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AN UPDATE ON THE MicroRNAs AND THEIR TARGETS IN UNICELLULAR RED ALGA *PORPHYRIDIUM CRUENTUM*

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Abstract

MicroRNAs (miRNAs) are small, non-coding and regulatory RNAs about ≈ 21 nucleotides in length. The miRNAs are reported in large number of higher eukaryotic plant species. But very little data of miRNAs in algae is available. *Porphyridium cruentum* is unicellular red alga famous as a source for polyunsaturated fatty acids, proteins and polysaccharide contents. The present study is aimed to update the microRNAs and their targets in this important algal species. A comparative genomics approach was applied to update the miRNAs in *P. cruentum*. This effort resulted in a total of 49 miRNAs belonging to 46 families in *P. cruentum*. Their precursor-miRNAs were observed with a range of 40 to 351 nucleotides (nt). The mature miRNA sequences showed a range of 17-24 nts. The minimum free energies by stem loop structures of these miRNAs are found with an average of -32 Kcalmol^{-1}. A total of 13 targets, including important proteins like; Ribulose-1,5-bisphosphate carboxylase oxygenase, Light-harvesting complex I, Oxygen-evolving enhancer protein, Phycobiliproteins, Granule-bound starch synthase and Carbonic anhydrase were also predicted for these miRNAs.

Key words: Comparative genomics, microRNAs, *Porphyridium cruentum*.

Introduction

MicroRNA (miRNA) is a special regulatory member of the small RNAs that are involved in post-transcriptional gene silencing. Gene regulation by miRNAs is an impressive advancement and most exciting area of present regulatory RNA research. They are endogenous, non-coding in nature and about ≈ 21 nucleotides (nt) in size. They participate as negative regulator at post-transcriptional stage of gene regulation (Ambros *et al.,* 2003). A self-folded stable hair-pin/ stem-loop secondary structure termed as precursor-miRNA (pre-miRNA) is produced from long single strand RNA known as primary miRNA. Later pre-miRNA generates functional, small mature miRNA. The mature miRNA integrate into argonaute proteins and advances into the RNA induced silencing complex (RISC) (Hammond *et al.,* 2000). The RISC complex having mature miRNA triggers post-transcriptional gene suppression of the messenger RNA (mRNA) either by inhibiting protein encoding or by activating mRNA degradation. This inhibition and degradation capability of the miRNA depends on the scale of complementarity between miRNA and its targeted mRNA (Bartel, 2004). The impartial pairing of the miRNAs with the mRNA targets causes its repression from protein synthesis. While,the complete pairing of miRNAs with their mRNA targets causes the mRNAs degradation (Kidner & Martienssen, 2005). miRNAs participate widely in life activities such as; growth and development (Bartel, 2004), foreign genes suppression, signal transduction, environmental stresses and as a defense against the attacking microbes in various living organisms (Gao *et al.,* 2011; Barozai *et al.,* 2012a, Bai *et al.,* 2012; Ali *et al.,* 2016).

Porphyridium cruentum (*Syn. Porphyridium purpureum)* (Bory de Saint-Vincent) (Drew & Ross, 1965) is an important member of the microalgae that is a major developing nutritional source. It is widely recognized for its remarkable richness in polyunsaturated fatty acids. The *P. cruentum* is also remarkable for its anticancer and immunomodulating properties. Only few studies are available about the miRNAs in algae. According to the latest microRNA Registry Database (Version Rfam 21.0 released June, 2014) (Griffiths-Jones, 2004), only one algae member i-e., *Chlamydomonas reinhardtii* is reported for miRNAs. This situation demands to focus and profile new miRNAs and their targets in *P. cruentum* that will act as preliminary data to manage and understand the *P. cruentum* at molecular level.

The research on *P. cruentum* conducted in the present study, produced a total of 49 new miRNAs belonging to 46 families. These new miRNAs were also annotated for various features like; their targets, minimum free energy (MFE), stem loop structures and GC contents.

