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Expression analyses of miRNA Up-MIR-843 and its target genes in *Ulva prolifera*

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Abstract

microRNAs (miRNA) families play a critical role in plant growth, development, and responses to abiotic stress. In this study, we characterized Up-miR-843 and its targets genes in *Ulva prolifera* responses to nitrogen depravation and heat stress. The data demonstrated that 184 target genes of Up-miR-843 could be successfully validated. N deficiency not heat stress stimulus induced increase in abundance of the Up-miR-843 while exhibited reverse expression of target genes, including cyclin A3 and cyclin L, which were strictly required for cell cycle progression. In addition, *U. prolifera* with highly expression of Up-miR-843 showed improved biomass, and photosynthesis compared with that under normal growth conditions. Thus, the N deprivation and heat responsive miRNAs might be a possible member mediating the expression of these target genes, which further regulated the growth of *U. prolifera*.

Key words: cyclins, nitrogen deprivation, microRNA, *U. prolifera*, heat stress

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1 Introduction

Ulva prolifera is one of the most common bloom-forming macroalgae, which broken out since 2008 in the Yellow Sea (Lin et al., 2011) and have caused both ecological and economic impacts to coastal environments and human activities. These blooms of opportunistic macroalgae are generally explained by eutrophication caused by the increased nutrient (Conley et al., 2009; Pérez-Mayorga et al., 2011), optimal light, temperature and so on (McGlathery, 2001; Taylor et al., 2001).

While physical forcing by winds and tides in shallow estuaries can lead to the rapid replacement of more saline, nutrient-poor marine water, higher surrounding temperature (Stumpf et al., 1993), *Ulva* still exhibited rapid nutrient uptake and subsequent growth. For example, *U. lettuce* maintained the ability to take up ammonium and nitrate under conditions of rapidly changing salinity (Lartigue et al., 2003), and nitrogen would be the limiting for the growth of *U. curvata* only at saturated light conditions (Coutinho and Zingmark, 1993). Therefore, we hold the view that *Ulva* might evolve self-regulating mechanisms to adapt to this abiotic stress.

miRNAs are a group of the non-coding small RNA, which play vital roles in mediating plant growth, development, abiotic stress adaptation and so on (Jones-Rhoades et al., 2006; Voinnet, 2009). During past decade, various of the miRNAs that are involved in transducing nutrient signaling, including starvation of nitrogen (Gao et al., 2016; Paul et al., 2015) have been documented, for example, miR-395, miR-399 mediated the regulation of sulfate and phosphate homeostasis, respectively (Chiou et al., 2006; Liang et al., 2010); and miR-169 oppositely regulated N starvation response via target NFYA (nuclear factor Y, subunit A) in *Arabidopsis thaliana* (Zhao et al., 2011). In addition, the small RNA-sequencing methods suggested that elevated temperature can alter the expression of miR-156/miR-157 and miR-172, mediating the growth and development of *Arabidopsis* (May et al., 2013), miR-396 protected sunflower from high-temperature stress via post-transcriptionally regulation of HaWRKY6 (Giacomelli et al., 2012). Therefore, it is safe to presume that plant cells must be capable of sensors (protein or other macromolecules) to adapt to the changes, while there has been less previous evidence for how these sensors participate in mediating development of *U. prolifera* adaptation to abiotic stress. In this study, we verified expression of miRNAs under N limitation or heat stress conditions based on the constructed small *U. prolifera* thalli RNA library (Huang et al., 2011), and investigated the changes of the candidate target genes, which may help elucidate the complex mechanisms as to how *U. prolifera* tolerate theses stressors.

2 Materials and methods

2.1 *Alga culture and treatments*

Floating *U. prolifera* was gifted by Jiacheng Li from Zhejiang University, China. The algal was cultured at 20°C in SPX-GB-250 intelligent illumination incubators (Botai, Shanghai, China) for 4 d in fresh distilled seawater. Then *U. prolifera* was cultured in prepared cultural medium, which was made up as reported (Breuer et al., 2012).

Light was provided by a halogen lamp at PAR of 100 μmol/(m·s) and light time was 12 h. The culture medium was renewed every day, temperature stress experiments were performed after pretreatment, and 36°C was set as the heat stress conditions.

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2.2 *Determination of growth rates and contents of chlorophylls*

Approximately 0.1 g algal was cultured under the pre- or N-culture medium at 20°C, or in the pre-culture medium at 36°C in the incubators and at the indicated time points, the algal material was reweighed.

