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Computational Identification of MicroRNAs and their Targets in two Species of Evergreen Spruce Tree (*Picea*)

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***Abstract*—**MicroRNAs (miRNAs) are small, non-coding and regulatory RNAs about 20 to 24 nucleotides long. Their conserved nature among the various organisms makes them a good source of new miRNAs discovery by comparative genomics approach. The study resulted in 21 miRNAs of 20 pre-miRNAs belonging to 16 families (miR156, 157, 158, 164, 165, 168, 169, 172, 319, 390, 393, 394, 395, 400, 472 and 861) in evergreen spruce tree (*Picea*). The miRNA families; miR 157, 158, 164, 165, 168, 169, 319, 390, 393, 394, 400, 472 and 861 are reported for the first time in the *Picea*. All 20 miRNA precursors form stable minimum free energy stem-loop structure as their orthologues form in *Arabidopsis* and the mature miRNA reside in the stem portion of the stem loop structure. Sixteen (16) miRNAs are from *Picea glauca* and five (5) belong to *Picea sitchensis*. Their targets consist of transcription factors, growth related, stressed related and hypothetical proteins.

***Keywords*—**BLAST, Comparative Genomics, Micro-RNAs, Spruce

International Science Index, Biotechnology and Bioengineering Vol:5, No:3, 2011 waset.org/Publication/2964

I. Introduction

A Spruce is an evergreen coniferous tree belongs to the genus *Picea* and family Pinaceae, found in the northern temperate and boreal (taiga) regions of the earth [1]. Due to its properties, the Spruce has wide importance in making of paper, medicines and food items [2].

MicroRNAs (miRNAs) are small about 20-24 nucleotide long, non-coding and endogenous RNAs [3]. They are conserved in plants and animals [4, 5]. They have vital role in gene regulation at mRNA level [6, 7]. Precursor miRNA (pre-miRNA) is the primary transcript of mature miRNAs (pri-miRNAs) that fold into a stable hair-pin / stem-loop structure. The loop of pre-miRNA is detached to create a short double-stranded RNA (dsRNA), a single strand of the dsRNA acts as mature miRNA [8]. A special RNaseIII-like endonuclease, Dicer-like enzyme (DCL) in plants involved to process the mature miRNA production [9], that also mostly integrate the mature miRNA into the RNA induced silencing complex (RISC) [8]. The RISC complex negatively regulates gene expression either by inhibiting translation elongation or by triggering messenger RNA (mRNA) destruction on the basis of the degree of complimentary of miRNA within its target [10, 11]. The animal-mRNA target has many weak miRNA complementary sites, so miRNA imperfectly match to these sites and suppress gene expression [7, 12].

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The miRNAs are involved in multipurpose functions in plant and animals like; growth [14] organogenesis [13, 14], transgene suppression [15] signaling pathway [16], environmental stresses [17, 18], disease development [19], and defense against the invading viruses [20].

Mostly miRNAs are conserved among animals and plants and also from animals to plants [5, 21, 22]. The conserved nature of these miRNAs becomes a logical approach for identification of new orthologues by comparative genomics in other species. Barozai et al. (2008), found 22 cotton miRNAs belonging to thirteen (13) miRNA families by homology search [23]. Zhang et al. (2006) identified 481 miRNAs belonging to 37 miRNA families in 71 different plant species from EST sequences in plants by homology search [24].

This research produced 21 new miRNAs in 20 pre-miRNAs from evergreen spruce tree (*Picea*). These miRNAs belong to 16 miRNA families (miR 156, 157, 158, 164, 165, 168, 169, 172, 319, 390, 393, 394, 395, 400, 472 and 861). The comparative genomics approach is applied on the Spruce ESTs using *Arabidopsis* pre-miRNAs as reference, with the same strategy described by Barozai et al. (2008), with little modification as described in the Fig-1 [23]. All 20 miRNA precursors form stable minimum free energy stem loop structure as their orthologues form in *Arabidopsis* and the mature miRNAs reside in the stem portion of the stem loop structure. Sixteen (16) miRNAs are from *Picea glauca* and five (5) belong to *Picea sitchensis.*

