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ORIGINAL ARTICLE

Profiling the Transcriptome of *Gracilaria changii* (Rhodophyta) in Response to Light Deprivation

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Abstract Light regulates photosynthesis, growth and reproduction, yield and properties of phycocolloids, and starch contents in seaweeds. Despite its importance as an environmental cue that regulates many developmental, physiological, and biochemical processes, the network of genes involved during light deprivation are obscure. In this study, we profiled the transcriptome of *Gracilaria changii* at two different irradiance levels using a cDNA microarray containing more than 3,000 cDNA probes. Microarray analysis revealed that 93 and 105 genes were up- and down-regulated more than 3-fold under light deprivation, respectively. However, only 50% of the transcripts have significant matches to the nonredundant peptide sequences in the database. The transcripts that accumulated under light deprivation include vanadium chloroperoxidase, thioredoxin, ferredoxin component, and reduced nicotinamide adenine dinucleotide dehydrogenase. Among the genes that were down-regulated under light deprivation were genes encoding light harvesting protein, light harvesting complex I, phycobilisome 7.8 kDa linker polypeptide, low molecular weight early light-inducible protein, and vanadium bromoperoxidase. Our findings also provided important clues to the functions of many unknown sequences that could not be annotated using sequence comparison.

Keywords cDNA microarray · Irradiance · *Gracilaria changii* · Light deprivation · Seaweed

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Introduction

Rhodophytes occupy a wide range of irradiance environments including irradiance extremes such as high latitude and high intertidal habitats subjected to long period of full sunlight and deepwater marine habitats with only 0.005% of surface irradiance (Graham and Wilcox 2000). Intertidal seaweeds must cope with both visible light and ultraviolet radiation and may face irradiance environments depleted in both light quantity and quality as coastal water may be turbid and with low light transmission. Red seaweeds cope with low irradiance by employing efficient light-harvesting mechanisms. *Chondrus crispus* that grew in deep waters was shown to have increased phycoerythrin content, and the efficiencies of photosynthesis increased with depth (Sagert et al. 1997).

The primary production of seaweeds displays a wide range of values due to variation in photosynthetic rates which depends on many variables (Figueroa et al. 2003); and light is among the most important ones. The growth of seaweeds at different irradiance levels is affected by variations in light-saturated photosynthetic rates (P_{max}) which is determined by the availability of CO_2 which is the primary substrate for ribulose-1,5-bisphosphate carboxylase/oxygenase (Mercado et al. 2000). Algae grown at high irradiance demonstrated higher P_{max} per chlorophyll (Chl) a than at low irradiance (Falkowski 1980; Richardson et al. 1983). *Gracilaria tenuistipitata* grown at high

irradiance had less cellular chlorophyll and a higher maximal photosynthetic rate per unit of Chl a than cells grown at low irradiance (Mercado et al. 2000).

Light also regulates the timing of seaweed growth and reproduction, agar yield and properties, starch content, and others. Increase in agar yield was reported in dim light, whereas starch content increases with light intensity (Rotem et al. 1986; Bird 1988). Ekman and Pedersén (1990) also recorded some improvement in agar properties after dark treatment. Despite the importance of light as an important environmental signal that regulates many important physiological and biochemical processes in seaweeds, little is known about the network of genes involved during light deprivation. In this study, we profiled the transcriptome of *Gracilaria changii* in response to light deprivation using a cDNA microarray containing more than 3,000 cDNA probes.

Materials and Methods

Seaweed Materials and Experimental Design

Fresh *G. changii* Xia & Abbott (Zhang, Abbott & Xia) were collected from mangroves at Morib (02°45.808′ N; 101°26.143′ E), Selangor, Malaysia. Mud and epiphytes were removed carefully from the seaweed samples. They were randomly checked for epiphytes under the dissecting microscope. The seaweeds were kept in artificial seawater (Marine Environment, Aqua Craft Inc., USA) at 30 ppt, which was close to the salinity at the collection site, for at least 3 h before they were used for further experiments. For treatments in the dark (D) and at a 12-h light and dark cycle with the light cycle under the illumination of two daylight fluorescent bulbs (Philips TL-D 18W/54, Japan) that provided 29.3 μmol photons/m^2s (L), we treated approximately 500 g of seaweed in 60 l of artificial seawater (pH 7) at 30 ppt at 24°C for 7 days, respectively. Each experiment was repeated three times at different dates, and they were referred to as biological replicates 1, 2, and 3, respectively, for cDNA microarray experiments. The thalli were collected after treatments and frozen in liquid nitrogen before storage at −80°C.