For chlorophylls detection, samples were ground in liquid nitrogen, suspended in extraction buffer (80% (v/v) acetone). After incubation on ice for 15 min, the samples were centrifuged at 6 000 r/min for 10 min at 4°C. Concentration (c) of chlorophylls (a, b) was determined according to the methods described by Arnon (1949). The equations were used as following:

$$c_{\text{Chl } a} = (12.71 \times A_{663} - 2.59 \times A_{645}) \times V / (W \times 1\,000),$$

$$c_{\text{Chl } b} = (22.88 \times A_{645} - 4.67 \times A_{663}) \times V / (W \times 1\,000),$$

where V represents the volumes of extraction buffer, and W represents the weight of algae samples used.

2.3 *Gene expression analysis*

Ulva prolifera was harvested at different time point and grinded in liquid nitrogen. RNA was extracted using TRIzol reagents (Thermo Fisher Scientific, Waltham, MA, USA), and total 2 μg RNA was reversed using Hifair™ II 1st Strand cDNA Synthesis Kit (Yeasen, China). For miRNA detection, total 2 μg RNA was reversed using miRNA First Strand cDNA Synthesis (Sangon Biotech, China). Then the expression of genes was detected through Applied Biosystems™ Power™ SYBR™ Green Mix (Thermo Fisher Scientific) and calculated as described by Liao et al. (2017). The specific primer pairs used for qRT-PCR are listed in Table 1, 18S rRNA and U4 snoRNA were used as internal controls for mRNA and miRNAs, respectively. All tests were made for three times in a same way with independent sample data.

2.4 *Identification of the Up-MIR-843 target genes*

To characterize the target genes interacted with Up-MIR-843, an online tool referred to as psRNATarget (Plant microRNA Potential Target Finder; http://plant grn.noble.org/psRNA Target/) was run to scan against cDNA databases using the mature Up-MIR-843 sequence as query. Since the cDNA database for *U. prolifera* was not totally published, the similar cDNA library used for prediction contained *Arabidopsis thaliana*, transcript, JGI genomic project, Phytozome 11, 167 TAIR10. The target gene functions were defined based on BLASTx search results in NCBI (https://www. ncbi. nlm. nih. gov/).

2.5 *Statistical analysis*

The mean SE of the three independent experiments was considered as each value. All data were analyzed by SPSS 20.0 (Windows, USA). Use Duncan's test and ANOVA to determine significance. A probability level of 5% (P< 0.05) was regarded as statistically significant.

3 Results

3.1 *Effect of temperature and N starvation on the growth of U. prolifera*

To verify the effect of abiotic stress on the growth of *U. prolifera*, algal was cultured under different conditions, the weight of which was weighed every day. The results demonstrated that compared with normal culture condition, the weight of *U. prolifera* maintained stability over heat or N deprivation stress, however, *U. prolifera* had notably decreased chlorophyll *a* and chlorophyll *b* contents exposure to N starvation condition not to heat stress (Fig. 1).

3.2 *Up-miR-843 was conversed across green algae and embryophyte*

Based on the sole published constructed and sequenced small RNA libraries from *U. prolifera* (Huang et al., 2011), firstly we verified expression of miRNAs using qRT-PCR assay, and miRNAs within count less than 500 were excluded in the study. The chosen miRNAs were identified to search the homologues in miRbase database (http://www.mirbase.org/search.shtml). As shown in Table 2, among the 60 candidate miRNAs, only 13 miRNAs matched the corresponding other plant miRNAs. The homologs of miR-1 048, miR-2 119, miR-2 679, miR-2 916 and miR-390 in *U. prolifera* were found in *Physcomitrella patens*, *Glycine max*, *Medicago truncatula*, *Populus euphratica*, respectively. miR-3 911 and miR-3 519 matched the homologs in *Arachis hypogaea*. Five miRNAs homologs, miR-443, miR-827, miR-1858, miR-1874, and miR-1877 were found in *Oryza sativa.*

Among these identical miRNAs, the mature sequence of Up-miR-843 is 19 nt-long in length (5'-TTTAGGTAGAGCCTCATGA-3'), which was identified to be identical to Ath-miR-843 (Fig. 2), which suggested that the miR-843 members may be conserved across green algal and embryophyte.