Materials and Methods

Prediction of potential miRNAs for *P. cruentum*: Similar methodology with some modification as reported earlier (Barozai *et al.,* 2011a) was applied to predict the miRNAs from *P. cruentum* ESTs. Total 5744 known plant miRNA sequences, both precursors and matures were downloaded from the microRNA Registry Database (Version Rfam 20.0 released June 2013) (Griffiths-Jones, 2004), and subjected to BLAST search against publicly available 386,903 *P. cruentum* (taxid: 35688)) ESTs from the dbEST release 010113 at http://blast.ncbi.nlm.nih.gov/Blast.cgi using BLASTN (Altschul *et al.,* 1990). The raw sequences were saved in FASTA formats. The repeated ESTs from the same gene were removed. The initial candidate miRNAs with 0-4 mismatches in the mature sequences were identified using Clustal W (1.83), a multiple sequence alignment tool with default parameters, publicly available at http://www.ebi.ac.uk/clustalw/ (Larkin *et al.,* 2007).

Elimination of protein coding miRNAs: The initial applicant miRNA sequences of *P. cruentum*, predicted by the mature source miRNAs were checked for protein coding. The FASTA format of the applicant sequences were subjected against protein database at NCBI using BLASTX with default parameter (Stephen *et al.,* 1997) and the protein coding sequences were removed.

Creation of self-fold secondary structures: The non-protein coding, having 0-4 mismatches with the source plant miRNAs and single tone initial candidate miRNA sequences of *P. cruentum* were subjected to self-fold secondary structures. A publicly available Zuker folding algorithm at http://www.bioinfo.rpi.edu/applications/mfold/rna/form1.cgi, known as MFOLD (version 3.2) (Zuker, 2003), was used to create the secondary structures. The MFOLD parameters were used as published by various researchers for the identification of miRNAs in various plant and animal species (Bai *et al.,* 2011; Barozai, 2012a, b). The secondary stem loop structures generated by MFOLD were subjected for the physical inspection to meet either the lowest free energy ≤-18Kcal/mol or ≤ the lowest free energy of the source miRNAs. Ambros *et al.,* (2003) threshold values were applied to finalize the *P. cruentum* miRNAs. The stem region of the stem-loop structures of the *P. cruentum* miRNAs were inspected for the mature sequences either with at least 16 or equal to the source miRNAs base pairs forming a Watson-Crick or G/U base pairing between the mature miRNA and the opposite passenger strand (miRNA*).

Convergence and phylogenetic analysis: The convergence and phylogenetic analysis was carried out for one of the conserved miRNA, mir-164 (Rhoades, 2011). Simply, the *P. cruentum* miRNA, pcr-mir-164, for its conserved behavior in different plant species was checked for convergence and phylogenetic investigation. The *P. cruentum* miRNA, pcr-mir-164 alignment was created with *Arabidopsis lyrata (aly), Arabidopsis thaliana (ath), Vitisvinifera (vvi), Oryza sativa (osa), Zea mays (zma)* by the publicly accessible weblogo: a sequence logo generator (Crooks *et al.,* 2004) and Clustal W (Larkin *et al.,* 2007) to produce cladogram tree using neighbor joining clustering method respectively. The results were saved.

Profiling potential targets: More stringent criterion was adopted by applying dual scheme to profile the potential targets for the *P. cruentum* miRNAs. In the first scheme, the newly identified *P. cruentum* miRNAs were subjected to psRNATarget (http://bioinfo3.noble.org/psRNATarget) with default parameters (Dai & Zhao, 2011) for profiling their potential targets. The *P. cruentum* miRNAs not producing potential targets through psRNATarget, were subjected to the second scheme as described by Barozai, (2012c). Briefly in this scheme, the *P. cruentum* mature miRNA sequences were subjected as queries through the NCBI BLASTn program. The parameters were adjusted as, Database; Reference mRNA sequences (refseq_rnat), organism; *P. cruentum* (taxid: 35688) and Program Selection; highly similar sequences (megablast). The mRNA sequences showing ≥75% query coverage were selected and subjected to RNAhybrid, a miRNA target prediction tool (Kruger &Rehmsmeier, 2006) for the confirmation of the targets. Only targets having stringent seed site located at either positions 2-7 or/and 8-13 from the 5' end of the miRNA along with the supplementary site and the minimum free energy (MFE) of the hybridization was -20 kcal/mol were selected. The Gene Ontology analysis was conducted on AmiGO website.