II. Materials and Methods

A. Identification of Candidate Sequences

A little modified methodology described by the Barozai et al. [23], as illustrated in (Fig-1.), was used. The *Arabidopsis* pre-miRNAs instead of mature sequences were used as reference. The candidate sequences were identified containing Pre-miRNA sequences of *Picea glauca* (pga) and *Picea sitchensis* (psi), using previously known *Arabidopsis* pre-miRNAs from the microRNA Registry Database (Version Rfam16.0 released Sept 2010) [25], and subjected through blastn [26] against publicly available *Picea* ESTs database at http://blast.ncbi.nlm.nih.gov/Blast.cgi. Adjusted blast parameter settings were as follows: expect values were set at 1000; low complexity was chosen as the sequence filter, database (others. *Picea*) program selection (somewhat similar sequence) and all other parameters were used as default. The FASTA formats of all the candidate sequences were saved. The repeated ESTs created were found out by BLAST against the *Picea* ESTs Database using blastn with default parameters. The repeated ESTs from the same gene were removed and single tone EST was created for each miRNA.

B. Prediction of Picea Initial Candidate's Pre- miRNAs

Clustal W (1.83), a multiple sequence alignment tool with default parameters, publicly available at http://www.ebi.ac.uk/clustalw/, was used to align each *Arabidopsis* pre-miRNA against the corresponding single tone ESTs and an initial candidate miRNAs were created. The

International Science Index, Biotechnology and Bioengineering Vol:5, No:3, 2011 waset.org/Publication/2964

initial candidate *Picea* miRNAs were checked for mature sequences with their orthologues of *Arabidopsis* in the range of 0-4 mismatches.

C. The Validation of Picea miRNAs as a Non-protein Encoding Sequences

The initial candidate *Picea* miRNA sequences were subjected for protein homology search. The sequences in FASTA format were BALST against protein database at NCBI using blastx with default parameter [27].

D. Creation of Hairpin Structures

Zuker folding algorithm, MFOLD (version 3.2) [28], publicly available at http://www.bioinfo.rpi.edu/applications/mfold/rna/form1.cgi., was used to create the hairpin structure of the initial candidate's sequences. The parameters were adjusted as RNA sequence (linear), folding temperature (37^0C), ionic condition (1MNaCl with no divalent ions), percent sub-optimality number (5); maximum interior/bulges loop size (30), and all others with defaults values. The lowest free energy structures were selected for manual inspection. The threshold values used to select a miRNA were same as described by Zhang et al. [24]. The stem portion of the hairpin checked for the mature sequences with at least 11 base pairs involved in Watson-Crick or G/U base pairing between the mature miRNA and the opposite strand (miRNA*).

E. Sequence and Structural Features Filtration

To validate the miRNAs through the sequence and structural features filter, the GC content, Core mfe, hairpin mfe and Ch_ratio were calculated as described by Li et al. [29] with a little modification for Core mfe calculation, as described by Barozai et al. [23]. The mfe for core and hairpin structures were calculated by MFOLD (version3.2) [28], publicly available at http://www.bioinfo.rpi.edu/applications/mfold/rna/form1.cgi. The parameters were adjusted same as described earlier. For Ch_ratio calculation, the core mfe divided by the hairpin mfe, and the quotient is referred to as the ch_ratio.

F. Conservation Analysis of Picea miRNAs

The *Picea* miRNA (pga-MIR390) conservation studies with rice (*Oryza sativa*), *Brassica napus* and *Arabidopsis* orthologues was done by the publically available Weblogo [30]. The results were saved.

G. Prediction of Picea miRNA Targets

The *Picea* miRNAs targets were predicted using the NCBI blastn program [26], by subjecting the mature miRNA sequences as queries. The parameters were adjusted as, Database; Nucleotide collection (nr/nt), organism; *Picea* (taxid:3328) and Program Selection; highly similar sequences (megablast). The sequences showing 60% query coverage were selected and subjected to RNA-hybrid, a miRNA target prediction tool [31] for the confirmation of the targets. The results were saved.

