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SHORT REPORTS

First global transcriptome analysis of brown algae *Macrocystis integrifolia* (Phaeophyceae) under marine intertidal conditions

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Abstract

To understand the physiological responses of the brown macroalga *Macrocystis integrifolia* during the marine tidal cycle, two RNA libraries were prepared from algal frond samples collected in the intertidal zone (0 m depth) and subtidal zone (10 m depth). Samples collected from intertidal zone during low tide was considered as abiotic stressed (MI0), while samples collected from subtidal zone was considered as control (MI10). Both RNA libraries were sequenced on Illumina NextSeq 500 which generated approx. 46.9 million and 47.7 million raw paired-end reads for MI0 and MI10, respectively. Among the representative transcripts (RTs), a total of 16,398 RTs (39.20%) from MI0 and 21,646 RTs (39.24%) from MI10 were successfully annotated. A total of 535 unigenes (271 upregulated and 264 downregulated) showed significantly altered expression between MI0 and MI10. In abiotic-stressed condition (MI0), the relative expression levels of genes associated with antioxidant defenses (vanadium-dependent bromoperoxidase, glutathione S-transferase, lipoxygenase, serine/threonine-protein kinase, aspartate Aminotransferase, HSPs), water transport (aquaporin), photosynthesis (light-harvesting complex) protein were significantly upregulated, while in control condition (MI10) most of the genes predominantly involved in energy metabolism (NADH-ubiquinone oxidoreductase/NADH dehydrogenase, NAD(P)H-Nitrate reductase, long-chain acyl-CoA synthetase, udp-n-acetylglucosamine pyrophosphorylase) were overexpressed.

Keywords *Macrocystis integrifolia* (Phaeophyceae) · Abiotic stress · Transcriptome analysis · De novo assembly · Differential gene expression

Introduction

Brown algae (Kingdom: Chromista; class: Phaeophyceae) are probably the most abundant photosynthetic inhabitants of the intertidal zone. Like other organisms of the intertidal zone, brown alga is also subjected to recurring, harsh changes in the environment associated with life in the interface of terrestrial and marine habitats. These changes include, but are not limited to, desiccation, osmotic pressure, temperature, nutrients, light, and tidal flow (Kupper et al. 2008). However, little is known about the physiological processes in the brown algae which allow them to adapt to such abiotic stress conditions. In macroalgae, the intensity of the effects generated by the abiotic stresses is related to the vertical distribution range of these species in the intertidal zone (López-Cristoffanini 2013). Several species of brown macroalga are economically very important. The economic interest of brown macroalga is related mainly due to their use in the industrial production of polysaccharides (McHugh 2003; Bixler and Porse 2011; Synytsya et al. 2015). For example, alginate, a brown algal cell wall polysaccharide, is used both for pharmaceutical purposes (Tonnesen and Karlsen 2002; Thanh-Sang Vo et al. 2012) and in the food industry (Jensen 1993; Hafting et al. 2012; Fleurence 2016; Chapman 2015). Economically important *Macrocystis integrifolia* is one of four morphotypes of kelp (large brown algae) in the genus *Macrocystis* which lives in intertidal zone along the Pacific

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coast of North America, Peru and Chile (Alveal 1995) and is moderately tolerant to abiotic stresses. However, to date, the cellular and molecular responses, as well as abiotic stress tolerance factors of brown algae are not well-studied. Hence, the main objective of this study is to analyze the effect of abiotic stresses in brown algae *M. integrifolia* under intertidal condition using global transcriptome data.

Data description

Sample collection and maintenance

Samples from six individual plants of *M. Integrifolia,* three of each condition were collected at two sampling points in the rocky intertidal zone of Punta San Juanito, Ica, Peru (Table 1). Samples collected from intertidal zone (0 m) during low tide after 2 h of air exposure as well as from subtidal zone (10 m). Small pieces of tissues (approx. 2 cm^2) were cut from fresh fronds and were promptly cleaned with ethanol for removing epiphytes and immediately frozen in liquid nitrogen until further analysis. In this study, the samples from 0 and 10 m were marked as MI0 and MI10, respectively. MI10 represented the control samples, while MI0 represented the natural abiotic-stressed samples.