Results and Discussion

The potential *P. cruentum* miRNAs: The present study is a comparative genomics based approach applying various bioinformatics tools. This is in agreement with the previous reports by many research groups (Barozai & Husnain, 2011; Barozai & Wahid, 2012; Barozai *et al.,* 2012b; Barozai *et al.,* 2014a, b; Barozai & Din, 2014; Behlil *et al.,* 2016; Barozai & Din, 2017; Jahan *et al.,* 2017; Shah *et al.,* 2017), where the homology based search by applying comparative genomics is found a valid and logical approach to find interesting findings in various organisms at genomics level. Here, a total of 49 novel miRNAs were identified from the computational and bioinformatics analyses of 386,903 *P. cruentum* ESTs. The 49 putative *P. cruentum* miRNAs belong to 46 miRNA families, (pcr-miR; 156 (a,b), 159, 164, 165, 171, 390, 394, 413, 419 (a,b), 447, 834, 838, 839, 843, 852, 855, 900, 951 (a,b), 1039, 1062, 1072, 1088, 1092, 1103, 1104, 1107, 1155, 1161, 1165, 1846, 1848, 2091, 2097, 2111, 2112, 2867, 2926, 3440, 3442, 3630, 4224, 5503, 5523, 5819, 5836 and 6104). On the basis of the present available algal miRNA literatures, the miRNA families; (pcr-miR; 159, 164, 165, 171, 390, 394, 413, 447, 834, 838, 839, 843, 852, 855, 900, 951 (a,b), 1039, 1062, 1072, 1088, 1092, 1103, 1104, 1107, 1155, 1161, 1165, 1846, 1848, 2091, 2097, 2111, 2112, 2867, 2926, 3440, 3442, 3630, 4224, 5503, 5523, 5819, 5836 and 6104 are reported for the first time in *P. cruentum*. The two miRNA families; miR-156 (a,b) and 419 (a,b) were also reported by Gao *et al.,* (2016). The absence of the 44 miRNA families from Gao *et al.,* (2016) work is may be due to different varieties, condition and methodology. The pcr-miR-156, 419 and 951 families were observed with maximum two members and rest of all the families were observed with only one member. These newly identified potential 49 *P. cruentum* miRNAs were observed in the arm regions of the stem-loop secondary structures. These potential 49 *P. cruentum* miRNAs were considered as a valid candidate after justifying the empirical formula for biogenesis and expression of the miRNAs, suggested by Ambros *et al.,* (2003). The 49 potential *P. cruentum* miRNAs rationalize to the criteria B, C and D, on the basis of Ambros *et al.,* (2003) report, just the criterion D is sufficient for homologous sequences to confirm as potential miRNAs in other species.

Characterization of the miRNAs in *P. cruentum:* Characterization of the novel potential candidate miRNAs is now considered as a crucial step for the validation of computationally predicted miRNAs (Wang *et al.,* 2012; Bibi *et al.,* 2017; Gul *et al.,* 2017). The minimum folding free energy (MFE) of miRNA is

the first vital and valid term of characterization. The newly identified potential *P. cruentum* pre-miRNAs have shown minimum folding free energies (MFEs) in a range from -9.0 to -124 Kcal mol^{-1} with an average of -32 Kcal mol^{-1}. The 49 *P. cruentum* miRNAs distribution on basis of their MFEs are as; 20 (41%) have -9 to -21, 14 (29%) have -22 to -30, 8 (16%) have -32 to -48 and 7 (14.3%) have -52 to -124 Kcal mol^{-1}. The mature miRNA sequences of newly identified miRNAs in *P. cruentum* are observed in the stem region of the precursor self-folded structures, as shown in Figure 1. The profiled miRNAs in *P. cruentum* were observed with an average of 52% GC content. Many researchers (Barozai, 2012d; Bai *et al*., 2012; Wang *et al*., 2012) reported almost similar findings for various plant and animal species. The newly identified *P. cruentum* miRNAs characterization in terms of source miRNAs, pre-miRNAs length, the minimum free folding energies (MFEs), mature miRNAs, number of nucleotides mismatches, mature sequence length, source EST, mature sequence arm and GC percentage are tabulated in Table 1.