H. Minimizing False Positives

Minimizing of false positives is very crucial step in bioinformatics based identification of novel miRNAs. Various steps were taken to remove false positives, as described by Barozai et al. [23]. The known pre-miRNAs were used for orthologues discovery. The long length of pre-miRNAs made them more suitable for the new miRNAs orthologues identification on conservation basis with the mature sequences in a range of 0-4 mismatches. To create a single representation from a gene, removal of the repeated ESTs from the same gene was done. The protein coding *Picea* pre-miRNAs were removed in third step. In the fourth step, the sequence and structure filter was applied on *Picea* pre-miRNAs to authenticate them. In the fifth step the stem-loop structures parameters were used to validate them as miRNAs. All these steps have been taken to remove the false positives and confirm the real nature of our identified miRNAs in *Picea*.

III. Result And Discussion

A. Identification of Pre-miRNA Sequences in Picea

Arabidopsis pre- miRNA sequences from the miRNA Registry (Version Rfam16.0 released Sept. 2010)[25], was subjected to a basic local alignment search tool (BLAST) search against the known expressed sequence tags (ESTs) of the *Picea* publicly available at http:/www.ncbi.nlm.nih.gov . Total 21 new miRNAs in 20 pre-miRNAs from spruce tree (*Picea*) were identified after filtration and completion of the process (Fig.-2). These miRNAs belong to 16 miRNA families (miR 156, 157, 158, 164, 165, 168, 169, 172, 319, 390, 393, 394, 395, 400, 472 and 861). Maximum three miRNAs are from miR 156 family followed by two in miR 157 and 395 each. Sixteen (16) miRNAs are from *Picea glauca* and five (5) belong to *Picea sitchensis.* The miRNA

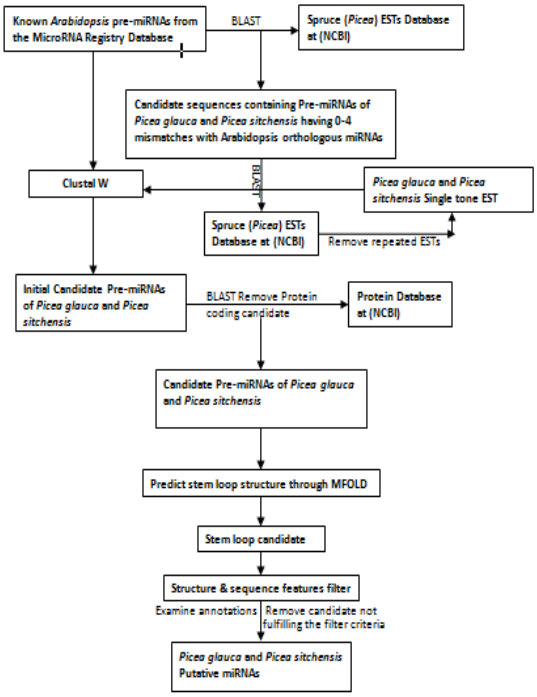

Fig. 1 Flow-chart for the computational identification of *Picea glauca* and *Picea sitchensis* Putative miRNAs using *Arabidopsis* Pre-miRNAs as reference through bioinformatics tools.

families; miR 156, 160, 163 and 166 are reported in *Picea glauca* and miR159, 163 and 172 are reported in *Picea sitchensis* [24, 32].The miRNA families; miR160, 166, 395-397, 482, 535, 947, 950, 1311, 1863 and 3693-3712 are reported in *Picea abies* [33]. So, this paper

International Science Index, Biotechnology and Bioengineering Vol:5, No:3, 2011 waset.org/Publication/2964

reported miRNA families; miR 157, 158, 164, 165, 168, 169, 319, 390, 393, 394, 400, 472 and 861 for the first time in the *Picea*. All the novel *Picea* miRNAs considered as a valid candidate after fulfilling the empirical formula for biogenesis and expression of the miRNAs, suggested by Ambrose et al. [34]. The novel *Picea* pre-miRNAs fulfilled the criteria B, C and D. According to Ambrose et al. [34] only the criterion D is enough for homologous sequences to validate as new miRNAs in different species. Meyers et al. (2008) further confirmed it in favor of plants miRNA annotation describing that the conservation of both the stem-loop secondary structure and the mature miRNAsequence is by itself sufficient for confident annotation of orthologous miRNAs [35].