RNA extraction and preparation of cDNA library for RNAseq

Total RNA was extracted from the samples MI0 and MI10 using RNAeasy mini kit (Quiagen) and treated with RNase free DNase (Quiagen) to remove the residual genomic DNA. RNA quantity and quality were assessed with NanoDropTM 1000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA) and 2100 Bioanalyzer (AGILENT). Library preparation was performed by Illumina TruSeq RNA library protocol. Briefly, 1 g of Total RNA was subjected to Poly A purification of mRNA. Purified mRNA was fragmented for 2 min at elevated temperature (94 °C) in the presence of divalent cations and reverse transcribed with Superscript III Reverse transcriptase by priming with Random Hexamers. Second strand cDNA was synthesized in the presence of DNA Polymerase I and RnaseH. The cDNA was cleaned up using HighPrep PCR (MAG-BIO, Cat# AC-60050) and Illumina adapters were ligated to the cDNA molecules after end repair and addition of A base. SPRI cleanup was performed after ligation. Each of the two libraries was amplified using 8 cycles of PCR for the enrichment of adapter-ligated fragments. The prepared library was quantified using Qubit and validated for quality by running an aliquot on High Sensitivity Bioanalyzer Chip (Agilent). Based on the population observed in the profile,

Table 1 MixS information of *Macrocystis integrifolia*

Item	Definition
General feature of classification	
Classification	Eukaryota; Heterokonta; Phaeophyceae; Laminariales; Laminariaceae; *Macrocystis integrifolia*
Investigation type	Eukaryote transcriptome
Project name	Bioproject PRJNA322132
Environment	
Geographic location	Zona Rocosa de Punta San Juanito, Ica, Perú
Latitude, longitude	15°14′43.2″S, 75°15′31.9″W
Collection date	2015-04
Isolation environment	Ocean
Depth	0 and 10 m of water depth
Biome	ENVO:01000320 (marine environment)
Collector (s)	Erika Salavarría and Sujay Paul
Sequencing	
Sequencing	Illumina NextSeq 500; Paired-end (2 × 151 bp Max)
Assembly	
Method	De novo assembly
Program	trinityrnaseq_r20140717
Finishing strategy	High-quality transcriptome assembly
Data accessibility	
Sequence information	Genbank accession SRA426960

the libraries were sequenced on Illumina NextSeq 500 at Genotypic Technology Private Limited, Bangalore, India.

Data filtering, de novo transcriptome assembly, and functional annotation

The Illumina NextSeq 500 paired-end raw reads were first quality checked using FastQC (Andrews, 2010) and then processed by the in-house script for adapters and low-quality bases trimming towards 3′- end. De novo assembly of Illumina NextSeq 500 processed data was performed using trinityrnaseq (Grabherr et al. 2011) with default K-mer = 25. Transcripts having length ≥ 300 bp have been considered, followed by clustering of these transcripts with 95% identity using CD-HIT (Li and Godzik 2006) which resulted into COG's. The assembled transcripts were then annotated using NCBI BLAST 2.2.31 (Altschul et al. 1990) with the protein sequences of brown algae *Ectocarpus siliculosus* available at UniProt Protein Database. The same Uniprot protein database was used for Gene Ontology (GO) annotation and transcripts were assigned GO subcategories under biological process (BP), molecular function (MF) and cellular component (CC). Differential gene expression (DGE) between the samples was performed by employing a negative binomial distribution model with DeSeq v1.8.1 tool (Anders and Huber 2010). Briefly, transcripts of both MI0 and MI10 samples were combined to the size ≥ 300 bp and were clustered together using CD-HIT at 95% identity. Consequently, master control transcriptome data (unigenes) were generated. The RPKM measurement (readings per kilobase of transcribed per million mapped readings) was then performed using "Bowtie2 tool" to generate the read count profile. Transcripts having log2 (fold change) value ≥ 1 and ≤ − 1 were considered to be upregulated and downregulated, respectively. Moreover, transcripts showing significant variation in fold change expression (corrected *P* value ≤ 0.05) in these sets were also identified. The overall transcriptome assembly and annotation procedure, as well as DGE analysis performed in this study, have been graphically represented in Supplementary Figure S1 and S2.