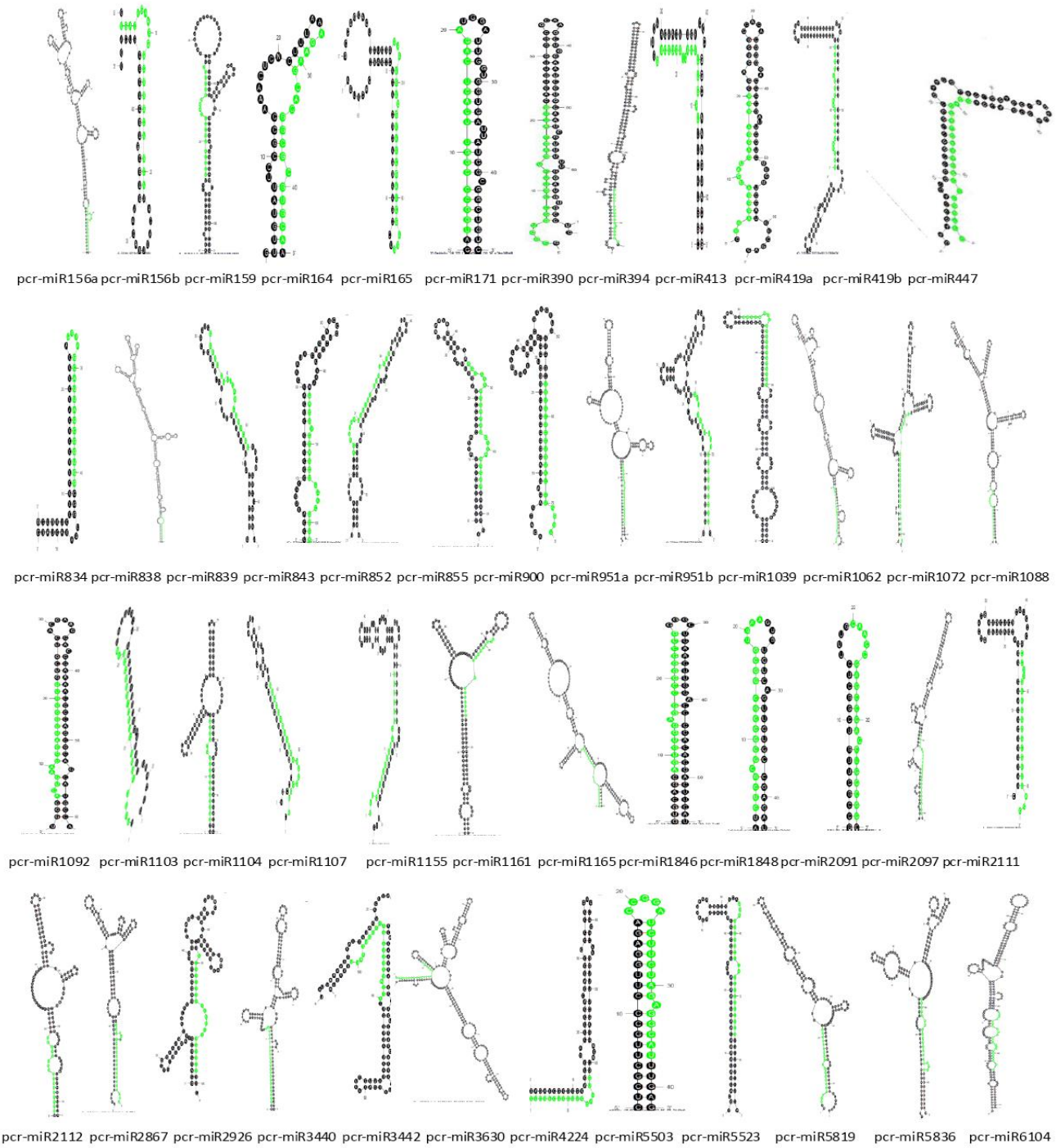

Fig. 1. The new *Porphyrdium cruentum* miRNA secondary structures.
The *Porphyrdium cruentum* pre-miRNAs secondary structures were predicted using Mfold algorithm. These structures are clearly showing that mature miRNAs in stem region of the stem-loop structures, highlighted in green.

Table 1. The newly identified *Porphyridium cruentum* miRNAs characterization.