Preparation of cDNA Array

Polymerase chain reaction (PCR) amplification was performed on cDNA clones selected randomly from a previous study (Teo et al. 2007). Each PCR reaction contained 1× DyNazyme II buffer (10 mM Tris–HCl pH 8.8, 1.5 mM $MgCl_2$, 50 mM KCl, and 0.1% (*v*/*v*) Triton X-100), 0.2 μM T3 and T7 primer mixture, 0.2 mM deoxyribonucleotide triphosphate mix, one unit DyNazyme II DNA polymerase (Finnzymes, Espoo, Finland), and 20 ng plasmid DNA in a total volume of 80 μl. PCR was carried out in 96-well plates using the following steps: 94°C for 4 min; 35 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min; and a final extension at 72°C for 7 min. The PCR products were precipitated in 2.5 vol of 100% ethanol and 0.1 vol of 3 M sodium acetate pH 5.2, at −20°C for 30 min before centrifugation at 3,000×*g*, 4°C for 30 min. Ethanol was removed, and DNA was rinsed with 100 μl 70% ethanol. The pellet was then air-dried and resuspended in 20 μl distilled water.

The purified PCR products were resolved using 1% (*w*/*v*) agarose gel. Only PCR products with a minimum concentration of 200 ng/μl that showed single bands were arrayed on amion-silane coated glass surface (GAPII; Corning, NY, USA) using GeneTac G3 gridder (Genomic Solutions, Ann Arbor, MI, USA). The array contained 3,326 cDNA probes and 23 artificial control genes (Universal Lucidea Scorecard, Amersham Biosciences, Piscataway, NJ, USA).

RNA Extraction, Labeling, and Hybridization

Total RNA was isolated using the method described by Chan et al. (2004). A total of 5 μg total RNA was amplified in vitro using MessageAmp™ II aRNA Amplification Kit following the instructions of the manufacturer (Ambion, Austin, TX, USA). For each hybridization, 1 μg of amplified RNA (aRNA) and 0.5 μl mRNA spike mix from Lucidea Universal Scorecard (Amersham Biosciences, Piscataway, NJ, USA) were reverse-transcribed using CyScribe First-Strand cDNA labeling Kit (Amersham Biosciences, Piscataway, NJ, USA) according to the instructions provided by the manufacturer. Experimental RNAs from both seaweed samples, D and L, were labeled with Cy5 and Cy3, respectively. The purified labeled RNA samples were mixed in equal amount and dried using SpeedVac™ concentrator (Eppendorff, Hamburg, Germany). The pellet was resuspended in 7.5 μl nuclease-free water, heat-denatured at 95°C for 2 min, and added to equal amount of hybridization buffer provided by the CyScibe GFX™ purification kit (Amersham Biosciences, Piscataway, NJ, USA) and 15 μl formamide. The labeled RNAs were added to cDNA array, covered with cover slip, and sealed in VersaArray Hybridization Chamber (BioRad, San Jose, CA, USA). The sealed chamber was incubated at 42°C for 16 h. The arrays were washed in 1× Sodium Chloride-Sodium Citrate Buffer (SSC) with 0.2% (*w*/*v*) sodium dodecyl sulfate (SDS) for 10 min and subsequently washed twice in 0.1× SSC with 0.2% (*w*/*v*) SDS for 10 min at 42°C. An additional washing in distilled water at 42°C for 10 min was performed to remove the residues on the slide surface. Finally, the slide was dried by centrifugation at 160×*g* at room temperature for 2 min. In total, 18

hybridizations were performed for each experiment including three biological replicates with three technical replicates and dye swaps.