A total of approx. 46.9 million and 47.7 million raw paired-end reads were generated from Illumina NextSeq 500 platform for MI0 and MI10 samples, respectively. The raw paired-end sequences data in FASTQ format was deposited in the National Centre for Biotechnology Information's (NCBI) Short Read Archive (SRA) database under the accession number SRA426960. Raw reads were subjected to quality control using FastQC and the results showed that the average per sequence Phred quality score was above 30 in both the reads indicating high-quality sequencing run. Post quality filtering for low-quality regions, adaptors and sequencing tags, a total read count of approx. 42.4 million for MI0 and 42.3 million for MI10 were withdrawn for further processing. De novo assembly of Illumina NextSeq 500 processed data was performed using trinity at a hash length of 25, which generated 43,952 and 57,516 transcripts for MI0 and MI10, respectively (Table 2). A total of 11,756 and 12,524 transcripts with more than 1 kb in length as well as 25,982 and 30,709 transcripts with more than 500 bp in length were generated in MI0 and MI10 (Table 2). Higher N50 values (1159 and 986 for MI0 and MI10, respectively) also indicate the high quality of sequence assembly.

It is always challenging to predict accurate annotations for the transcripts from the non-model organism. In this study, representative transcripts (RTs) were annotated using NCBI BLAST 2.2.31 with the protein sequences of brown alga *Ectocarpus siliculosus*. Transcripts greater than 30% identities were considered to be suitable to assign annotation based on high degree of sequence identity. GO annotation revealed that the annotated RTs represent various genes which are involved in various metabolic pathways (Fig. 1). In the molecular function (MF) category, the most frequently occurring terms were ATP binding (18.64%) and zinc ion binding (8.12%) followed by DNA binding (6.12%). Ubiquitin-dependent protein catabolic process (1.60%) and translation (1.59%) were the most frequently occurring terms in biological processes (BP) category followed by transmembrane transport (1.52%).The most frequently occurring terms in cellular component (CC) category was integral component of the membrane (11.56%) and nucleus (6.02%) followed by membrane (3.31%) (Fig. 1a). In control condition (MI10), the most frequently occurring terms under MF category were found also ATP binding (18.53%) followed by zinc ion binding (7.06%) and metal ion binding (5.20%). Translation (3.31%), followed by protein folding (1.94%) and intracellular protein transport (1.57%) were found to be the most represented terms under BP category. The most

Table 2 De novo assembly summary of sample MI0 and MI10 using Trinity tool

Samples	MI0	MI10
Tool used	Trinity	
Hash/k-mer length	25	
Transcripts/isotigs(contigs) generated	43,952	57,516
Maximum transcript/isotig length	10,538 bp	10,627
Minimum transcript/isotig length	301 bp	301 bp
Average transcript/isotig length	869.4 bp	784.3 bp
Median transcript/isotig length	2329 bp	441.5 bp
Total transcript length	38,210,788 bp	45,109,937 bp
Transcripts ≥ 500 bp	25,982	30,709
Transcripts > 1 kb	11,756	12,524
Transcripts > 10 kb	2	1
N50 value	1159	986

