

UBC gene family and their potential functions on the cellular homeostasis under the elevated $p\text{CO}_2$ stress in the diatom *Phaeodactylum tricornutum*

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ABSTRACT

Ocean acidification (OA) as a result of more and more anthropogenic CO_2 release, has already been referred to a severe ecological environmental issue. OA would destroy the balance of ocean carbonate buffering system and have negative effects on marine primary producers. Diatom *Phaeodactylum tricornutum* is one of the most important primary producers in the ocean, and it is susceptible to the elevated $p\text{CO}_2$ stress. Under the elevated $p\text{CO}_2$ stress, endoplasmic reticulum-associated degradation (ERAD) and its important components Ubiquitin-conjugating enzymes (UBCs) are pivotal to sustain cellular homeostasis. However, systematic investigation regarding phylogenetic relationships of UBC gene family, expression profiles under the elevated $p\text{CO}_2$ stress and their potential functions on the cellular homeostasis of *P. tricornutum* remain poorly understood. In this study, a genome-wide analysis of PtUBC gene family was performed. It was shown that 18 PtUBC genes were unevenly distributed to the 14 chromosomes of total 33 chromosomes in *P. tricornutum*. Phylogenetic analysis showed that 18 PtUBC proteins were divided into 5 groups and each of them contained different conserved motifs. Besides, lots of *cis*-acting elements related to diverse stress responses were identified from PtUBC genes. Remarkably, transcriptomic analysis revealed that 3 PtUBC genes (*PtUBC15*, *PtUBC16* and *PtUBC7*) were downregulated under the exposure to elevated $p\text{CO}_2$ level, while the other 15 PtUBC genes did not have significant expression. Meanwhile, the model of endoplasmic reticulum-associated degradation (ERAD) mechanism was displayed, explaining that the misfolded/ unfolded proteins under the elevated $p\text{CO}_2$ stress would be accumulated and then degraded via the ERAD mechanism to sustain the cellular homeostasis. The downregulated PtUBC genes might have a negative effect on the ERAD mechanism. Overall, this study provided an important foundation for further understanding of possible functions of PtUBC genes, especially on the cellular homeostasis, and the regulatory mechanism of PtUBCs on the diatom response to different environmental stresses.

1. Introduction

The endoplasmic reticulum (ER) is an important subcellular organelle that plays significant important roles in the biosynthesis, folding, modification, and transport of proteins. To ensure high precision of protein folding, modification and maturation in the ER, ER-protein quality control (ERQC) system is of vital importance in the correct folding and modification of proteins to maintain cellular homeostasis (Volpi et al., 2017). However, some environmental stresses can break the balance between the protein-folding demand and folding capability, giving rise to an accumulation of misfolded/ unfolded proteins in the ER.

Endoplasmic reticulum-associated degradation (ERAD) is one of pathways to sustain ER homeostasis. It is responsible for the removal of misfolded/unfolded proteins in the ER for proteasomal degradation (Hwang and Qi, 2018). The ERAD mechanism comprises substrate recognition, targeting, *retro*-translocation, polyubiquitination, and finally degradation by the 26S proteasome (Cui et al., 2012). Among them, ubiquitination plays a critical role in the ERAD. It was already known that UBC has pivotal influences on the stability of some ERAD substrates in certain stress tolerance (Cui et al., 2012). Ubiquitination is a process that the ubiquitin covalently attaches to a specific target protein, effecting the protein stability and biological function in

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eukaryotic cells. It contains several consecutive processes. Firstly, a ubiquitin molecule chain selects and labels short-lived and misfolded peptides and protein substrates. Subsequently, the 26S proteasome decomposes the substrates. Finally, the ubiquitin molecules are recycled for a further ubiquitination cycle (Liu et al., 2020). Among them, E2 ubiquitin-conjugating (UBC) enzymes play crucial roles in the selection of lysine amino acid to form ubiquitin chains and target substrates, serving as major determinants.