The arrays were scanned by using Affymetrix 428™ Microarray scanner (Affymetrix, CA, USA). Independent TIFF images from each channel (Cy5 and Cy3, respectively) were analyzed using Affymetrix® Jaguar™ software that performed grid alignment and subtraction of background intensities. TIGR Microarray Data Analysis System (Marine Information and Data Archive System) in TM4 suite (www.tigr.org/software/tm4) was used for normalization and data analysis. The raw data was first converted to *mev* and *ann* format by using TIGR ExpressConverter ver. 1.7 before dye swap, total intensity, and Lowess normalization were performed. The normalized data were analyzed using TIGR Multiple Experiment Viewer (MeV). The expression ratio of individual genes in each treatment was expressed as $\log_2$ ($\text{intensity}_{\text{treatment}}/\text{intensity}_{\text{control}}$). One-class *t* test ($P$=0.01, permutation=100) and adjusted Bonferroni *P* value correction was applied to reduce false discovery rate. The candidate genes with $\log_2$ ($\text{intensity}_{\text{treatment}}/\text{intensity}_{\text{control}}$)≥ 1.58 (or 3-fold) were identified as differentially expressed genes that were significant in each experiment.

Real-Time PCR

Real-time PCR was conducted to validate the results obtained from microarray analysis. Total RNA (5 μg) was treated with RNase free-DNase (Invitrogen, CA, USA) and reverse-transcribed to first-strand cDNA using StrataScript™ QPCR cDNA Synthesis Kit (Stratagene, CA, USA). PCR primers were designed using Primer3 (http://frodo.wi.mit.edu) for the cDNAs encoding light-harvesting complex I polypeptide (DV969011, 5′-AGC ACC TGC CAT CTC AGT AG-3′ and 5′-CGA GGC TTT CTT CCA GGT A-3′); low molecular mass early light-inducible protein (DV964113, 5′-TTT CGT CGC ATC CTC ATC-3′ and 5′-AGC ACA CCC CAG TCC TTT-3′); 14-3-3 protein (DV963521, 5′-CCC GTC TCA TCG TCT CAC-3′ and 5′-CCA TTG ACT CCT CTT TCT GC-3′); hedgehog protein precursor (DV963453, 5′-ACG ACG ACG AAG TCT GTT TC-3′ and 5′-AGC ACT CTC TCC AGC CTT G-3′); and endogenous control (DV965260, 5′-ACC ACC ACC ACC TCA TAC C-3′ and 5′-CCT TCT CAA CCT CCA GAC CT-3′). Real-time PCR was performed using Brilliant® II SYBR Green® QPCR Master Mix (Stratagene, CA, USA) according to the instructions of the manufacturer in Mx3005P real-time PCR machine (Stratagene, CA, USA) using the following steps: 95°C for 10 min; 40 cycles at 95°C for 30 s, 58°C for 60 s and 72°C for 60 s. The PCR reactions were repeated in triplicates, and their average threshold cycle numbers were used to calculate the fold change ($\log_2$) in expression.

Results

In this study, a custom-made cDNA microarray containing 3,326 cDNA probes that represented more than 2,117 tentative unique genes from *G. changii* was used to identify genes that were expressed at light-deprived condition. These cDNA probes were prepared from the same set of cDNA clones that have been used to generate expressed sequence tags in a previous study (Teo et al. 2007). For the identification of genes that were expressed during light deprivation, the labeled aRNA from both treatments (D and L) were compared using cDNA microarray. Analyses were conducted on seaweed collected from the same site at the same time in pair to reduce the variations due to natural environment and developmental stages. Hybridizations were repeated for three biological replicates, three technical replicates, and dye swap. Thalli from *G. changii* were pooled for RNA extraction to reduce individual variations.