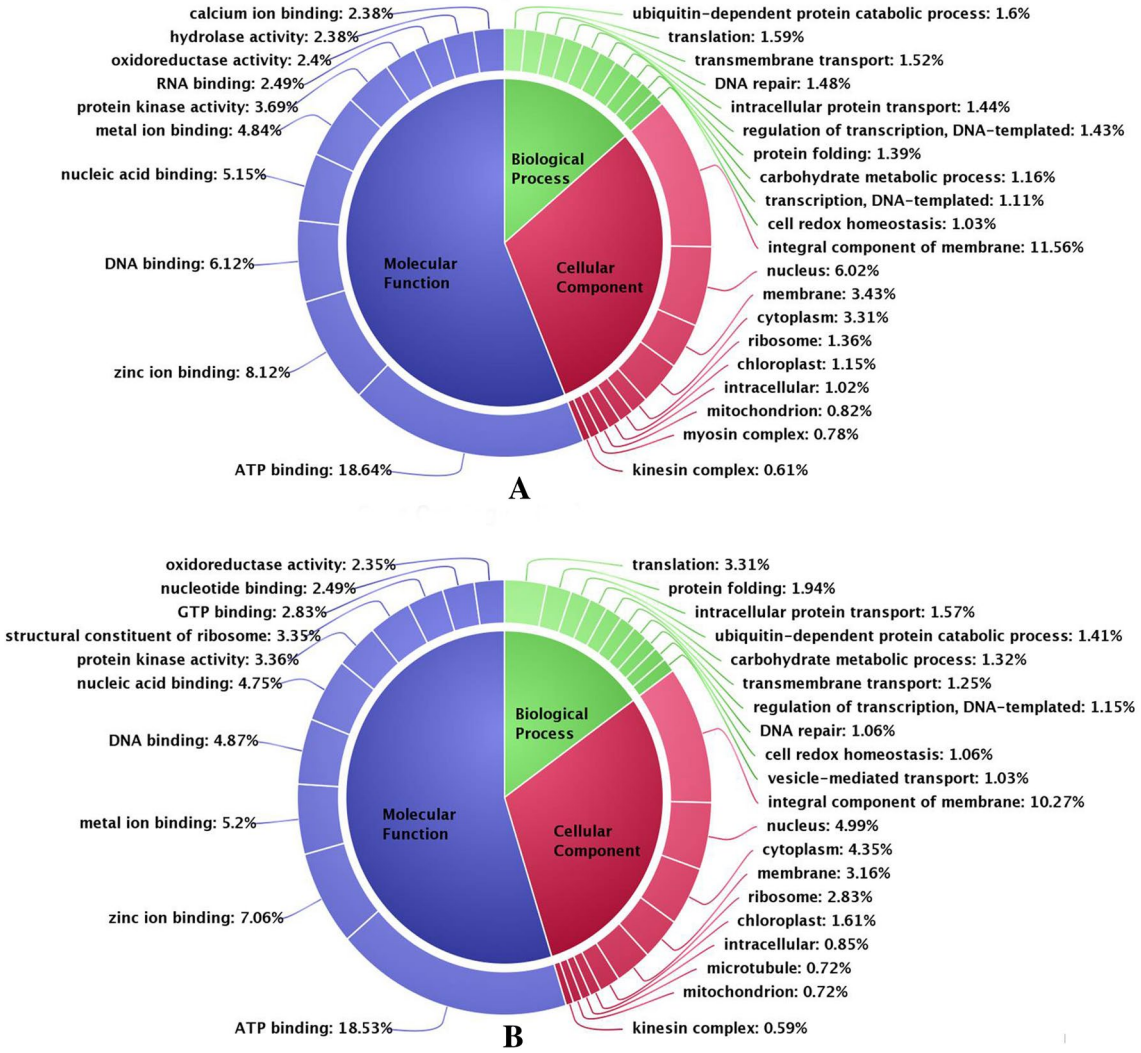

Fig. 1 Gene ontology (GO) analysis. **a** MI0, **b** MI10

frequently occurring terms in CC category were also integral component of membrane (10.27%) and nucleus (4.99%) followed by cytoplasm (4.35%) (Fig. 1b).

Differential gene expression (DGE) analysis

In the transcriptomic analysis, the differentially expressed genes between MI0 and MI10 were estimated by clusters or hierarchical groupings considering q value < 0.05, FDR ≤ 0.05 and minimum twofold change (log $2 \geq +1$ or ≤ -1). A total of 9519 unigenes expressed differentially between MI0 and MI10 among which 271 upregulated and 264 downregulated genes are significantly altered. To find out the possible molecular mechanisms involved in the stress responses, we manually classified and examined several genes that exhibited major significant changes in the stress conditions (MI0) compared to the MI10 control (Table 3). Many of these genes altered were related to the synthesis of chaperone proteins (molecular chaperones HSP70/HSC70, HSP70 superfamily), electron transport (Rieske (2Fe-2S), essential components in physiological processes including enzymes for carbon fixation (fructose-bisphosphate aldolase, glycine dehydrogenase), alginate and cellulose synthesis (mannuronan C-5-epimerase, UDP glucose 6-dehydrogenase), amino acid metabolism (shikimate kinase), transmembrane transport systems (aquaporins), genes involved in nitrogen fixation (glutamine synthetase), processes related to photosynthesis (light-harvesting complex protein), classical pathways to stress (vanadium-dependent bromoperoxidase), aprataxin synthesis (FHA-HIT protein), fatty acid metabolism (lipoxygenase), oxidative stress (alkaline phosphatase, HSPs, glutathione S-transferase), phytohormone biosynthesis (S-adenosylmethionine synthase, gibberellin

Table 3 List of some significantly altered genes in MI0 as compared to MI10 in *M. integrifolia* during abiotic stress response