P. cruentum miRNAs	Source miRNAs	PL	MFE	MS	NM	ML	SE #	MSA	GC%
pcr-miR156a	hci-miR156b	113	-62.29	GGACAGAAGAGAGCGAGCGA	04	20	HS829859	3’	60
pcr-miR156b	har-miR156c	50	-16.90	TGACAGAAGGGCGGGCAGCA	03	20	HS943066	3’	65
pcr-miR159	osa-miR159a	104	-24.90	TTGCATGCGCCAGGAGCTGC-	02	20	HS914235	5’	65
pcr-miR164	aly-miR164a	45	-8.70	TAAAGAAGCAGGGCGCGTGCA	04	21	HS876904	3’	57
pcr-miR165	aly-miR165a	63	-15.90	GAATGTTGTATCGATCGAGG	02	20	HS973678	3’	45
pcr-miR171	osa-miR171a	48	-15.70	AGATGGCCCGGTTCACTCAGA	04	21	HS945787	5’	57
pcr-miR390	ppt-miR390c	71	-28.30	CGCTGGCCGTTCTGAGCATTG	02	21	HS802432	5’	62
pcr-miR394	osa-miR394	147	-48.50	TTGGCATTCTGTCCACTGGT	04	20	HS653979	3’	50
pcr-miR413	osa-miR413	53	-18.60	TTAGTTTCACTTGTTCTGGTC	04	21	HS965235	3’	38
pcr-miR419a	ppt-miR419	65	-23.10	TGATGGATGATGACGATGTCG	03	21	HS942239	5’	48
pcr-miR419b	osa-miR419	97	-29.90	AGATGAATGCTGACGCTGTAT	04	21	HS949863	5’	43
pcr-miR447	ath-miR447a	68	-20.40	ATGAGGACGAAAGGTTTTGTTG	04	22	HS591443	5’	41
pcr-miR834	aly-miR834	54	-32.40	AGGTAGCAGTAGCGGTGGCGG	04	21	HS621397	3’	67
pcr-miR838	aly-miR838	351	-123.96	TTTTCTTCTTCTTCTTGTCGA	04	21	HS916110	3’	33
pcr-miR839	ath-miR839	64	-13.30	GCCCAGCCTTTCATCGTTCGC	04	21	HS738952	3’	62
pcr-miR843	ath-miR843	62	-17.20	AATAGGTCGAGCTTGACTGGA	04	21	HS830372	3’	48
pcr-miR852	aly-miR852	76	-24.90	GAACAAAGGCGCGTGTCTTCG	04	21	HS957418	3’	57
pcr-miR855	ath-miR855	73	-9.20	AGGAAAAGGAAAGGAAAAGGAA	03	22	HS785849	5’	36
pcr-miR900	ppt-miR900	74	-28.00	TGTGCTCCTGTACCTGGGACG	04	21	HS629489	3’	62
pcr-miR951a	pab-miR951	201	-41.38	TGTTCTTGACGTGTGTACCACC	03	22	HS959589	3’	50
pcr-miR951b	pde-miR951	79	-27.43	CTTTCTTGAACTCTGGACCAC	04	21	HS947173	3’	48
pcr-miR1039	ppt-miR1039	113	-15.10	GGTTTGGGTCTTTGTCT-CTG	04	20	HS774456	3’	50
pcr-miR1062	ppt-miR1062	220	-69.27	--CATGACGTGGTGTTTGCAGC	04	20	HS690100	5’	55
pcr-miR1072	ppt-miR1072	142	-20.48	TGAATTGTGTTATTTCAAACTTGA	03	24	HS877495	3’	25
pcr-miR1088	smo-miR1088	230	-86.24	GTGTGCGCTTTTTCTTCTGCC	04	21	HS863106	5’	52
pcr-miR1092	smo-miR1092	61	-20.40	GGCCATGAATGCATTGGTGTT	04	21	HS619654	5’	48

Table 1. (Cont'd.).