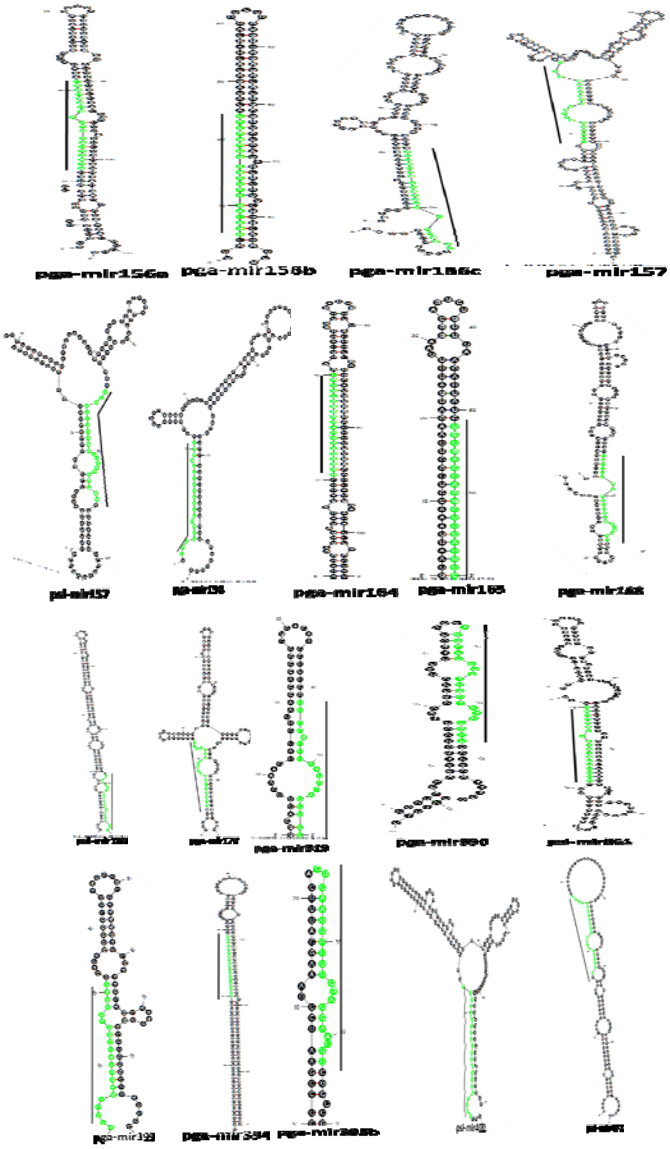

Fig. 2a The *Picea glauca* (pga) and *Picea sitchensis* (psi) pre-miRNAs stem-loop structures are illustrated, folded through Zuker folding algorithm, MFOLD (version 3.2). The mature sequences (miRNAs) can be seen in the stem region, highlighted in green and parallel lines.

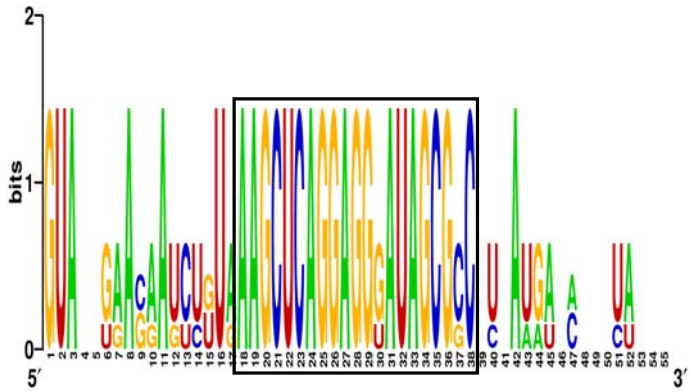

Fig. 3.The *Picea* miRNAs Conservation studies.
Alignment of pre-miRNAs (MIR-390) of *Picea*, *Arabidopsis*, Rice (*Oryza sativa*) and *Brassica napus*, using Weblogo, showing conserved nature of mature sequence highlighted in a rectangle box.

In many plant and animal miRNAs are expressed in pre-miRNAs cluster. The current research also result pga-miRNA 395a in pre-miRNA cluster (Fig-2b) that contains two mature sequences. Similar miRNA is reported as clusters in various plants species [36].