Microarray analysis revealed that 93 and 105 genes were up- and down-regulated more than 3-fold under light deprivation, respectively. The results obtained from real-time PCR analysis supported the data generated using cDNA microarray analysis (Fig. 1). The genes encoding low mass light inducible protein, light harvesting complex I polypeptide, and 14-3-3 were found to be down-regulated under light deprivation, whereas the gene encoding sonic hedgehog protein precursor was shown to be up-regulated under light deprivation when analyzed using both cDNA microarray and real-time PCR. The transcripts that accumulated under light deprivation include vanadium chloroperoxidase, thioredoxin, ferredoxin component, and reduced nicotinamide adenine dinucleotide (NADH) dehydrogenase (Table 1, Supplementary Table 1). Among the

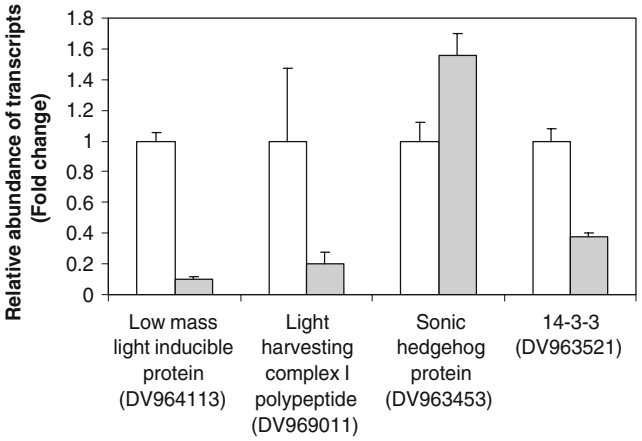

Fig. 1 Real-time PCR validation of cDNA candidates. *White and grey bars* represent the relative ratios of transcripts under light and dark conditions, respectively

Table 1 Transcripts with putative functions that were up-regulated at light deprivation

GeneBank accession number		Putative identity	*E* value	Log_2 (fold change)
DV968026	ref|ZP_00091527.2|	COG0627: Predicted esterase [*Azotobacter vinelandii*]	2.00E−13	1.7642862
DV967766	ref|NP_864925.1|	Vanadium chloroperoxidase [*Rhodopirellula baltica* SH 1]	4.00E−26	2.0116818
DV963441	gb|AAM62670.1|	Putative DnaJ protein [*Arabidopsis thaliana*]	2.00E−17	1.6070563
DV963453	gb|AAM94006.1|	Sonic hedgehog protein precursor [*Griffithsia japonica*]	5.00E−21	1.8055617
DV965012	pir||JC7180	14-3-3 [*Lentinula edodes*]	1.00E−87	3.6438563
DV962386	ref|NP_961104.1|	MbtG [*Mycobacterium avium* subsp. paratuberculosis str. k10]	3.00E−21	1.9111907
DV963402	dbj|BAB03163.1|	Phosphoprotein phosphatase [*Arabidopsis thaliana*]	2.00E−47	1.9385995
DV965228	gb|AAO20260.1|	Thioredoxin [*Chlamydomonas reinhardtii*]	7.00E−28	1.9393371
DV968094	gb|AAP80819.1|	Ferredoxin component [*Griffithsia japonica*]	5.00E−48	2.3985493
DV965678	ref|ZP_00351527.1|	COG2319: FOG: WD40 repeat [*Anabaena variabilis* ATCC 29413]	1.00E−18	1.941897
DV963514	gb|AAK44123.2|	Putative casein kinase II, alpha chain 2 CK II [*Arabidopsis thaliana*]	1.00E−128	1.9688776
DV966166	dbj|BAD00144.1|	Cyclin C [*Homo sapiens*]	5.00E−12	2.7395394
DV963232	ref|NP_594261.1|	Putative ATP-dependent RNA helicase [*Schizosaccharomyces pombe*]	1.00E−154	3.5313814
DV969651	ref|NP_244214.1|	RNA helicase [*Bacillus halodurans* C-125]	2.00E−28	4.607899
DV963442	ref|NP_916686.1|	Putative NADH dehydrogenase ubiquinone chain PSST precursor [*Oryza sativa* japonica cultivar-group]	3.00E−59	2.0747678
DV963496	gb|AAM65847.1|	NADH dehydrogenase, putative [*Arabidopsis thaliana*]	7.00E−64	2.7176003
DV968034	ref|XP_470714.1|	Putative ribonucleoprotein [*Oryza sativa*]	7.00E−05	1.9400574
DV963404	gb|AAS38843.1|	Similar to seryl-trna synthetase, cytoplasmic EC 6.1.1.11 [*Schizosaccharomyces pombe*]	7.00E−43	2.2108967
DV966115	ref|XP_519766.1|	PREDICTED: similar to 40S ribosomal protein S20 [*Pan troglodytes*]	9.00E−43	3.6147099
DV963543	ref|ZP_00314195.1|	COG1098: Predicted RNA binding protein contains ribosomal protein S1 domain [*Clostridium thermocellum* ATCC 27405]	6.00E−16	3.6147099
DV964091	sp|P53465|ACT1_LYTPI	Actin, cytoskeletal 1 LPC1	9.00E−69	2.376376
DV964066	gb|AAM93981.1|	Alpha 1 type VII collagen precursor [*Griffithsia japonica*]	2.00E−31	2.4718688