P. cruentum miRNAs	Source miRNAs	PL	MFE	MS	NM	ML	SE #	MSA	GC%
pcr-miR1103	smo-miR1103	53	-17.00	TAGAGGGCACCACCTTTCCAAT	03	22	HS757103	5'	50
pcr-miR1104	smo-miR1104	96	-20.67	GGGCGCTGTTCTTTTTCCTTC	04	21	HS766861	5'	52
pcr-miR1107	smo-miR1107	58	-16.80	TGTGCTGGTTCCAATTCGGGA	03	21	HS782657	3'	52
pcr-miR1155	cre-miR1155	82	-21.60	TA-TCCTGCTCGAGGAAGGAGA	03	21	HS724557	5'	52
pcr-miR1161	cre-miR1161a	140	-34.19	TC--GGCGTTCTCAACAGC	03	17	HS880307	3'	59
pcr-miR1165	cre-miR1165	220	-51.64	ACGGTCCGCTTGTACGGATTCT	04	22	HS898883	5'	55
pcr-miR1846	osa-miR1846a	56	-24.20	AGTGTGGAGGCCGGGGCCGGG	03	21	HS818806	5'	81
pcr-miR1848	osa-miR1848	43	-17.10	TCTCGCGCGCGCGCGTGAA	04	21	HS820756	5'	76
pcr-miR2091	osa-miR2091	40	-22.90	GCAACCGAGCCGAGCTGGCGG	04	21	HS925447	3'	76
pcr-miR2097	osa-miR2097	141	-44.72	AGAGATGGGACGGGCAGCGATG	02	22	HS887040	3'	64
pcr-miR2111	ath-miR2111a	63	-18.50	CGCCTTGGGAAGCGGATTACC	04	21	HS870320	3'	62
pcr-miR2112	aly-miR2112	123	-27.32	TGCAAATGCGGATATCAAGTT	04	21	HS686245	5'	38
pcr-miR2867	osa-miR2867	135	-47.30	TTTGCCATCCCACACGTCCAGT	03	22	HS934022	3'	55
pcr-miR2926	osa-miR2926	101	-29.04	ACGTCTTCTACGTTGGTGCT	03	20	HS899514	3'	50
pcr-miR3440	aly-miR3440	158	-44.64	TGGAAATGGTCAAAGAGAACCGCA	03	24	HS974866	5'	46
pcr-miR3442	aly-miR3442	107	-9.30	AGCATAGTTAGAGCTCTGAAT	04	21	HS957005	3'	38
pcr-miR3630	han-miR3630	257	-83.99	TGTGGGAATCTCTCTGA-GCTT	01	22	HS900430	5'	45
pcr-miR4224	aly-miR4224	78	-29.20	ACGCTGAGCTTCCTCATCTCG	04	21	HS943942	3'	57
pcr-miR5503	osa-miR5503	42	-15.60	ACCGGATCTT-CTAGAGGCATT	03	21	HS626839	3'	48
pcr-miR5523	osa-miR5523	87	-17.60	TGAGGTGGCACATATTTACTCG	03	22	HS965542	3'	45
pcr-miR5819	osa-miR5819	149	-61.22	AGTACG-GGGGAACGGCGGCG	02	20	HS756030	5'	75
pcr-miR5836	osa-miR5836	167	-47.07	CGATCAACCGGACCATGGGAT	03	21	HS948347	3'	57
pcr-miR6104	cca-miR6104	201	-24.35	TAAGACAAATAGAACAAAAAAA	04	22	HS936059	3'	18

The newly identified *Porphyridium cruentum* miRNAs were characterized in terms of 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, SE=Source EST, MSA=Mature Sequence Arm and GC percentage

Fig. 2. *Porphyridium cruentum* miRNA Conservation study
Alignment of miRNAs (miR-164) of *Porphyridium cruentum* with *Arabidopsis lyrata (aly),Arabidopsis thaliana (ath), Vitis vinifera (vvi), Oryza sativa (osa), Zea mays (zma),* using Weblogo, showing conserved nature of mature sequence highlighted in a rectangle box.

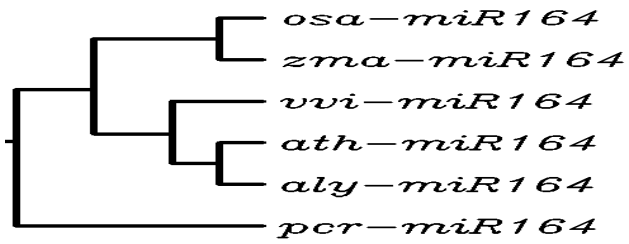

Fig. 3. *Porphyridium cruentum* miRNA Phylogenetic tree study
Alignment of miRNAs (miR-164) of *Porphyridium cruentum* with *Arabidopsis lyrata (aly), Arabidopsis thaliana (ath), Vitisvinifera (vvi), Oryza sativa (osa), Zea mays (zma),* is done with the help of Clustalw and cladogram tree was generated using neighbor joining clustering method. The Phylogenetic tree showed that on the basis of pre-miRNA sequences, the *Porphyridium cruentum* is unique and not closed to *Arabidopsis lyrata (aly), Arabidopsis thaliana (ath), Vitisvinifera (vvi), Zea mays (zma), Oryza sativa (osa).*