3.3 *Up-miR-843 targets different genes functional in various biological process*

Based on running an online tool (http://plantgrn.noble.org/psRNATarget/), the genes putatively targeted by miRNAs were predicted. Results indicated that totally 184 genes (including

Table 1. Sequences of primers for qRT-PCR assays

Gene		Sequence of primers (5′ to 3′)
Up-miR-843	forward	GCGCAGTTTAGGTAGAGC
	reverse	GTCCAGTTTTTTTTTTTTTTTCATGAG
Ath-miR-843	forward	GCAGTTTAGGTCGAGCTTCA
	reverse	GGTCCAGTTTTTTTTTTTTTTTCCA
Cyclin L	forward	TGTGGTGCTGTACGTTGGAG
	reverse	ACCGCAGACGTTTCTCAGTT
Cyclin A3	forward	CGCTCATTGTCCACATCCCT
	reverse	GGCATACTGCTCTAGCGTCC
18S rRNA	forward	CGCAACTCCCGACTCACGAAGG
	reverse	ACCGGACCATGTGGCCCAGAAT
SnoRNA U4	forward	CAAAAGGCCCGACAGAAAT
	reverse	GTGAGGTCTAACCGAGTCGC

Note: Up, *Ulva prolifera*; Ath, *Arabidopsis thaliana.*

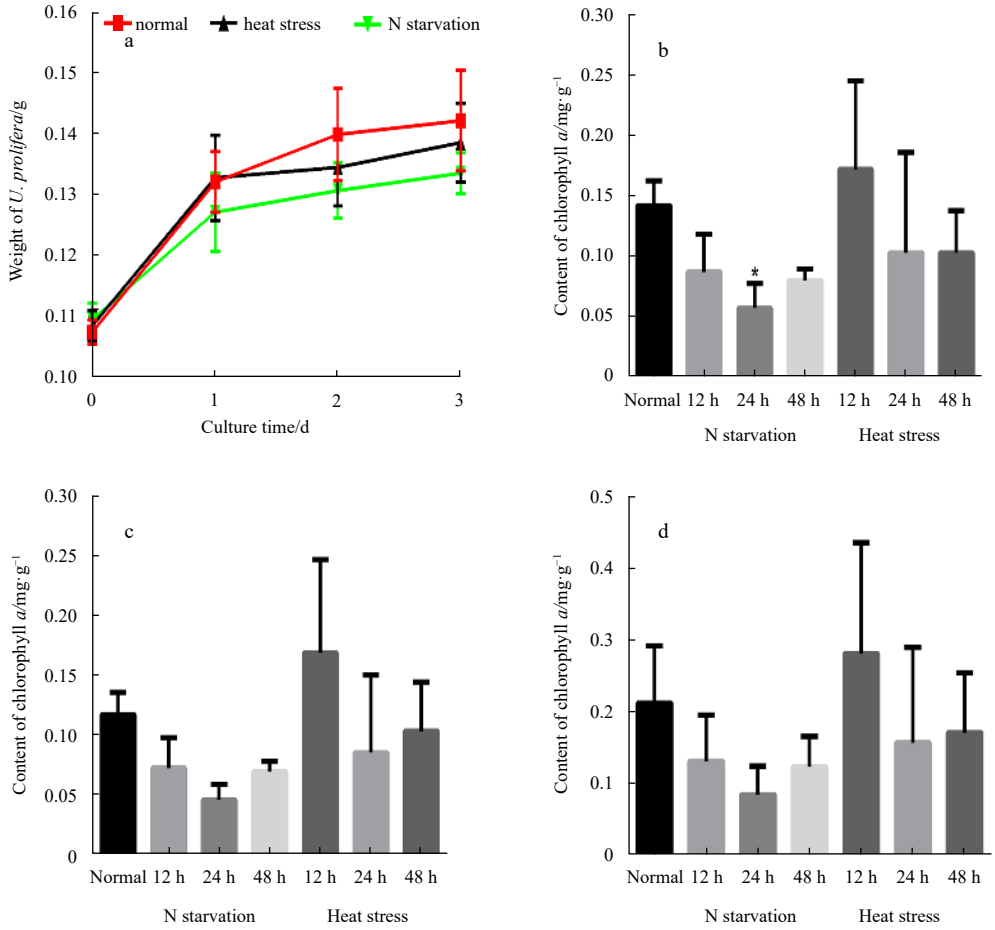

Fig. 1. Growth rates and contents of chlorophyll in *U. prolifera*. a. Growth ratio of *U. prolifera* under N depravation or heat stress. b–d. Contents of chlorophyll (*a*, *b*) and total chlorophyll in *U. prolifera*. $^{*}P<0.05$, vs. normal. Normal represents *U. prolifera* cultured in normal culture medium at 20°C, N starvation represents *U. prolifera* cultured in N deficiency culture medium at 20°C, and heat stress represents *U. prolifera* cultured in normal culture medium at 36°C.