B. *MicroRNAs Characterization*

The newly identified *Picea* pre-miRNAs have minimum folding free energies (mfe) ranges from -7.6 to -80.6 Kcal mol^{-1} with an average of about -35.9 Kcal mol^{-1}, according to MFOLD [30], that is much lower than folding free energies of tRNA (-27.5 Kcal mol^{-1}) and ribosomal RNA (rRNA) (-33Kcal mol^{-1}). The pre-miRNAs length ranges from 43-210 nt with an average of 119 nt. The mature miRNA sequences length ranges from 19-22nt. Majority (47.6%) of the miRNAs have 21nt length, followed by 21nt (28.6%), 22nt (14%) and 19ts (9.5%). Majority (47.6%) of the miRNAs have 1 mismatches with their homologs, followed by 2 (19%), 0 (14.3%), 3 (9.5%) and 4 (9.5%) mismatches. Almost equal miRNAs are present on the 3' (52.4%) and 5' (47.6%) arms of the pre-miRNAs. The *Picea* miRNAs characterization such as source miRNAs, minimum free folding energies (mfe), pre-miRNAs length, mature miRNAs, mature sequence length, number of mismatches, source ESTs, mature sequence arm and GC percentage are summarized in Table I. All the mature sequences of *Picea* miRNAs are in the stem region of the stem-loop structures, as shown in (Fig.-2).The predicted miRNA stem-loop structures show that there are at least 11-20 nucleotides engaged in Watson-crick or G/U base pairings between the mature miRNA and the opposite arms (miRNAs*) in the stem region and the hairpin precursors do not contain large internal loops or bulges. These findings are same as described by many researcher groups [21, 23, 24].

TABLE II SEQUENCE AND STRUCTURAL FEATURES FILTER
Comparison of *Picea*, *Arabidopsis* and Li et al., Reference values of GC- content, core mfe, hairpin mfe and ch-ratio

Reference	GC-contents	Core-mfe	Hairpin-mfe	Ch_ratio
Li et al.	30-60	-42 to -17 (99%)	-50 to -24(99%)	50-96(99)
Arabidopsis	36-51	-54 to -23	-79 to -48	42-93
Picea	33-54	-50 to -17.80 (82%)	-58 to -21 (85%)	41-90 (82%)

TABLE I CHARACTERIZATION OF THE NOVEL IDENTIFIED *PICEA* miRNAs

Picea miRNAs	Source miRNAs	PL	MFE	MS	NM	ML	SE	MSA	GC%
pga-MIR 156a	aly-MIR156a	122	-55.40	TGACAGAAGAGAGTG**G**GCAC	1	20	DR551211	5′	49.18
pga-MIR 156b	aly-miR156a	88	-56.40	TGACAGAAGAGAGTG**G**GCAC	1	20	DR583780	5′	54
pga-MIR 156c	aly-MIR156a	140	-28.13	TGACAGAAGAGAGTG**G**GCAC	1	20	DR583146	3′	45.13
pga-MIR 157	aly-MIR157a	176	-39.25	TTGACAGAAGA**G**AGAGAGCAC	1	21	EX306319	5′	51.59
psi-MIR 157	aly-MIR157a	129	-23.39	TTGACAGAAGA**G**AGAGAGCAC	1	21	GT123085	3′	51.93
pga-MIR 158	aly-MIR158a	120	-17.64	C**T**TTT**C**TCTA**T**AATTT**A**GGAAA	4	22	DV986159	5′	33.33
pga-MIR 164	aly-MIR164a	108	-58.40	TGGAGAAGCAGGGCACGTGC	0	20	DR579554	5′	52.77
pga-MIR 165	aly-MIR165a	70	-31.80	TCGGACCAGGCTTCATT**T**CC	1	19	CO475880	3′	41.42
pga-MIR 168	aly-MIR168a	140	-28.70	CCC**CA**C**A**TGCATCAACTGAAT	3	21	DR576491	3′	45.71
psi-MIR 169	aly-MIR169b	137	-39.80	CAGCCAAGGATGACTTGCC**TA**	2	21	DR522589	3′	45.33
pga-MIR 172	aly-MIR172a	132	-43.78	AGAATC**C**TGATGATGCTGCA	1	20	GO370794	3′	51.2
pga-MIR 319	aly-MIR319a	43	-7.60	TTGGACTGAAGGGAGCTCC	0	19	DR560122	3′	60
pga-MIR 390	aly-MIR390a	74	-15.10	AAGCTCAGGAGG**T**ATAGCGGC**G**	2	22	EX378532	3′	41.89
pga-MIR 393	aly-MIR393a	80	-12.10	ATCCAAAGGGATCGCATTGAT	0	21	EX366327	5′	47.05
pga-MIR 394	aly-MIR394b	156	-80.60	**C**TGGCATTCTGTCCACCTCC	1	20	CO234072	5′	49.35
pga-MIR 395a	aly-MIR395b	210	-79.50	CTGAAGTGTTTGGGGG**A**ACTC CTGAAGTGTTTGGGGG**A**ACTC	1 1	21 21	EX395401	5′ 3′	50 50
pga-MIR 395b	aly-MIR395d	49	-17.80	CTGAAGTGTTTGG**A**GGAACT**T**	2	21	EX396486	3′	45.73
psi-MIR-400	aly-MIR400	140	-34.09	GACTTA**C**AATA**C**T**T**T**G**ATGAA	4	21	ES249022	5′	51.96
psi-MIR-472	aly-MIR472	140	-21.50	AT**GT**TTTTCC**A**ACTCCGCCCAT	3	22	CO219526	5′	39.16
psi-MIR-861	aly-MIR861	123	-26.10	GTTT-GAGAGATATG-ATCAT	2	21	DR516681	3′	35.77