The pre-miRNAs length of the profiled miRNAs in *P. cruentum* ranges from 40-351 nt with an average of 108 nt and mature miRNA sequences ranges from 17 nt to 24 nt with an average of 21 nt. Majority (55%, i.e., 27 out of 49) of the miRNAs are 21 nt in length, followed by 22 nt (20%), 20 nt (18%), 24 nt (4%) and 17 nt (2%). These findings for pre-miRNAs and mature sequences in *P. cruentum* are within agreement to prior published data in other plant species (Barozai *et al.*, 2013a, b; Din & Barozai, 2014a, b, Din *et al.*, 2014). The 55 % novel identified *P. cruentum*'s miRNA sequences have a difference of 4, 33% have a difference of 3, 11% have 2 and 2% have a difference of 1 with the corresponding homologous of source miRNAs. These values are matched with the previously reported values in different plants, where the mature sequences have a difference of 0-4 nucleotides (Wang *et al.*, 2012; Barozai, 2013). The 59% (29 off 49) *P. cruentum* miRNA sequences were found at 3' arm, while the remaining 41% are at 5' arm as shown in Fig. 1. The secondary stem-loop structures of the *P. cruentum* pre-miRNAs were observed with at least 16 nucleotides engaged in Watson-Crick or G/U base pairings between the mature miRNA and the opposite arms (miRNAs*) in the stem region except few, where the source miRNAs have also less base pairings and these precursors do not contain large internal loops or bulges. Many groups (Barozai *et al*., 2008; Barozai *et al*., 2015; Baloch *et al*., 2015a, b; Din *et al*., 2016) observed similar results in various research papers. Furthermore, the newly identified *P. cruentum* miRNAs sequences were observed with no significant similarity with known proteins. This confirms them as non-coding RNAs.

Convergence and Phylogenetic analysis: The newly identified *P. cruentum* miRNA; pcr-mir-164, due to its conserved nature was investigated for convergence and phylogenetic. The *P. cruentum* miRNA; pcr-miR-164 was observed in convergence with *Arabidopsis lyrata (aly), Arabidopsis thaliana (ath), Vitisvinifera (vvi), Oryza sativa (osa), Zea mays (zma)* as shown in Fig. 2. The Phylogenetic investigation of the same pre-miRNA has shown that the *P. cruentum* miRNA is unique and not closely related to *Arabidopsis lyrata (aly),Arabidopsis thaliana (ath), Vitisvinifera (vvi), Oryza sativa (osa), Zea mays (zma)* as illustrated in Fig. 3. This is shows a diverged nature of pre-miRNA in algae.

Potential targets of the identified miRNAs in *P. cruentum:* Profiling the potential *P. cruentum* miRNAs targeted genes is a vital step for validation of the computationally identified miRNAs. Total 13 targeted genes were profiled for the potential *P. cruentum* miRNAs. Different *P. cruentum* miRNAs targeting same proteins were predicted here. The profiled targeted genes are involved in anabolism, catabolism and photosynthesis. The detail description is mentioned in Table 2. Almost all of these targets are already reported as miRNA targets in other plants (Bai *et al.*, 2012; Barozai, 2012a, b).

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is a key enzyme in photosynthesis and photorespiration, where it catalyzes the fixation of CO_2 and O_2, respectively. Due to its rate-limiting property in photosynthesis, it is the prime focus of improving the plant productivity (Whitney & Andrews, 2001). The eighteen (18) *P. cruentum* miRNAs (pcr-miR156, 394, 419, 447, 834, 838, 900, 1062, 1072, 1092, 1107, 1161, 2112, 2867, 3440, 3442, 4224 and 5523) are predicted to target this important enzyme. These *P. cruentum* miRNAs are the potential resource to modify Rubisco expression and ultimately plant productivity.

Light-harvesting complex I (LHC) is a multi-unit protein; encoded by nuclear genes. Translated proteins of these genes, are migrated from the cytosol to the ER with the help of an amino acids terminal signal sequence. Finally these proteins are embedded in the membrane of thylakoids in plants, algae, and cyanobacteria (Chitnis, 2001). It oxidizes plastocyanin or cytochrome c by transferring solar energy into biomass. Therefore, the LHC gene family is an important source of metabolic engineering for the biofuel synthetic processes. The newly identified *P. cruentum* miRNAs; pcr-miR156, 164, 171, 900, 1039, 1062, 1088, 1165, 1848, 2111, 5503 and 5819 may target the Light-harvesting complex I. Thus these miRNAs are important resources to fine tune the LHC regulation in biofuel engineering.