Table 2. Expression profile of homologs of miRNAs in *U. prolifera*

U. prolifera miRNA family (sequence, 5′ to 3′)		Homologs (sequence, 5′ to 3′)	
miR-1023	AGAGAACTCGGGTGGAGGCAG	eca-miR-8924	UGGGACUCGGGUGGAGGCUCUCCUG
miR-1026	TGAAATGACTTGAGAGGTAGCA	mdo-miR-12374-3p	AGCUGCCCUUGCUUUGUCAUUUU
miR-1037	CCTTATTGGATTGGATGG	no matches	
miR-1043	ATGCGCGTGAATGATGAAGGCCT	osa-miR-1877	AGAUGACAUGUGAAUGAUGAGGGG
miR-1048	TGAACATGAGAGTGGACGA	ppt-miR-1048-5p	UAGAACAUGAGUGUAGACGA
miR-1061	TGTTAGCATGGAATAATG	no matches	
miR-2119	CAAAGGAGTGGTAGGGGA	gma-miR-2119	UCAAAGGGAGUUGUAGGGGAA
miR-2626	AAGGTCGGGATGAGGTGTT	bbe-miR-92d-3p	UAUUGCACUUAUCCUGGCCUGU
miR-1091	CGGGTGACGGAGGATTAGC	sma-miR-8475-5p	UAUCUCCUUCUAUUCCCCGG
miR-1110	GCAGGGCGGTGGTCATGGA	gga-miR-7467-5p	GCCAGCGCGGUGGUGAUGGUGCC
miR-1113	TGACAGTCCTAAGGTAGC	smo-miR-1113	UGAGCAGUCAUAAGGUAGCCU
miR-1127	TAGCTTTGTTCGGAATTAC	no matches	
miR-1140	ACGCCTACAATCAGTCGGAGC	dre-miR-2194	GUAAUGCUUCGACUGAUUGGUG
miR-1154	CTTAGTCGATCCTAAGGCAT	no matches	
miR-2634	TTTATTCTCGTTGGTTTC	no matches	
Up-miR-2651	TGAACATTGTTGAGACGAGTGACTT	cin-miR-4018a-5p	CGGAACAUUGUUGGAACGGG
miR-2678	TGAACATTGTTGAGACGAGTGACTT	cin-miR-4018a-5p	CGGAACAUUGUUGGAACGGG
miR-2679	CGGTTCATTTCGGAACGGGTG	mtr-miR-2679a	CUUUUCACUUUCGAACGGGUG
miR-2680	TCCTCGGACCTACGTGTGGT	no matches	
miR-2866	TCAGTTTGTTAGAGCATC	gga-miR-1727	AAGCUGCUCUAAUGAACUGAAGG