UBC enzymes contain a conserved UBC domain (approximately 140–200 amino acids) (Schumacher et al., 2013). The UBC domain mainly harbors four α -helices, an anti-parallel β -sheet and a short 3_{10} helix (Wenzel et al., 2011). Based on the additional fragments to the catalytic core in the UBC domain, the family of UBC enzymes are divided into four classes. Class I consists only of UBC domain, class II consists of UBC domain and N-terminal extension, class III consists of UBC domain and C-terminal extension, and class IV consists of UBC domain, C-terminal and N-terminal extensions (Wijk and Timmers, 2010). UBCs, a multigene family, has been observed in many organisms, such as *Arabidopsis thaliana* (Kraft et al., 2005), *Saccharomyces cerevisiae* (Michelle et al., 2009), *Zea mays* (Jue et al., 2015), *Vitis vinifera* (Gao et al., 2017) and *Solanum lycopersicum* (Sharma and Bhatt, 2017). The UBC enzymes play indispensable roles in biological processes of plants, such as plant growth, response to stress, etc. (Jue et al., 2015). For instance, AtUBC13 was reported to function on the epidermal cell differentiation and iron deficiency responses (Li and Schmidt, 2010; Wen et al., 2006). AtUBC2 plays a key role in tolerance response to UV stress and activating a floral repressor gene (Xu et al., 2009). Under the circumstance of heat shock and cadmium chloride (CdCl_2), the expression of LeUBC1 increases evidently, suggesting that LeUBC1 responds to these two stresses (Feussner et al., 1997). UBC37 and UBC44 genes in *Solanum lycopersicum* regulate plant responses to ABA and auxin hormones. UBC44 gene also responds to cytokinin hormone in leaf tissues (Sharma and Bhatt, 2017). UBC genes in *Gracilaria lemaneiformis* has an impact on the heat stress (Li et al., 2014). In *Zea mays*, ZmUBC genes are obviously upregulated to response salt and drought circumstances, respectively (Jue et al., 2015). However, very few studies were focused on the UBC gene family in microalgae. For example, CrUBC2, a UBC gene in *Chlamydomonas reinhardtii*, can increase DNA damage tolerance and a protein substrate of CrUBC13–CrUBC2 involves in lipid accumulation (Fei et al., 2017). It was shown that studies on structure and function of the UBC gene family in plants are abundant, but the identification of UBC enzymes and the functional characterization in microalgae, especially in diatom are still very limited.

Phaeodactylum tricornutum is an ideal model diatom that was widely used for the study of biological processes and ecotoxicology (Dell'acquila and Maier, 2020). For example, ecotoxicological tests on *P. tricornutum* exposure to nano particles, amoxicillin (Broccoli et al., 2021), polyethylene glycol (Pastorino et al., 2022) and Zinc oxide nanoparticles and potassium dichromate (Pastorino et al., 2021) were previously performed. Because it is able to make approximately 20% of global primary photosynthetic production, considerable carbon dioxide uptake and oxygen production capacity (Cruz de Carvalho et al., 2022), *P. tricornutum* plays pivotal functions in maintaining the balance of ecosystem. With the rapid development of industrialization, anthropogenic CO_2 has been pumped into the atmosphere quickly, resulting in global warming and ocean acidification. The surface seawater takes in additional atmospheric CO_2 , breaking the balance of ocean carbonate buffering system and greatly influencing primary producers in the ocean (Eberlein et al., 2017). CO_2 has been referred as a pivotal component of biological processes around the biosphere. It has close associations with the critical physiological processes of photosynthesis, metabolism, cellular homeostasis and chemo-sensing system (Cummins et al., 2019). The growth and physiological processes of several microalgae are susceptible to elevated $p\text{CO}_2$ stress. For example, the specific growth rate of *Microcystis aeruginosa* was significantly reduced at the 10,000 p.p.m. CO_2 (Sandrini et al., 2014). On the one hand, the dissolved inorganic carbon

(C_i) concentration was increased by the elevated CO_2 , on the other hand, elevated CO_2 resulted in the very low light availabilities and the weak competition for light in *Monoraphidium* (Ji et al., 2017). In *Skeletonema marinoi*, elevated $p\text{CO}_2$ could obviously restrain the growth and photosynthesis (Zhang et al., 2021). A previous study has revealed *P. tricornutum*'s response to elevated carbon concentrations via transcriptional regulatory network (Levering et al., 2017). Nonetheless, studies on the overall responses of UBC enzymes to elevated $p\text{CO}_2$ stress in *P. tricornutum* have not been performed so far.

In this study, the UBC gene family was firstly analyzed at the genome-wide level in *P. tricornutum* and 18 PtUBC genes were identified. Then the comprehensive phylogenetic analyses of gene structure, motif composition and *cis*-acting element were performed. The chromosomal localization of the UBC genes were also analyzed. The expression patterns and potential functions of PtUBC genes under elevated $p\text{CO}_2$ stress were finally investigated based on the transcriptomics data. Our study provided detailed information of the PtUBC gene family in *P. tricornutum*. The investigation on the potential function of PtUBCs under the elevated $p\text{CO}_2$ stress will provide valuable information for understanding the ERAD mechanism, especially on the regulatory mechanism under the stress of elevated $p\text{CO}_2$.