Where,
PL=Precursor miRNA Length, MFE=Minimum Free Energy, MS=Mature Sequence, NM= Number of Mismatches (represented in bold & enlarged font size), ML=Mature sequence Length, MSA=Mature Sequence Arm, SE=Source EST, GC%=GC percentage

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

International Science Index, Biotechnology and Bioengineering Vol:5, No:3, 2011 waset.org/Publication/2964

The *Picea* miRNAs showed conservation with *Arabidopsis*, rice (*Oryza sativa*) and *Brassica napus* miRNAs. The miR390 miRNA family was observed with more conserved nature than others, as illustrated in (Fig-3). Similar findings were reported in many plants [23, 24].

C. Sequence and Structural Features Filter

The sequence and structural features filter is introduced by Li et al. [38] in animals' miRNA validation and by Barozai et al. in plant [23]. It is useful to filter the false positive and validate candidates. The filter is composed of four indices, namely GC content, core minimum free energy (mfe), hairpin mfe and the ratio of core mfe to hairpin mfe (ch_ratio).

As presented in (Table-II), identified miRNAs of *Picea* have a range of GC content (33 to 54%), core mfe (-50.10 to -17.80 kcal mol^{-1}, 82%), hairpin mfe (-58 to -21kcal mol^{-1}, 85%) and ch_ratio (41.0 to 90.0, 82%). The GC content and ch_ratio are within the range given by Li et al. [31] and Barozai et al. [23].

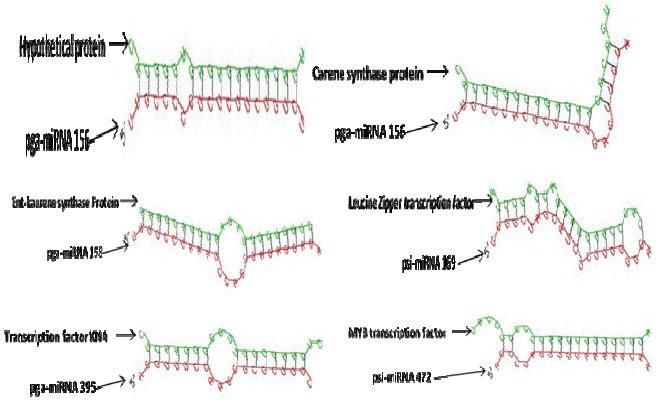

Fig. 4 The *Picea* miRNAs targets.
The novel Picea miRNAs targets were identified with the help of blastn and RNA-hybrid tools. Some of the RNA hybrid results for

D. Picea miRNA Targets

The finding of miRNAs targets is an important step for validation of miRNAs identified through homology basis. The conserved nature of miRNAs in different organisms suggests their conserved function [24]. Their targets are mostly transcription factors. The novel identified *Picea* miRNA families also targeted (Table-III) the transcription factors like; MADS-box transcription factor, Leucine Zipper transcription factor, KN4 transcription factor and MYB transcription factor. The ATAF1-like protein, Carene synthase protein, Hypothetical proteins, Ent-kaurene synthase Proteins, Ascorbate oxidase-like protein, Thaumatin-like protein and Peroxidase-like protein are also enlisted in the *Picea* targets as shown in Fig-4. These findings further validate the identified miRNAs, as the same findings were given by number of researchers [21, 23, 24].