genes that were down-regulated in the dark were those encoding light harvesting protein (LHP), light harvesting complex I, gamma subunit, phycobilisome 7.8 kDa linker polypeptide and low-molecular-weight early light-inducible protein, mastigoneme-like proteins, polyubiquitins, adenosine 5′-phosphosulfate kinase, and vanadium-dependent bromoperoxidase (Table 2, Supplementary Table 2). The two transcripts encoding LHPs that reduced in the dark, DV969011 and DV963357, shared 64% and 55% identity to light harvesting complex I polypeptide from *Griffithsia japonica* and LHP from *Galdieria sulphuraria*, respectively. Both phycobilisome and low-molecular-mass early light-inducible protein showed high homology to their homologs from *G. japonica*.

Our findings also provided important clues to the functions of more than 130 unknown sequences that could not be annotated using sequence comparison (Supplementary Tables 1 and 2) since only 50% of the differentially expressed transcripts have significant matches to the nonredundant peptide sequences in the database.

Discussion

Light is an important environmental stimulus that regulates many physiological processes and biochemical pathways in seaweeds. In all eukaryotic oxygenic photosynthetic organisms, light harvesting chlorophyll a- or b-binding proteins function in the collection and transfer of light energy to the reaction centers of photosystem II and I. LHPs are important components that optimize photosynthetic function and minimize photooxidative damage in response to light quantity and quality (Staubar et al. 2003). In addition, there are three basic types of biliprotein-phycoerythrin, phcocyanin, and allophycocyanin that absorb light. Light energy that is absorbed by phycoerythrin migrates first to phycocyanin then to allophycocyanin and finally to chlorophyll. The phycobilisome is composed of two domains: a core and a number of rods. The organization of phycobilisome is established by polypeptides called linkers that are versatile and interact with phcocyanin and phycoerythrin to assemble the rods. In the core, they

Table 2 Transcripts with putative functions that were down-regulated at light deprivation