2. Materials and methods

2.1. Identification and bioinformatic analysis of PtUBC genes in *P. Tricornutum*

The whole genome and protein information of *P. tricornutum* were retrieved from the Ensembl (<http://protists.ensembl.org/index.html>). The Hidden Markov Model (HMM) profile of UBC domain (PF00179) was retrieved from the Pfam database (<http://pfam.xfam.org/>) (Finn et al., 2016). The website hmsearch (<http://www.hmmsearch.org/>) (Eddy, 1998) was used to identify UBC proteins from *P. tricornutum* genome with a threshold of E-value $< 1 \times 10^{-5}$. Then all the candidate proteins were further confirmed by the Pfam, CDD (<https://www.ncbi.nlm.nih.gov/cdd/>), and SMART (<http://smart.embl.de/smart/batch.pl>) (Letunic et al., 2021) database.

Bioinformatic analysis was carried out as follows. The signal peptide was predicted on SignalP-5.0 (<https://services.healthtech.dtu.dk/service.php?SignalP-5.0>). Transmembrane domains were predicted on different websites (<https://services.healthtech.dtu.dk/service.php?TMHMM-2.0>, <https://topcons.cbr.su.se>, <https://dgpred.cbr.su.se/index.php>). The N-glycosylation, ubiquitination and phosphorylation modifications of putative UBC proteins were respectively validated by <https://services.healthtech.dtu.dk/service.php?NetNGlyc-1.0>; <https://services.healthtech.dtu.dk/service.php?NetPhos-3.1>; <https://bdiup.biocuckoo.org/>. The subcellular localization of protein was predicted on website (<http://cello.life.nctu.edu.tw/>). Further characterization, including the analysis of amino acid length, molecular weights (MWs), and theoretical isoelectric points (pIs) of the identified UBCs were predicted by ExPASy (Artimo et al., 2012).

2.2. Phylogenetic relationship, conserved motif and gene structure analysis of PtUBCs

The alignment of the full-length PtUBCs was performed via ClustalW software with parameters in default. MEGA 7.0 was used to construct an unrooted phylogenetic tree with the neighbor-joining method and 1000 bootstrap replicates. Subsequently, the tree was further improved by iTOL (Letunic and Bork, 2021). Multiple Em for Motif Elicitation (MEME) online program (<http://meme-suite.org/>) was used to predict the conserved motifs (Bailey et al., 2015). The parameters were following: any number of repetitions, optimum motif length of 6–50 residues, time 18,000 s, max size 6,000,000, and searching for 10 motifs, with default parameters. Online Gene Structure Display Server GSDS2.0 (<http://gsds.gao-lab.org/>) was used to analyze the gene structures (Hu

et al., 2015). Finally, TBtools software was used for the assembling and editing of the phylogenetic trees, conserved motifs and gene structures (Chen et al., 2020).

2.3. Chromosomal locations, cis-acting regulatory element and gene duplications analysis of PtUBC genes

The DNA sequence of each PtUBC was downloaded from the whole genome database of *P. tricornutum*. MapChart software (Voorrips, 2002) was used to map all PtUBC genes on chromosomes. The cis-acting regulatory elements on the promoter of each PtUBCs (1,500 bp sequences upstream of the coding sequences) were analyzed by PlantCARE databases (Lescot et al., 2002). The analysis of gene duplication events was carried out via a multiple collinear scanning toolkit MCScanX (Wang et al., 2012).

2.4. Analysis of PtUBC genes expression profiles under elevated pCO₂ stress

Strand-specific RNA sequencing (ssRNA-seq) data was obtained from the previous paper (Huang et al., 2019). Differential expression of the PtUBC genes in response to the elevated pCO₂ stress were analyzed. After the growth of 15 generations (10 days) in elevated pCO₂, the *P. tricornutum* cells were collected for the analysis of ssRNA-seq data. Ambient air of about 400 ppmv (low CO₂) was set for the low CO₂ medium, while bubbled with pre-mixed air-CO₂ mixtures (1,000 ppmv, high CO₂) was for the high CO₂ medium. Three replicated experiments were performed. The heatmap was improved via TBtools software (Chen et al., 2020).

