likeCleavagedno-miR414AT5G4780.12.5copper transcription	dno-miR414	AT4G29180.1	2.5	root hair specific 16	Cleavage
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and markingand a sequence probability protection of the pro	dno-miR414	AT4G30600.1	2.5	signal recognition particle recentor subunit alpha-like	Cleavage
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GO and KEGG pathway analysis:

To further understand the regulatory functions of miRNAs, the target genes were subjected to Gene Ontology (level 2) and KEGG pathway enrichment analysis, using Blast2Go v5.2. The results suggested that *D.nobile* miRNAs were involved in regulation of 14 broadly defined biological processes and 3 basic molecular functions. The target genes were also found to be part of 9 different types of cellular components (**Figure 3**). Pathway enrichment analysis of target genes based on KEGG database demonstrated the participation of identified miRNAs in 34 different metabolism

networks (**Figure 4**). These networks are involved in various important pathways such as purine metabolism, antibiotic synthesis, caffeine metabolism, pentose phosphate pathway and TCA cycle.

Phylogenetic Analysis:

Phylogenetic analysis was carried out to understand the relationship between the identified miRNAs in *D. nobile* with the other plant species available in miRNA database for same family identification (**Figure 5**).

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Statistics of KEGG Pathway Analysis

Number of genes



Figure 4: KEGG pathway analysis reports of the target genes showing the number of genes belonging to each pathway

No miRNAs have been reported for *D. nobile* in miRBase. Maximum likelihood method was used for carrying out three different phylogenetic analyses for three identified miRNA families and their representative members. miR390 is a conserved miRNA family and its members have reported in many important species including *Arabidopsis*, *Brassica* and rice, whereas miR528a and miR528b have been reported only in *Zea mays*. miR414 have been reported only in three species in miRBase viz. *A. thaliana*, *Oryza sativa* and *Physcomitrella patens*.



Figure 5: Neighbour joining Phylogenetic trees constructed using stem-loop precursor sequences for three different groups of miRNAs i.e. miR390, miR414 and miR528a & b. Entries marked with green dots have been identified from *D. nobile* ESTs.

Discussion:

Identification and annotation of genetic modulators help in deciphering the critical roles played by such components in regulation of specific biological processes and their associated cellular properties. miRNA's are considered as one such group of regulatory molecules which inhibit gene expression by cleavage



mediated target mRNA degradation or translational repression. Before this study, no comprehensive work was done on identification of putative miRNAs from Expressed Sequence tags of D. nobile. In this research we considered all the important criteria such as the MFEI values, mismatch inhibition and sequence length which have been used for miRNA identification in other angiospermic species. The MFEI values of the 14 identified miRNAs in our work were mostly in the range of 0.5 to 1.0, among which 13 of them have MFEI values even greater than 0.9. As compared to the miRNAs identified in some other plants from EST sequences [33-36], this is a comparatively higher range of MFEI values, and a higher value of MFEI indicates greater thermodynamic stability of the secondary structure of the miRNAs, and hence lesser chance of encountering false positives. The G+C% of most of the miRNAs was found to be in the range of 41-47%, however only the members of miR528 family presented a G+C% value greater than 50. Among the predicted targets 13% genes are sequence specific transcription factors, 33% genes with various catalytic functions and 54% genes act as sequence specific DNA-binding, metal ion binding or protein binding factors. In the gene ontology analysis, the two main categories represented among the biological processes are cellular processes and metabolic processes (18% and 16% genes respectively). 22% of the target proteins have been found to be part of the nucleus, 18% proteins are present in various cell organelles and 13% proteins act as integral part of the cell membrane.