TABLE III THE PICEA miRNAs FAMILIES THEIR TARGETED PROTEINS AND GENBANK ACC

***Picea* miRNA**	**Genbank Acc.**	**Target**
156/157	AF051222	ATAF1-like protein
	FJ609174	Carene synthase protein
	BT103576	Hypothetical protein
158	GU059905	Ent-kaurene synthase Protein
	BT106252	Hypothetical protein
164	HM202603	Thaumatin-like protein
	BT118986	Hypothetical protein
165	BT122669	Hypothetical protein
168	AF158540	MADS-box transcription factor
169	DQ258691	Leucine Zipper transcription factor
173	HM199360	Peroxidase-like protein
319	BT110042	Hypothetical protein
390	BT118273	Hypothetical protein
393	GU059905	Ent-kaurene synthase Protein
394	BT108248	Hypothetical protein
395	DQ258473	Transcription factor KN4
400	BT113317	Hypothetical protein
472	EF601073	MYB transcription factor
861	BT122261	Hypothetical protein

IV. Conclusions

Twenty one (21) novel putative miRNAs are identified in two species (*P. glauca* and *P. sitchensis*) from ESTs sequences based on homology search. Their targeted proteins are also identified. These findings will be helpful to understand the gene regulation concept in the evergreen coniferous plant *Picea*. It also strengthens the bioinformatics approach for new pre-miRNAs identification from plant species whose genome is not yet sequenced. The ESTs based identification confirmed the miRNAs expression.

References

[1] Sunset Western Garden Book, (1995): 606–607.
[2] Sigurgeirsson, A. and Szmidt, A. E. Phylogenetic and biogeographic implications of chloroplast DNA variation in Picea. Nordic Journal of Botany 13(3): (1993) 233–246.
[3] Mica E., Gianfranceschi L. and Pe M. E., Characterization of five microRNA families in *maize*. J. Expl. Bot. 57(11): (2006) 2601-2612.
[4] Bonnet E., Wuyts J., Rouze P. and Van-de-Peer Y. Detection of 91 potential conserved plant microRNAs in *Arabidopsis thaliana* and *Oryza sativa* identifies important target genes. PNAS USA. 101: (2004) 11511–11516.
[5] Weber M.J. New human and mouse microRNA genes found by homology search. FEBS J.272: (2005) 59–73.
[6] Bartel D. P. MicroRNAs: genomics, biogenesis, mechanism, and function.Cell.116: (2004) 281–297.
[7] Carrington J.C. and Ambros V. Role of microRNAs in plant and animal development. Sci. 301: (2003) 336–338.
[8] Hammond S. C., Bernstein E., Beach D .and Hannon G.J. An RNA-directed nuclease mediatesposttranscriptional gene silencing in *Drosophila* cells. Nat. 404: (2000) 293–296.
[9] KuriharaY. and Watanabe Y. *Arabidopsis* micro-RNA biogenesis through Dicer-like 1protein functions. PNAS USA.101: (2004) 12753–12758.
[10] Aukerman M. J. and Sakai H. Regulation of flowering time and floral organ identity by a microRNA and its APETALA2-Like target genes.Plant Cell.15: (2003) 2730–2741.
[11] Tang G., Reinhart B. J., Bartel D. P. and Zamore P .D. A biochemical framework for RNA silencing in plants. Genes Dev. 17: (2003) 49–63.
[12] Novina C. D. and Sharp P.A.The RNAi revolution. Nat. 430: (2004) 161–164.
[13] Kidner C. A and Martienssen R. A. The developmental role of microRNA in plants .Curr Opin Plant Biol.8: (2005) 38–44.
[14] Chen X. A microRNA as a translational repressor of APETALA2 in *Arabidopsis* flower development. Sci. 303: (2003) 2022–2025.
[15] Allen E., Xie Z., Gustafson A. M., and Carrington J. C., microRNA-directed phasing during transacting siRNA biogenesis in plants. Cell. 121: (2005) 207–221.