to be continued

Continued from Table 2

U. prolifera miRNA family (sequence, 5′ to 3′)		Homologs (sequence, 5′ to 3′)	
miR-2880	ACGGTAATGCCGTTCGGAAGTG	no matches	
miR-2910	TCTTAGTTGGTGGAGCGATTTGT	oan-miR-1406-3p	CCUCUCUCCACCACCUAAAUGA
miR-2914	GTGGTGGTAACGGGTGACGGA	hpo-miR-10030-5p	GAUAAUAAACACCACUUGCAGCAGU
miR-2915	TCCCCTGTAGCTCAGTTGGTA	gga-miR-1692	UGUAGCUCAGUUGGUAGAGU
miR-2916	GGGGCTCGAAGACGATTAGAT	peu-miR-2916	UGGGGACUCGAAGACGAUCAUAU
miR-2948	TAGTGCGAGTAGATGGGCAAGAATC	ghr-miR-2948-5p	UGUGGGAGAGUUGGGCAAGAAU
miR-3476	TGACTGGAGGATGTTGGCTGC	cja-miR-616	UCACUGGAGGAUUUUGAGCAGA
miR-3511	CCGGGCAACGAATGCAGA	ahy-miR-3511-5p	GCCAGGGCCAUGAAUGCAGA
miR-3519	TCAAACAATGACAGCATCTGATCCA	ahy-miR-3519	UCAAUCAAUGACAGCAUUUCA
miR-390	AAATAGTAGGGATAGCACC	gma-miR-390b-5p	AAGCUCAGGAGGGAUAGCACC
Up-miR-395	CTGAAGATCGGAGGACCAC	mmu-miR-7005-5p	CCUGGGGAUGGGAGGACCAGCA
miR-398	TGTGTTCATCACGGACAGCACCCTG	ppy-miR-485-5p	AGAGGCUGGCCGUGAUGAAUUC
miR-414	TATCTCATCGTCATCGCCA	hpo-miR-10078-5p	AUCUCAUCCUGAUCGCCA
miR-419	TATGACTGATGACGTGTT	no matches	
miR-437	AAAGGTAGAAGTTTGCTT	aca-miR-5397-5p	UACGCAGAAGUUUGCUUCAUCA
miR-443	ATACATGCAAAAAATCTGGA	osa-miR-443	AUCACAAUACAAUAAAUCUGGA
miR-533	CTGACACAGGTGCTGCATGGCTGTC	rno-miR-324-3p	CCACUGCCCCAGGUGCUGCUGG
miR-827	TTAGAGGACGATCAGCAAC	osa-miR-827	UUAGAUGACCAUCAGCAAACA
miR-1531	ACGTCCACTCTGGGAAGACGTTGT	cpo-miR-12077-3p	AACUGAGGCCACUCUGGGUAGA
miR-1520	TCAGAACTGAAACGGACAA	eca-miR-9145	UUAGCGUUUGAGUUCUGACAA
miR-1441	ACCGAAGTGGAGAAAGGTTT	mmu-miR-7086-5p	AAGAGGAGAAAGGUUUGGGCA
miR-1318	TCAGGAGATGATGACATAGAAC	ppc-miR-2234a	UGAUAUGUCAUCAUCUUCCUUU
miR-1221	TTGAAAGGGACTTGCAGTGGTACAC	bta-miR-2367-3p	UUGAAAGGCACUUACAGGAAG
miR-1217	TGGAACATGTTGGCAAATTGC	ptc-miR-6463	UGGAUGAUCAUGUUGGCAACC
miR-1168	ACACGGACAAGGCGAAGAA	no matches	
miR-1165	TACCTACAAGCGGTCGGAGCC	no matches	
miR-169	TAGCCAAGATATTGACGTTGGCCTG	dme-miR-977-3p	UGAGAUAUUCACGUUGUCUAA
miR-1583	TATTGGGCATGATTCGGTTGACAT	no matches	
miR-1858	TAGAGGAGGACGGAGTGAGTAGGCT	osa-miR-1858a	GAGAGGAGGACGGAGUGGGGC
miR-1874	TAGGAAGTAGGTGTAACCCGCATG	osa-miR-1874-3p	UAUGGAUGGAGGUGUAACCCGAUG
miR-1875	ACAGATGGAATGACTGCAACAGAAG	cpa-miR-8145	AUAAACAGAUAGAAUGACAGCCUU
miR-1877	AATGACATGTGTAGGATAGGGG	osa-miR-1877	AGAUGACAUGUGAAUGAUGAGGGG
miR-1884	TGTGACGGCGTGCCTTTTGCAT	no matches	
miR-1919	ACGAGAGTATTTGGAAGG	no matches	
miR-952	AACGAGGATCCATTGGAG	no matches	
miR-947	ACATCGGAATGTGTATACTAGTGTC	cpi-miR-9588-5p	CCUCAGAUGUGUCUACUAGUCU
Up-miR-916	CAAGAGGTCATCGGTTCGAC	ppc-miR-8333-5p	AUCAAGAGUUCAUCGUUUUGAC
miR-904	TCTTGTCACATGGAATGTAGGGGCA	gma-miR-1520m	AAUCAGAACAUGACAUGUGACAAU
miR-843	TTTAGGTAGAGCCTCATGA	Ath-miR-843	UUUAGGUCGAGCUUCAUUGGA
miR-833	TGTTTTTGACTCGGTATAGA	no matches	

Note: Up, *Ulva prolifera*; eca, *Equus caballus*; mdo, *Monodelphis domestica*; osa, *Oryza sativa*; ppt, *Physcomitrella patens*; gma, *Glycine max*; bbe, *Branchiostoma belcheri*; sma, *Schistosoma mansoni*; gga, *Gallus gallus*; Ath, *Arabidopsis thaliana*; cpi, *Chrysemys picta*; cpa, *Carica papaya*; dme, *Drosophila melanogaster*; ptc, *Populus trichocarpa*; ppc, *Pristionchus pacificus*; mmu, *Mus musculus*; cpo, *Cavia porcellus*; rno, *Rattus norvegicus*; aca, *Anolis carolinensis*; hpo, *Heligmosomoides polygyrus*; ppy, *Pongo pygmaeus*; ahy, *Arachis hypogaea*; cja, *Callithrix jacchus*; ghr, *Gossypium hirsutum*; smo, *Selaginella moellendorffii*; peu, *Populus euphratica*; hpo, *Heligmosomoides polygyrus*; oan, *Ornithorhynchus anatinus*; mtr, *Medicago truncatula*; cin, *Ciona intestinalis*; dre, *Danio rerio*. The miRNAs marker with gray could alignment to homologs in plants.