Gene/protein name	Regulation	Fold change	*p* value	Function
Vanadium-dependent bromoperoxidase	UP in MI0	6.8914	0.0222098808	Antioxidant defenses
Urease accessory protein UreF	UP in MI0	22.4184	0.0317399721	Urea cycle
Glutathione *S*-transferase (EC 2.5.1.18)	UP in MI0	9.5782	0.0096688523	Glutathione synthesis and antioxidant defenses
Light harvesting complex protein	UP in MI0	16.4412	0.0017643654	Photosynthesis
Rieske (2Fe-2S) region	UP in MI0	39.0188	0.0003381751	Photosystem 2, electron transport, light sensing and carotenoid synthesis
Aquaporin	UP in MI0	30.4891	0.0091832762	Transportation system (activation of water and ion transporters)
Molecular chaperones HSP70/HSC70, HSP70 superfamily	UP in MI0	12.3671	0.0040478809	Response to heat and oxidative stress (desiccation). Synthesis of chaperones
Branched-chain alpha-keto acid dehydrogenase E1 beta subunit	UP in MI0	6.2733	0.0350976028	Branched-chain amino acids
Serine/threonine-protein kinase CTR1	UP in MI0	11.8631	0.0415284001	Branched-chain amino acids, antioxidant defenses
Aspartate aminotransferase (EC 2.6.1.1)	UP in MI0	11.1982	0.0075979684	Branched-chain amino acids, antioxidant defenses
Similar to ubiquitin specific protease 34	UP in MI0	18.3831	0.0151442253	Recycling proteins
Similar to ATP synthase mitochondrial F1 complex assembly factor 2	UP in MI0	6.5190	0.0458993508	Energy metabolism
Monophenol monooxygenase	UP in MI0	27.0267	0.000428844	Betalaina biosynthesis
Alkaline phosphatase	UP in MI0	19.2381	0.0022184014	Pathway: NAD/NADH phosphorylation and dephosphorylation(responses to oxidative stress)
Oxidoreductase (arsenate reductase. glutaredoxin)	UP in MI0	10.3163	0.0069709376	Arsenate detoxification
DNA helicase (EC 3.6.4.12)	UP in MI0	2.6621	0.0090710198	DNA helicases, utilize the energy from ATP hydrolysis
5-Methyltetrahydropteroyltriglutamate–homocysteine *S*-methyltransferase	UP in MI0	12.4255	0.0092518168	Methionine metabolism, seleno compound metabolism, L-metionine biosynthesis
DEAD box helicase (EC 3.6.4.13)	UP in MI0	9.9354	0.0363002914	A nucleoside triphosphate + H_2O = a nucleoside diphosphate + phosphate catalytic mechanism
Lactoylglutathione lyase, putative	UP in MI0	9.8005	0.0141131854	Pyruvate metabolism, methylglyoxal degradation I
Ubiquitin-conjugating enzyme, putative	UP in MI0	5.5672	0.04170213	Protein ubiquitylation pathway
Ankyrin 3.6.1.15 (NTPase)	UP in MI0	7.3078	0.02370033	Oxidative phosphorylation
Gamma-glutamyl transpeptidase (EC 2.3.2.2)	UP in MI0	8.8739	0.03232579	Glutatione metabolism
Monogalactosyldiacylglycerol synthase, family GT28 (EC 2.4.1.46)	UP in MI0	6.9442	0.03697278	Galactolipid biosynthesis I
Serine/threonine dehydratase (EC 4.3.1.-) L-serine ammonia-lyase	UP in MI0	6.4735	0.02875467	Serine metabolism, threonine metabolism
Patatin(EC. 3.1.1.4)	UP in MI0	8.9673	0.04265161	Phospholipase metabolism
Acetoacetate–CoA ligase (EC 6.2.1.16)	UP in MI0	6.7772	0.04515125	Valine and leucine degradation
Carbohydrate kinase 2.7.1.14	UP in MI0	5.6828	0.04656553	Aerobic respiration I–III
Dihydroorotate dehydrogenase (quinone), mitochondrial (DHOdehase) (EC 1.3.5.2)	UP in MI0	5.3654	0.04693179	Pyrimidine metabolism
Lipoxygenase	UP in MI0	18.02	0.001319908	Fatty acid metabolism and antioxidant defenses
ABC transporter-like	DOWN in MI0	0.1472	0.03404662	Cell signaling
NADH-ubiquinone oxidoreductase chain 1/NADH dehydrogenase (Ubiquinone) (EC 1.6.5.3)	DOWN in MI0	0.0026	1.40E−07	Aerobic respiration I–III
Shikimate kinase	DOWN in MI0	0.1832	0.04200643	Aromatic amino acids
Udp-*n*-acetylglucosamine pyrophosphorylase	DOWN in MI0	0.0557	0.00209323	Energy metabolism
Galactose-4-epimerase, UDP	DOWN in MI0	0.932	0.00831223	UDP-D-galactose biosynthesis