GeneBank accession number		Putative identity	*E* value	Log_2 (fold change)
DV963383	ref|ZP_00327160.1|	COG0337: 3-dehydroquinate synthetase [*Trichodesmium erythraeum* IMS101]	4.00E−32	−2.604862
DV968082	ref|ZP_00351610.1|	COG2303: Choline dehydrogenase and related flavoproteins [*Anabaena variabilis* ATCC 29413]	2.00E−10	−2.4253058
DV968104	ref|NP_624642.1|	Putative decarboxylase [*Streptomyces coelicolor* A32]	2.00E−44	−1.9946862
DV964139	gb|AAF94318.1|	Phosphoserine aminotransferase [*Vibrio cholerae* O1 biovar eltor str. N16961]	5.00E−46	−1.5866843
DV963581	gb|AAO16166.1|	Phosphotidyl inositol-specific phospholipase C [*Listeria monocytogenes*]	5.00E−05	−1.9237643
DV965009	ref|NP_914822.1|	Putative O-linked GlcNAc transferase [*Oryza sativa* japonica cultivar-group]	4.00E−52	−1.831123
DV966048	gb|AAR90852.1|	Pyruvate phosphate dikinase [*Rhodospirillum centenum*]	0	−1.7848319
DV965565	gb|AAP97123.1|	Adenosine 5′-phosphosulfate kinase [*Porphyra purpurea*]	2.00E−68	−1.750722
DV969640	ref|NP_001004157.1|	Scavenger receptor class F, member 1 [*Mus musculus*]	3.00E−11	−2.9343407
DV966072	gb|AAK39876.1|	Heat shock protein 70KD [*Guillardia theta*]	2.00E−50	−2.7381477
DV965143	gb|AAM46061.1|	Vanadium-dependent bromoperoxidase [*Corallina officinalis*]	1.00E−119	−1.7744403
DV964113	gb|AAM94016.1|	Low molecular mass early light-inducible protein HV60 [*Griffithsia japonica*]	6.00E−59	−3.075942
DV969011	gb|AAN39005.1|	Light-harvesting complex I polypeptide [*Griffithsia japonica*]	4.00E−56	−2.4481726
DV963406	gb|AAP80835.1|	Phycobilisome 7.8 kDa linker polypeptide [*Griffithsia japonica*]	2.00E−28	−2.196892
DV963357	emb|CAB75583.1|	Light-harvesting protein [*Galdieria sulphuraria*]	8.00E−48	−1.9751966
DV966022	dbj|BAD36767.1|	Oxygen-evolving enhancer [*Cyanidioschyzon merolae*]	2.00E−78	−2.1793237
DV963197	gb|AAM33652.1|	Mastigoneme-like protein [*Chlamydomonas reinhardtii*]	6.00E−27	−3.5932302
DV965021	gb|AAM33652.1|	Mastigoneme-like protein [*Chlamydomonas reinhardtii*]	6.00E−27	−1.8365012
DV966003	sp|O04354|CYB5_BOROF	Cytochrome b5 [*Borago officinalis*]	4.00E−19	−2.8460872
DV963521	pir||JC7180	14-3-3 [*Lentinula edodes*]	7.00E−33	−1.8923244
DV963551	emb|CAA62869.1|	Cathepsin L [*Paramecium tetraurelia*]	3.00E−54	−1.8365012
DV963236	ref|ZP_00007578.2|	COG0737: 5′-nucleotidase2′,3′-cyclic phosphodiesterase and related esterases [*Rhodobacter sphaeroides* 2.4.1]	1.00E−27	−1.7951802
DV962087	ref|XP_463787.1|	Putative senescence-associated protein [*Oryza sativa* japonica cultivar-group]	6.00E−12	−1.7241969
DV963630	gb|AAM93968.1|	Gbp1 [*Griffithsia japonica*]	3.00E−63	−1.6977305
DV968041	ref|NP_198787.1|	Pentatricopeptide PPR repeat-containing protein [*Arabidopsis thaliana*]	5.00E−07	−1.6322683
DV968015	ref|NP_477515.2|	Ubiquitin conjugating enzyme E2, J2 isoform 2 [*Homo sapiens*]	1.00E−52	−2.2980156
DV963443	gb|AAM93956.1|	Protein translation factor [*Griffithsia japonica*]	2.00E−39	−1.7564429
DV968035	gb|AAA72126.1|	Polyubiquitin	1.00E−161	−1.6825179
DV963172	gb|AAK27413.1|	Elongation factor 1 alpha long form [*Monosiga brevicollis*]	0	−1.6724253
DV963478	emb|CAH04327.1|	S12e ribosomal protein [*Curculio glandium*]	3.00E−37	−1.9232606
DV968042	ref|NP_997927.1|	Ribosomal protein S15a [*Danio rerio*]	5.00E−56	−1.8506224
DV968047	gb|AAC15656.1|	60S Ribosomal protein P2 [Cryptochiton stelleri]	3.00E−14	−1.6532685
DV963656	gb|AAP80719.1|	Histone H2B protein [*Griffithsia japonica*]	5.00E−52	−2.3048546
DV962086	sp|P11237|TBA1_NAEGR	Tubulin alpha-123 chain	3.00E−74	−1.9109489
DV966107	ref|XP_470047.1|	Putative clathrin assembly protein [*Oryza sativa* japonica cultivar-group]	2.00E−42	−1.7243655
DV963619	gb|AAD31847.1|	Water channel protein MipI [*Mesembryanthemum crystallinum*]	2.00E−11	−2.0588937
DV965077	pir||T14363	Probable H+-exporting ATPase EC 3.6.3.6 chain B, vacuolar - red alga *Cyanidium caldarium*	9.00E−76	−1.7797194