some genes encoding proteins unknown) were interacted by this *U. prolifera* miRNA, whilst genomic database for *U. prolifera* was still unpublished, so we used *Arabidopsis thaliana* cDNA database as cardiant target library. The target genes with the accurate annotation were as shown in Table 3, which contains genes encoding calmodulin-binding receptor-like cytoplasmic kinase 3, NIMA-related kinase 7, Protein phosphatase 2A regulatory B subunit family protein, peptide-N-glycanase 1, F-box and associated interaction domains-containing protein, Cyclin family protein and so on. Among which, we chose cyclins as the cardiants basing on the RNA-seq previously reported (He et al., 2018) before. Table 4 indicated all the cyclins in the *U. prolifera*.

3.4 *Up-miR-843 and its target genes under abiotic stress*

Expression patterns of Up-miR-843 and its target genes upon N starvation or high temperature stress were investigated in more detail. Results indicated Up-miR-843 showed down-regulated transcripts abundance after 12 h, while all target genes displayed reverse expression patterns to miRNAs upon N starvation, such as upregulated cyclin A3 expression over 24 h whilst increased levels of cyclin L over 12 h, the promoted transcripts under stresses were gradually repressed along the exposed time (Fig. 3).

Fig. 2. Stem-loop structure of Ath-miR-843 and base pairing characterization between miR-843 and its target genes. a. Secondary stem-loop structure initiated by the Ath-miR-843 precursor, the Ath-miR8-43 mature sequence is highlighted by red color. b-c. Base pairing characterization between miR-843 and its target genes. Since the cDNA library of *U. prolifera* was unpublished, the genes putatively targeted by Up-miR-843 were predicted from *Arabidopsis thaliana* cDNA database.

Therefore, Up-miR-843 regulates the target genes post-transcriptionally and establishes putative miRNA/target modules that mediate plant nitrogen starvation and high temperature stimulus.

4 Discussion

To our knowledge, abiotic stress conditions such as extreme temperature, salinity, nutrient deprivation, would inhibit root development, photosynthesis, and further thereby causing the damage of the overall plant growth and productivity (Suzuki et al., 2014; Zhang, 2015). The mechanism underlying such responses results from the differential production of several transcripts and their associated proteins. As one class of post-transcriptional regulators of gene expression, microRNAs (miRNAs) have been proven to play a central role in many biological processes including organ development, phase transition, and abiotic stress tolerance (Hackenberg et al., 2015; Niu et al., 2016; Vidal et al., 2010; Bi et al., 2007). For instance, miR-169, miR-398a decreased in *Arabidopsis* seedlings, whereas pri-miR-156, miR-447c were induced under N limitation (Hsieh et al., 2009), and nine miRNA families (miR-160, miR-167, miR-168, miR-169, miR-319, miR-395, miR-399, miR-408, and miR-528) were identified to respond to low nitrogen in maize roots (Xu et al., 2011). In our study, we found that 12 h of N limitation decreased the levels of *U. prolifera* miRNA (Up-miR-843), whereas it was restored after 48 h exposure to nitrogen deficiency. Hence, this miRNA was identified to be identical to *Arabidopsis Thaliana* homolog miRNA (Ath-miR-843), indicating the miR-843 members are conserved across green algal and embryophyte. Furthermore, as previously reported, low levels of miR-843 contributed to the tolerance of plants to nutrient deficiencies (Lu et al., 2014), we also found N deficiency inhibited the expression of miR-843, which provided the evidence that Up-miR-843 might play an important role in the development of *U. prolifera* under nitrogen deficiency stimulus.

Expect for the nutrient deficiency mentioned above, temperature is also a critical factor controls plant growth and development (Patel and Franklin, 2009). For most plant species, heat stress induced protein denaturation (Hasanuzzaman et al., 2013), while *U. prolifera* started to proliferate followed the increased temperature from 9.9°C to 20.3°C (Wang et al., 2015), even maintained the stability of contents of chlorophylls and growth rates at 36°C, which is of interest to know whether *U. prolifera* developed unique adaptations. To our knowledge, temperature response connected non-coding RNAs has been observed in different plant species, such as heat stress induced the expression of mature-miR-160a, miR-166a in spring barley plants (Kruszka et al., 2014), and enhanced levels of miR-162b, miR-171b in *Oryza sativa* (Li et al., 2015). In this study, the decreased trends of miR-483 was found but no significant difference was verified, suggesting that *U. prolifera* could burden heat stress more than other plants, and miRNAs might not the mediator in this process.