Transcription factors (TFs) are the master regulators of gene expression patterns in eukaryotes, and are responsible for facilitation of growth and development in plants [37]. dno-miR414 identified in this study has been shown to target several transcription factors including those from MYB as well as TCP family of TFs. Members of MYB DNA-binding domain superfamily protein are involved in many important biochemical and physiological processes in plants [38]. Furthermore, previous studies have also reported that miR414 can target the MYB family transcription factors in Allium cepa, Solanum tuberosum and Brachypodium distachyon [39-41]. The plant-specific TCP (TEOSINTE BRANCHED 1, CYCLOIDEA, PCF 1 and 2) transcription factor family is involved in plant development throughout its vegetative phase, i.e. from seed germination until the formation of flowers and fruits [42]. Members of a few other families of transcription factors have also been found to be probable targets of dno-miR414, such as ERF, GATA and WRKY family of transcription factors. The ERF (Ethylene responsive) transcription factors are responsible for establishment of floral meristem and tissue repair processes [43]. GATA transcription factors (binding to GATA rich sequences) are the DNA motifs that have been mostly implicated in lightdependent gene regulation in plants [44], and the WRKY family of transcription factors has a significant role in regulation of abiotic stress responses in plants [45]. Our results also show that dnomiR414 and dno-miR528a may also target several genes which encode various F-box proteins. These proteins are characterized as components of the SCF ubiquitin-ligase complexes (Skp I, Cullin, and an F-box protein), in which they bind substrates for ubiquitinmediated proteolysis **[46]**. Protein ubiquitination is considered as a critical post-translational modification process that is employed by eukaryotes in order to regulate various types of cellular processes **[47]**. Another important gene found to be targeted by dno-miR528 family is the co-chaperone that assists in protein folding mediated by HSP70 or HSP90 **[48]**. The KEGG pathway analysis also reveals involvement of miRNAs in regulation of genes associated with various significant metabolic pathways. Our findings have shown that ESTs can be a major source of functional information similar to previous reports of SSRs identified from ESTs **[49]**.

Conclusion:

We report the mining of miRNAs from EST data in *Dendrobium nobile*. We describe 14 potential miRNAs from 3 ESTs of *D. nobile*. They belong to 3 miRNA families (miR390, miR528 and miR414) linking to transcription factor regulation, signal transduction, DNA and protein binding, and various cellular processes covering 34 different metabolic networks in KEGG. These results help in the understanding of miRNA-mRNAs functional networks in *D. nobile*.

List of Abbreviations:

DCL1 - Dicer-like-1; AGO - Argonaute; RISC - RNA-induced silencing complex; EST - Expressed Sequence Tags; MFEI - Minimal Folding free Energy; AMFE - Adjust Minimal Folding free Energy; KEGG - Kyoto Encyclopaedia of Genes and Genomes; TCA - Tri-Carboxylic Acid; TF - Transcription Factor; MYB - Myeloblastosis; TCP - Teosinte Branched 1, Cycloidea, Proliferating Cell Nuclear Antigen Factor 1 and 2.; ERF - Ethylene Responsive Transcription Factor; SCF - Skp I, Cullin, and F-box

Declaration:

Ethics approval and consent to participate: Not applicable

Consent for Publication:

Not applicable

Availability of data and material:

A total of 38,589 previously identified mature micro-RNAs from different plants were retrieved from the miRBase database (http://www.mirbase.org/) (release 22.1). These sequences were defined as the query sequence set and used for identifying miRNAs in *D. nobile* Expressed Sequence Tags (ESTs). Publicly available 15,383 ESTs of the species were downloaded from National Centre for Biotechnology Information (NCBI)

(https://www.ncbi.nlm.nih.gov/). Non-redundant protein



sequences were used from the NR protein database of NCBI (ftp://ftp.ncbi.nlm.nih.gov/blast/db/).

Competing Interests:

The authors declare that they have no competing interests.

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Authors' Contributions:

DBK carried out data analysis and prepared the manuscript, PMB carried out most of the primary steps involved in data analysis, BD, KSB and HA thoroughly revised the compiled draft and put valuable inputs while carrying out the research, NA supervised the research work, designed the methodology and finally approved the manuscript. All authors have read and approved the manuscript.

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