attached phycobilisomes to the thylakoid membrane and interact with allophycocyanin to organize the core (Samsonoff and MacColl 2001). In this study, we have identified genes encoding two LHPs, phycobilisome 7.8 kDa linker polypeptide and low-molecular-weight early light-inducible protein that were down-regulated under light deprivation. The abundance of these transcripts might be reduced under extreme irradiance environment where the light availability is scarce and subsequently lower the photosynthetic rate in seaweeds growing at low irradiance (Mercado et al. 2000).

The carbon fixation in the dark in seaweed is only 1–7% of that in the light (Macler 1986). Unlike the carbon fixation via the reductive pentose phosphate pathway in light (Kremer 1978; Kremer and Kűppers 1977), fixation in the dark is primarily via phosphoenolpyruvate carboxylase (Kremer 1979). The activity of pyruvate phosphate dikinase may regulate the carbon fixation via the C4 dicarboxylic acid pathway. This enzyme is predominantly in the active form under light and is converted into inactive form as the light intensity decreases (Hatch and Slack 1969). In this study, we found that the transcript for this enzyme decreased in the dark. It is possible that this gene is also regulated at the transcriptional level.

The accumulation of transcripts encoding NADH dehydrogenase and ferredoxin implied light-dependent gene expression of proteins in the respiratory chain. The existence and involvement of NADH complex have been demonstrated in the transient nonphotochemical reduction of the plastoquinone pool after a light-to-dark transition (Burrows et al. 1998; Cournac et al. 1998; Shikanai et al. 1998). The mRNA of photosynthetic ferredoxin $NADP^+$ oxidoreductase isoforms pFNRI and pFNRII have also been observed in the dark in wheat (Gummadova et al. 2007). On the other hand, the transcript encoding thioredoxin, the major ubiquitous disulfide reductase that plays important biological role in maintaining proteins in their reduced states, was also found to accumulate in the dark in this study. This is in contrast with the findings of Nuruzzaman et al. (2008) that showed higher expression of rice thioredoxin gene under light treatment.

The transcripts encoding for 14-3-3 were found to be up-regulated (DV965012) and down-regulated (DV963521) under light deprivation. However, real-time PCR analysis of DV963521 confirmed that this gene was down-regulated in the dark, suggesting that highly similar sequences encoding for different isoforms of 14-3-3 may be differentially regulated during light deprivation in *G. changii*.

The present study also enabled us to identify a subset of genes that are regulated by light (Supplementary Tables 1 and 2). This information is especially important as *G. changii* has only a small number of annotated genes in the database with approximately 70%, and its transcripts have nonsignificant matches to peptide sequences in the database or match to either hypothetical or unknown proteins (Teo et al. 2007). Functional annotation of these seaweed sequences can be improved through further analysis of these co-expressed genes.

In conclusion, we have profiled the transcripts that were expressed under light deprivation using cDNA microarray, providing an overview of the network of genes operating under light deprivation. Many transcripts that were related to light harvesting, carbon fixation, and stress have been identified. The analysis of co-expressed genes regulated by light will enhance the functional annotation and elucidation of unknown seaweed sequences.

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