So why non-coding RNAs functioned differently in *U. prolifera* facing the N deficiency and heat stress stimulus, respectively. A total of 184 target genes of Up-miR-843 was identified as the effectors, especially two cyclins families. Hence, based on the transcriptome sequencing studies in our laboratory (He et al., 2018), we demonstrated that cyclin U4, L, A2, C, A3 in *U. prolifera* shared the differently similarity with *Selaginella moellendorffii, Brassica napus, Aphis gossypii, Brachypodium distachyon* and *Arabidopsis thaliana*, respectively, which is consistent with the cyclins family members in other plants (Mironov et al., 1999). However, only expression of cyclin L, A3 could be detected using qRT-PCR analysis in this study, which suggested cyclin L, A3 may be the two conversed cyclin genes during the evolution of plants. As previous report mentioned, the steady-state levels of cyclin A RNAs increased at or after the onset of S phase till G2 and occasionally M phases, and cyclin B RNAs only present in G2 and M phases (Ferreira et al., 1994; Kouchi et al., 1995; Meskiene et al., 1995), especially cyclin A3 genes occurred earlier in S phase (Reichheld et al., 1996), similarly, 24 h of N starvation not heat stress induced the expression of cyclin A3 and cyclin L. Based on the findings that cyclin L overexpressing could stimulate splicing and/or other steps of mRNA metabolism, further grant salt tolerance in *Saccharomyces* (Forment et al., 2002), we hold the hypothesis that miR-843 was a possible molecular mediating the expression of cyclin A3 and cyclin L, which reinforced the import-

Table 3. The targets of conversed mRNAs

Gene accession	Target protein
AT2G11520.1	calmodulin-binding receptor-like cytoplasmic kinase 3
AT4G24290.2, AT4G24290.1	MAC/perforin domain-containing protein
AT1G66430.1	PFKB-like carbohydrate kinase family protein
AT3G21650.1, AT3G21650.1	protein phosphatase 2A regulatory B subunit family protein
AT5G49570.1	peptide-N-glycanase 1
AT3G12200.1, AT3G12200.2	NIMA-related kinase 7
AT5G42480.1	chaperone DNAJ-domain superfamily protein
AT1G14750.2, AT1G14750.1	cyclin family protein
AT3G53402.1	conserved peptide upstream open reading frame 46
AT3G25500.1	formin homology 1
AT3G51240.2, AT3G51240.1	flavanone 3-hydroxylase
AT5G07220.1	BCL-2-associated athanogene 3
AT2G46180.1	Golgin candidate 4
AT1G08260.1	DNA polymerase epsilon catalytic subunit
AT3G06610.1	DNA-binding enhancer protein
AT5G40470.1	RNI-like superfamily protein
AT1G50270.1, AT4G35850.1, AT5G52850.1, AT5G04810.1	pentatricopeptide repeat superfamily protein
AT1G51320.1, AT1G60370.1	F-box and associated interaction domains-containing protein
AT4G20980.4, AT4G20980.3, AT4G20980.1, AT4G20980.2	eukaryotic translation initiation factor 3 subunit 7
AT5G56000.1, AT4G17750.1, AT5G56010.1	heat shock protein
AT2G38490.1	CBL-interacting protein kinase 22
AT5G09330.3, AT5G09330.4, AT5G09330.1, AT5G09330.2	NAC domain containing protein 82
AT3G08947.1, AT3G08943.1	ARM repeat superfamily protein
AT5G02590.1	tetratricopeptide repeat-like superfamily protein
AT3G58580.1	DNAse I-like superfamily protein
AT3G17152.1	plant invertase methylesterase inhibitor superfamily protein
AT1G64070.1	disease resistance protein family
AT4G33020.1	ZIP metal ion transporter family
AT1G59359.1, AT1G58684.1, AT1G58983.1	ribosomal protein S5 family protein
AT5G01700.2, AT5G01700.1	protein phosphatase 2C family protein
AT2G01110.1	sec-independent periplasmic protein translocase
AT3G63130.1, AT3G63130.2	RAN GTPase activating protein 1
AT4G24190.2, AT4G24190.1	chaperone protein htpG family protein
AT5G43960.2, AT5G43960.1	nuclear transport factor 2 family protein with RNA binding domain
AT2G44350.1, AT2G44350.2	citrate synthase family protein
AT5G49160.1, AT5G57300.1, AT5G57300.2, AT5G57300.3	methyltransferase
AT3G53090.2, AT3G53090.1	ubiquitin-protein ligase 7

Table 4. The valiant cyclins family in *U. prolifera* based on the BLASTx analysis