3. Results

3.1. Identification and bioinformatic characteristics of PtUBCs in *P. tricornutum*

A total of 18 PtUBCs were identified from *P. tricornutum* by HMM search and named from PtUBC1 to PtUBC18 (Table 1). Gene ID, signal peptide, transmembrane domains, subcellular localization and modifications of protein were presented in Table 1. Except PtUBC12 and

Table 1

Putative signal peptide, transmembrane domain, N-glycosylation modification, ubiquitination modification, phosphorylation modification and subcellular localization of 18 putative PtUBC proteins.

Name	Gene ID	SP	TMD	N-glycan/ Ubi-/P-	Subcellular localization
PtUBC1	Phatr3_J13114	No	No	Yes	Nucleus
PtUBC2	Phatr3_J37077	No	Yes	Yes	Mitochondrion
PtUBC3	Phatr3_J28433	No	No	Yes	Cytoplasm
PtUBC4	Phatr3_J47555	No	No	Yes	Cytoplasm
PtUBC5	Phatr3_J48512	No	No	Yes	Nucleus
PtUBC6	Phatr3_J22525	No	No	Yes	Nucleus
PtUBC7	Phatr3_J48810	No	No	Yes	Cytoplasm
PtUBC8	Phatr3_J48883	No	No	Yes	Cytoplasm
PtUBC9	Phatr3_J30092	No	No	Yes	Nucleus
PtUBC10	Phatr3_J30389	No	No	Yes	Cytoplasm
PtUBC11	Phatr3_J5608	No	No	Yes	Nucleus
PtUBC12	Phatr3_J6645	Yes	Yes	Yes	Mitochondrion
PtUBC13	Phatr3_J33325	No	No	Yes	Nucleus
PtUBC14	Phatr3_J10724	Yes	No	Yes	Nucleus
PtUBC15	Phatr3_J26750	No	No	Yes	Nucleus
PtUBC16	Phatr3_J20308	No	No	Yes	Cytoplasm
PtUBC17	Phatr3_J36198	No	No	Yes	Nucleus
PtUBC18	Phatr3_J27762	No	No	Yes	Nucleus

Abbreviations: SP, signal peptide; TMD, transmembrane domain; N-glycan-, N-glycosylation modification; Ubi-, ubiquitination modification; P-, phosphorylation modification. No, inexistence of the modification; Yes, existence of the modification.

PtUBC14, signal peptide was not found from other PtUBCs. It was predicted that PtUBC2 and PtUBC12 harbor transmembrane domain, while other PtUBC proteins were not equipped with TMDs. All PtUBC proteins might be modified by N-glycosylation, ubiquitination and phosphorylation. Based on the predictions of subcellular localization, ten proteins (PtUBC1, PtUBC5, PtUBC6, PtUBC9, PtUBC11, PtUBC13, PtUBC14, PtUBC15, PtUBC17 and PtUBC18) might locate in nucleus, and six proteins (PtUBC3, PtUBC4, PtUBC7, PtUBC8, PtUBC10 and PtUBC16) were predicted in cytoplasm, only two proteins (PtUBC2 and PtUBC12) were speculated in mitochondrion. According to the information from ExPASy, the length of PtUBC proteins were ranged from 146 (PtUBC16) to 414 (PtUBC8) amino acids, the molecular weights were from 16361.5 to 45525.2 Da, and the isoelectric points were from 4.38 to 10.55 (Supplementary Table 1).

3.2. Evolutionary relationship, gene structure and conserved motifs of PtUBCs

The UBC domain sequences of 18 PtUBCs were used to construct a phylogenetic tree by MEGA 7.0 using neighbor-joining method with 1,000 bootstrap replicates. According to the phylogenetic relationship of domains, the 18 PtUBC proteins were classified into 5 groups (Fig. 1). Group II and Group IV contained 6 PtUBCs, followed by group III with 3 PtUBCs and group I with 2 PtUBCs. Group IV had only one PtUBC.