International Science Index, Biotechnology and Bioengineering Vol:5, No:3, 2011 waset.org/Publication/2964

[16] Yoshikawa M., Peragine A., Park M. Y. and Poethig R. S., A pathway for the biogenesis of trans-acting siRNAs in *Arabidopsis.* Genes & Dev. 19: (2005) 2164–2175.
[17] Lu S., Sun Y. H., Shi R., Clark C., Li L. and Chiang V. L. Novel and mechanical stress responsive microRNAs in *Populus trichocarpa* that are absent from *Arabidopsis*. The PlantCell.17: (2005) 2186–2203.
[18] Sunkar R. and Zhu J. K. Novel and stress-regulated microRNAs and other small RNAs from *Arabidopsis.*The Plant Cell.16: (2004) 2001–2019.
[19] Johnson S. M., Grosshansm H., Shingara J., Byrom M., Jarvis R., Cheng A., Labourier E., Reinert K. L., Brown D. and Slack F.J. RAS is regulated by the let-7 microRNA family.Cell.120(5): (2005) 635-647.
[20] Bennasser Y., Le S.Y., Yeung M. L. and Jeang K. T., HIV-1 encoded candidate micro-RNAs and their cellular targets. Retro viro.1(1):(2004) 43.
[21] Reinhart B. J., Weinstein E. G., Rhoades M. W., Bartel B. and Bartel D. P. MicroRNAs in plants. Genes Dev. 16: (2002) 1616–1626.
[22] Mario A.V., Juan C.P. and Jean-Philippe V.C. A Family of MicroRNAs Present in Plants and Animals. Plant Cell.18: (2006) 3355-3369.
[23] Barozai M.Y.K., Irfan M., Yousaf R., Ali I., Qaisar U., Maqbool A., Zahoor M., Rashid B., Hussnain T., and Riazuddin S. Identification of micro-RNAs in cotton. Plant Physiol and Biochem. 46 (8-9): (2008) 739-51.
[24] Zhang B., Pan X., Cannon C. H., Cobb G. P. and Anderson T.A., Conservation and divergence ofplant microRNA genes. The Plant J. 46: (2006) 243–259.
[25] Griffiths-Jones S. The microRNA Registry. Nucleic Acids Res. 32D: (2004) 109–111.
[26] Altschul S. F. Gish W., Miller W., Myers E.W. and Lipman D. J. Basic local alignment search tool. J. Mol. Biol. 215: (1990) 403-410.
[27] Stephen F. A., Madden T. L., Schäffer A. A., Zhang J., Zhang Z., Miller W. and Lipman D. J. Gapped BLAST and PSI-BLAST, A new generation of protein database search programs. Nucleic Acids Res. 25: (1997) 3389-3402.
[28] Zuker M. Mfold web server for nucleic acid folding and hybridization prediction. Nucleic Acids Res. 31: (2003) 3406–3415.
[29] Li S. C., Pan C.U. and Lin W.C. Bioinformatic discovery of microRNA precursors from human ESTs and introns. BMC Gen. **7:** (2006) 164.
[30] Crooks G.E., Hon G., Chandonia J. M., Brenner S.E. WebLogo: A sequence logo generator, Gen. Res. 14: (2004)1188-1190.
[31] Kruger J and Rehmsmeier M. RNA hybrid: microRNA target prediction easy, fast and flexible Nucl. Acids. Res., 34(2): (2006) 451-454.
[32] Zhang B. H., PanX. P., WangQ. L., CobbG. P., Todd A. A. Identification and characterization of new plant microRNAs using EST analysis. Cell Research, 15(5): (2005) 336-360.
[33] Yakovlev I. A., Fossdal C. G., Johnsen O. MicroRNAs, the epigenetic memory and climatic adaptation in Norway spruce. New Phytol. 187: (2010) 1154-1169.
[34] Ambros V., Bartel B. and Bartel D. P. A uniform system for microRNA annotation. RNA. 9: (2003) 277–279.
[35] Meyers B. C., Axtell M. J., Bartel B., et al. Criteria for Annotation of Plant MicroRNAs. The Plant Cell. 20: (2008) 3186–3190.
[36] Aili L., and Long M. Evolution of plant microRNA gene families Cell Research 17: (2007)212-218.

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