Table 3 (continued)

Gene/protein name	Regulation	Fold change	*p* value	Function
Long-chain acyl-CoA synthetase	DOWN in MI0	0.1441	0.02197075	Energy metabolism
Casein kinase II alpha subunit	DOWN in MI0	0.0813	0.02875841	Branched-chain amino acids
Magnesium chelatase subunit H, putative chloroplast (EC 6.6.1.1)	DOWN in MI0	0.0006	4.834E−09	Chlorophyll metabolism
Mannose-6-phosphate isomerase	DOWN in MI0	0.0463	0.0170957	L-ascorbate biosynthesis I (L-galactose pathway) Smirnoff wheeler pathway
Transposase	ONLY in MI0		0.01483208	Replicative transposition mechanism
FHA-HIT protein	ONLY in MI0		0.00128322	Aprataxin synthesis
Gibberellin 2-beta-dioxygenase	ONLY in MI0		0.004783	Diterpenoid biosynthesis; gibberellin inactivation I
Isocitrate lyase	ONLY in MI0		1.63E−06	Gluconeogenesis
Polymorphic Outer membrane protein G/I family	ONLY in MI0		5.38E−06	Components of the membrane
Polymorphic outer membrane protein	ONLY in MI0		1.2758E−05	Components of the membrane
Light harvesting complex protein	ONLY in MI0		0.02008396	Photosynthesis
NAD(P)H-nitrate reductase (EC 1.7.1.1)	ONLY in MI10		8.64E−07	Nitrogen assimilation
Glycine dehydrogenase (decarboxylating)	ONLY in MI10		2.328782E−10	Photorespiration and carbon availability
Phosphoenolpyruvate carboxykinase (ATP)	ONLY in MI10		0.00097953	Branched-chain amino acids
Pyruvate dehydrogenase (EC 1.2.4.1)	ONLY in MI10		0.00083066	Branched-chain amino acids
Pyruvate kinase (EC 2.7.1.40)	ONLY in MI10		0.00013577	Pyruvate metabolism. Branched chain amino acids
Serine hydroxymethyltransferase	ONLY in MI10		0.03295779	Photorespiration and carbon availability
S-adenosylmethionine synthase	ONLY in MI10		0.00431818	Ethylene synthesis
Glyceraldehyde-3-phosphate dehydrogenase	ONLY in MI10		1.1215E−05	Pathway: glycolysis IV (plant cytosol)
Triosephosphate isomerase/glyceraldehyde-3-phosphate dehydrogenase	ONLY in MI10		8.47E−11	Gluconeogenesis. Photosynthesis
Aldehyde dehydrogenase	ONLY in MI10		2.9078E−05	Glycolysis/gluconeogenesis
Fructose-bisphosphate aldolase	ONLY in MI10		5.89E−10	Glycolysis/gluconeogenesis
L-Galactono-1,4-lactone dehydrogenase	ONLY in MI10		0.02630423	L-Ascorbate biosynthesis I (L-galactose pathway) Smirnoff-wheeler pathway
Formaldehyde dehydrogenase (EC 1.1.1.284)	ONLY in MI10		0.00160783	Methane metabolism, formaldehyde oxidation
Ribulose bisphosphate carboxylase large chain (RuBisCO large subunit) (EC 4.1.1.39)	ONLY in MI10		0.00013577	Involved in the first step of the calvin cycle for fixing carbon in photosynthetic systems, photorespiration

2-beta-dioxygenase), ascorbate biosynthesis (L-galactono-1,4-lactone dehydrogenase) and other processes (Table 3).

This is the first global transcriptome analysis of brown algae *Macrocystis integrifolia* (Phaeophyceae) under marine intertidal conditions. This study could be an important resource for subsequent genomic studies in brown algae to identify functional genes involved in different metabolic processes related to stress tolerance.

Data accessibility

The raw paired-end sequences data in FASTQ format was deposited in the National Centre for Biotechnology Information's (NCBI) Short Read Archive (SRA) database under the accession number SRA426960.

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Compliance with ethical standards

Conflict of interest The authors declare that they don't have any conflict of interest.

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