Using MEME, ten conserved motifs in the PtUBC proteins were displayed in Fig. 2A/2C. It was shown that except PtUBC8 and PtUBC13, all PtUBC proteins shared the motif 1. Apart from PtUBC5, PtUBC7 and PtUBC13, motif 4 was observed in all PtUBC proteins. Motif 1 and motif 4 were corresponded to UBC domain. In contrast, Motif 5 and Motif 8 existed only in PtUBC1, PtUBC2 and PtUBC12. Similarly, motif 9 was identified only from PtUBC8 and PtUBC13. Motif 10 was only presented in PtUBC1 and PtUBC8. Additionally, motif 7 was mainly found in PtUBC3, PtUBC11, PtUBC15, PtUBC18, PtUBC10, PtUBC17, PtUBC7 and PtUBC13. Motif 2, motif 3 and motif 6 were also present in 11–12 PtUBC proteins. Regardless, the PtUBC proteins from the same phylogenetic clade had the same or similar motif structure. The phylogenetic tree with 18 full-length sequences of PtUBC proteins was built, as presented in Fig. 2B. It was observed that all 18 PtUBC proteins were divided into three big clades. Exon-intron organization analysis of PtUBC genes was also performed, as shown in Fig. 2D. The number of exons in the PtUBC genes ranged from one to two. Among them, PtUBC5, PtUBC14, PtUBC18, PtUBC2 and PtUBC1 genes had only one exon, while the majority (13/18) contained two exons. PtUBC5, PtUBC14, PtUBC2 and PtUBC1 genes had no introns. Additionally, it was observed that PtUBCs with similar motif architectures had similar gene structures.

3.3. Chromosome localization of PtUBC genes and its cis-acting elements

Chromosome location of PtUBC genes was displayed via MapChart software. As shown in Fig. 3, 18 PtUBC genes were unevenly distributed to 14 chromosomes of the total 33 chromosomes. Only one PtUBC gene was found on chromosomes 3, 4, 6, 8, 10, 14, 20, 22, 23 and 25. Two PtUBC genes were mapped on chromosomes 9, 12, 18 and 19.

Cis-acting elements play a crucial role in gene transcription and expression. Cis-acting regulatory elements were shown in Supplementary Fig. 1 and Supplementary Table 2. The universal regulatory elements such as CAAT-box and TATA-box were observed in all 18 PtUBC genes. Besides, hormone-responsive boxes were revealed in the promoter regions of PtUBC genes, such as ABRE (abscisic acid responsiveness), TGA-element (auxin-responsive), AuxRR-core (auxin-responsive), P-box (gibberellin-responsive), TGACG-motif (MeJA-responsiveness) and CGTCA-motif (MeJA-responsiveness). Several stress-related boxes were also identified in the promoter region of PtUBC genes, including ARE for anaerobic induction, LTR for low-temperature, MBS (MYB binding site) involved in drought-inducibility. Cis-acting regulatory