Gene ID	BLASTx	
	Species	Gene
CL36416Contig1_3	*Selaginella moellendorffii*	cyclin U4
CL7758Contig1_3	*Brassica napus*	cyclin L
TRINITY_DN1459_c0_g2_i1_2_2	*Aphis gossypii*	cyclin A2
TRINITY_DN20783_c0_g1_i5_2_1	*Brachypodium distachyon*	cyclin C
TRINITY_DN4058_c0_g1_i2_1_2	*Arabidopsis thaliana*	cyclin A3

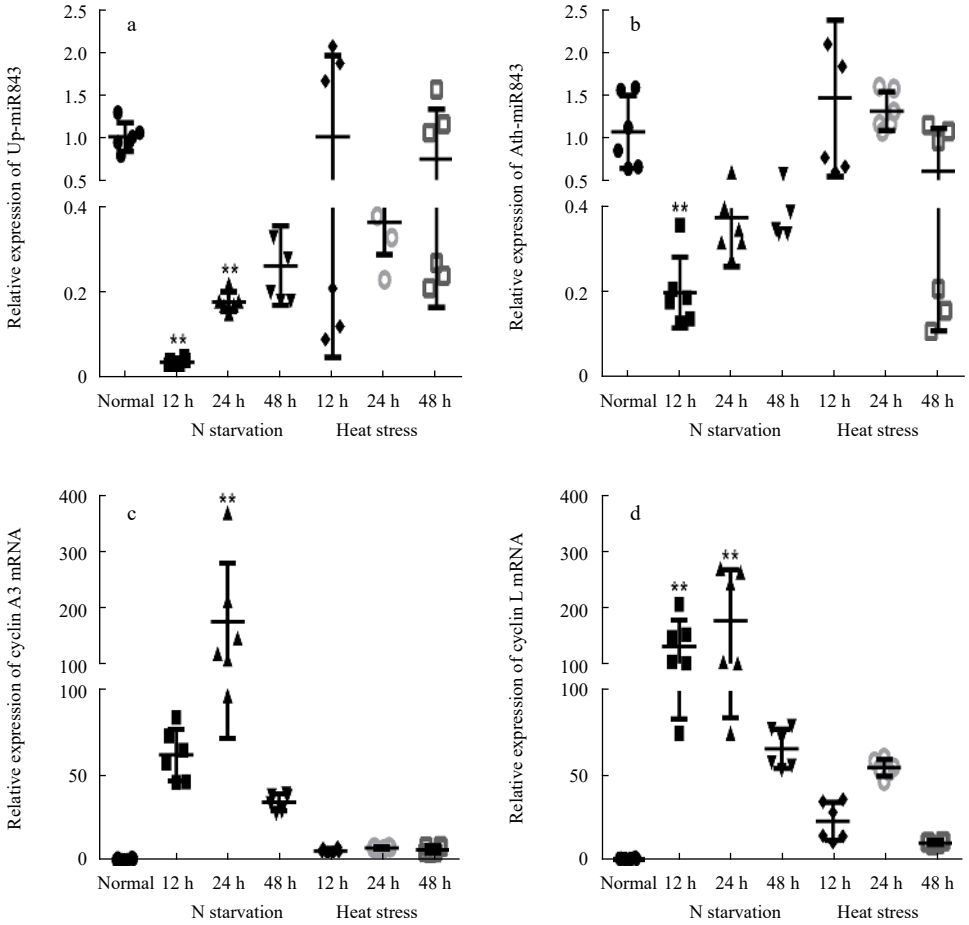

Fig. 3. Expression patterns of miR-843, and the target genes upon N starvation and heat stress. a, b. Expression patterns of Up-miR-843 and Ath-miR-843; and c, d. expression patterns of cyclin L and cyclin A3. Genes were detected at time points after N starvation or heat exposure for 12 h, 24 h, 48 h. $^{**}P<0.01$, vs. normal. Normal represents *U. prolifera* cultured in normal culture medium at 20°C, N starvation represents *U. prolifera* cultured in N deficiency culture medium at 20°C, and heat stress represents *U. prolifera* cultured in normal culture medium at 36°C.

ant regulatory function of alternative splicing for plant abiotic tolerance, however, the mediation function of miR-843 needed further study.

5 Conclusions

In conclusion, we analyzed *U. prolifera* small RNA expression profiles and found

Up-miR-843 differentially expressed under N deficiency not heat stress stimulus. Hence, this abiotic-responsive miRNA targeted different transcription factors, among which both cyclin A3 and cyclin L functioned in stress response. Therefore, we hypothesized that miRNA might be a possible member mediating the expression of these selected genes in *U. prolifera*, and the function of this tiny non-coding RNA in complex biological networks needed further study.

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