Table 2. Putative *Porphyridium cruentum* miRNA targets List of the potential targets.

miRNA	Target Acc. #	Target Description	Function
pcr-miR 156, 394, 419, 447, 834, 838, 900, 1062, 1072, 1092, 1107, 1161, 2112, 2867, 3440, 3442, 4224, 5523	DQ308439	Ribulose-1,5-bisphosphate carboxylase oxygenase	Catabolism
pcr-miR 156,164, 171, 900, 1039, 1062, 1088, 1165, 1848, 2111, 5503, 5819	U58680	Light-harvesting complex I mRNA	Photosynthesis
pcr-miR 156, 159, 165, 171, 394, 419, 838, 843, 1062, 1073, 1103, 1104, 1155, 1161, 1165, 1848, 2867, 2926, 3442, 3630, 4224, 5503, 5523, 5819,	AB545772	Oxygen-evolving enhancer protein	Photosynthesis
pcr-miR 156, 164	HM535635	Phycobiliproteins	Photosynthesis
pcr-miR 159, 165, 171, 390, 447, 834, 839, 951, 1062, 1092, 1104, 1107, 1165, 2111, 2112, 2867, 5523, 6104	U58679	Light-harvesting complex I mRNA	Photosynthesis
pcr-miR 159, 164, 390, 413, 839, 843, 852, 855, 900, 951, 1039, 1062, 1088, 1092, 1104, 1155, 1161, 1846, 1848, 2097, 2111, 2867, 3630, 4224, 5503, 5523	AB274917	Granule-bound starch synthase	Anabolism
pcr-miR 165, 413, 447, 1092, 1165, 2091, 2097, 2112, 2926, 3442	D86051	Carbonic anhydrase	Catabolism
pcr-miR 171, 390, 394, 413, 834, 2111, 2867, 3440, 3442, 4224, 5836	AJ843185	Carbonic anhydrase	Catabolism
pcr-miR 413, 852, 900,951, 1039, 1062, 1072, 1088, 1103, 1104, 1107, 1155, 1161, 1846, 2097, 2926, 5523	GQ921315	Assimilatory nitrate reductase-like (NR) gene	Catabolism
pcr-miR 419, 839, 1072, 1103, 1161, 1846, 2112, 3440, 3630, 4224, 5503	D86050	Carbonic anhydrase	Catabolism
pcr-miR 447, 838, 951, 1088, 1155, 1161, 2097, 2111, 2867, 2926,	DQ308459	photosystem I P700 chlorophyll A apoprotein	Catabolism
pcr-miR 834, 843, 900, 1039, 1062, 1072, 1092, 1103, 1165, 1848, 2091, 2111, 2112, 2926, 3630, 5836, 6104	AB040136	Carbonic anhydrase	Catabolism
pcr-miR 839, 843, 1039, 1846, 2091, 2926, 5836,6104	AB040135	Carbonic anhydrase	Catabolism

Another important protein of the chloroplast is oxygen evolving enhancer (OEE). The OEE is consist of three subunits, OEE 1 (33 kDa), OEE 2 (23 kDa) and OEE 3 (16 kDa). All these three OEEs are produced by nuclear-encoded genes PsbO, PsbP and PsbQ, respectively (Sugihara, 2000) and marginally bound to photosystem II (PSII) on the thylakoid membrane. The signaling peptide which directs OEEs to their proper section of chloroplast exists in the N-terminal end (Abbasi & Komatsu, 2004). Fathei *et al.,* (2012), observed an up regulation in OEE2 in salt-resistant and salt-sensitive barley genotypes during salinity. Previously, it was also reported in salt adaptation in cultured tobacco cells (Murota *et al.,* 1994), mangrove and rice (Sugihara *et al.,* 2000; Abbasi& Komatsu 2004). This protein is also observed to enhance its expression under biotic stress in tobacco (Perez-Bueno, 2004). The increased expression of OEE2 suggests its role to repair protein damage caused by dissociation and to keep oxygen evolving. In the present study, the newly identified *P. cruentum* miRNAs; pcr-miR156, 159, 165, 171, 394, 419, 838, 843, 1062, 1073, 1103, 1104, 1155, 1161, 1165, 1848, 2867, 2926, 3442, 3630, 4224, 5503, 5523, 5819 are found to target the oxygen evolving enhancer protein.

Conclusion

This research is resulted 49 novel miRNAs and their 13 targets for the first time in *P. cruentum* a unicellular red alga. These *P. cruentum* miRNAs are the significant resource to modify photosynthetic genes expression and ultimately plant productivity. And also these findings will also play significant role to elaborate the functional genomic resources in *P. cruentum*,

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