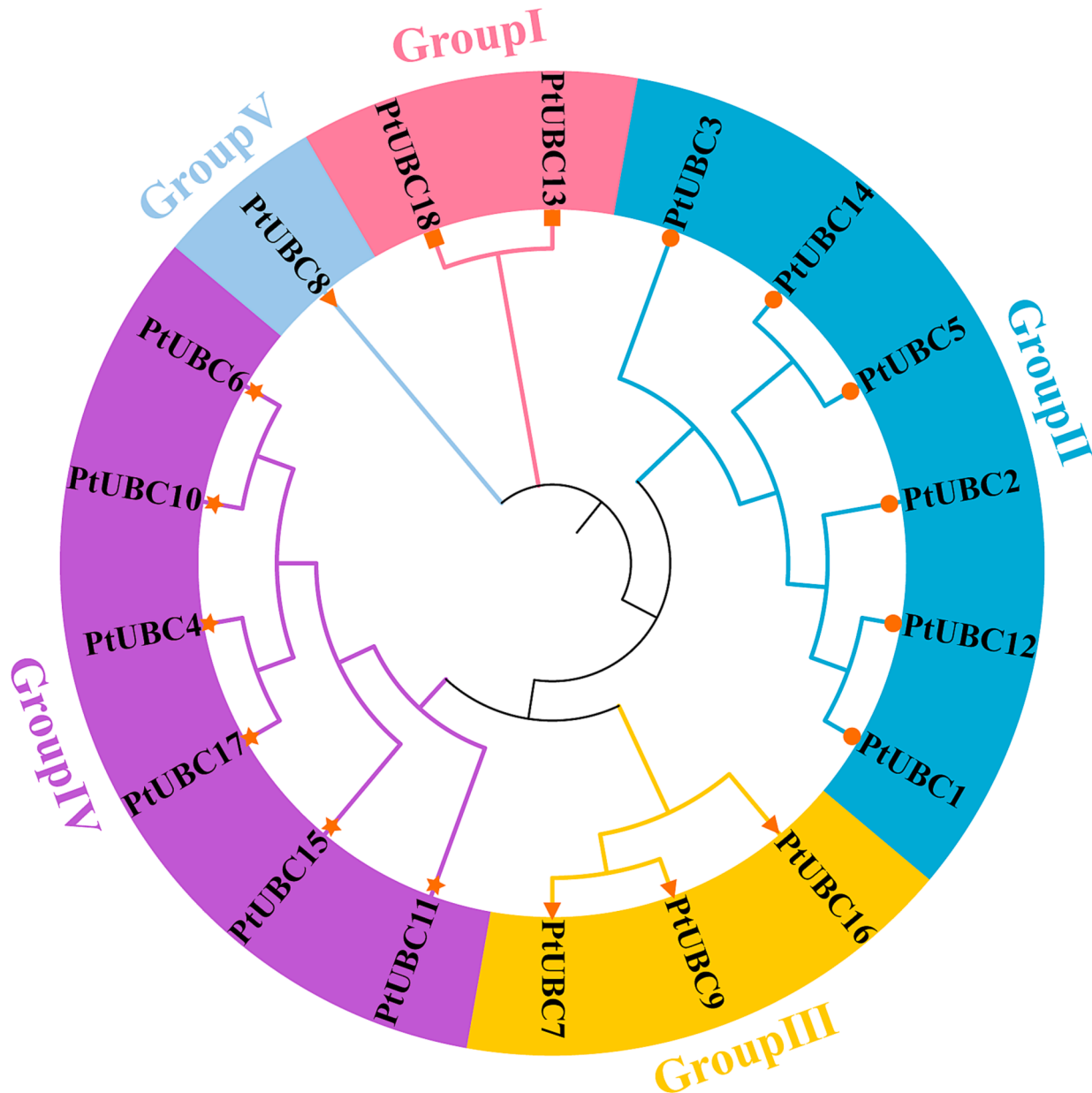


Fig. 1. Phylogenetic relationship of the 18 different PtUBCs in *P. tricornutum*. Multiple sequence alignment of PtUBC domain sequences is performed by ClustalW. MEGA 7.0 is used to construct the neighbor-joining (NJ) tree with 1,000 bootstrap replicates. Different groups were marked with different colors and labels.

elements related with light response, such as AE-box, GATA-motif, G-box, GT1-motif, CAG-motif, I-box, Sp1 and TCT-motif, were also identified from the promoters of PtUBCs. *Cis*-acting regulatory elements involved in endosperm expression (GCN4 motif), salicylic acid response (TCA-element), defense and stress responses (TC-rich repeats), circadian control (circadian), endosperm-specific negative expression (AACA-motif), zein metabolism regulation (O₂-site) and related to meristem expression (CAT-box) were also presented in the promoter regions of PtUBC genes. Based on the analysis of MCScanX, a total of 252 tandem-duplicated gene pairs were detected from the genome of *P. tricornutum*. However, PtUBC gene pairs were not found (Supplementary Table 4). According to the segmental-duplicated analysis, 69 pairs of segmental duplication were identified from the 33 chromosomes of *P. tricornutum* (Supplementary Table 3). However, segmental duplication was not observed among PtUBCs.

3.4. Expression pattern of PtUBC genes under the elevated *p*CO₂ stress

To analysis the expression of PtUBC gene family in response to the elevated *p*CO₂ stress, the expression profiles of 18 PtUBC genes were compared based on the ssRNA-Seq data. Among 18 PtUBC genes, 3 PtUBC genes (*PtUBC15*, *PtUBC16* and *PtUBC7*) were significantly downregulated ($\text{Log}_2\text{fold-change} < 0$). No gene was upregulated ($\text{Log}_2\text{fold-change} > 0$) under the elevated *p*CO₂ stress (Supplementary Table 5). As shown in Fig. 4, the normalized data was used to display the hierarchical clustering of PtUBCs gene expression. It was observed that the expression profile was divided into two patterns. Pattern I, the genes, including *PtUBC3*, *PtUBC9*, *PtUBC2*, *PtUBC17*, *PtUBC6*, *PtUBC4*, *PtUBC7*, *PtUBC18*, *PtUBC15*, *PtUBC16*, *PtUBC14* and *PtUBC10* genes, were low expressed under the treatment of high CO₂ (H-normalize) compared to low CO₂ condition (L-normalize). Pattern II, the genes including *PtUBC13*, *PtUBC11*, *PtUBC5*, *PtUBC12*, *PtUBC8* and *PtUBC1* genes, were highly expressed under the treatment of high CO₂ level compared to low CO₂ condition. More information concerning the

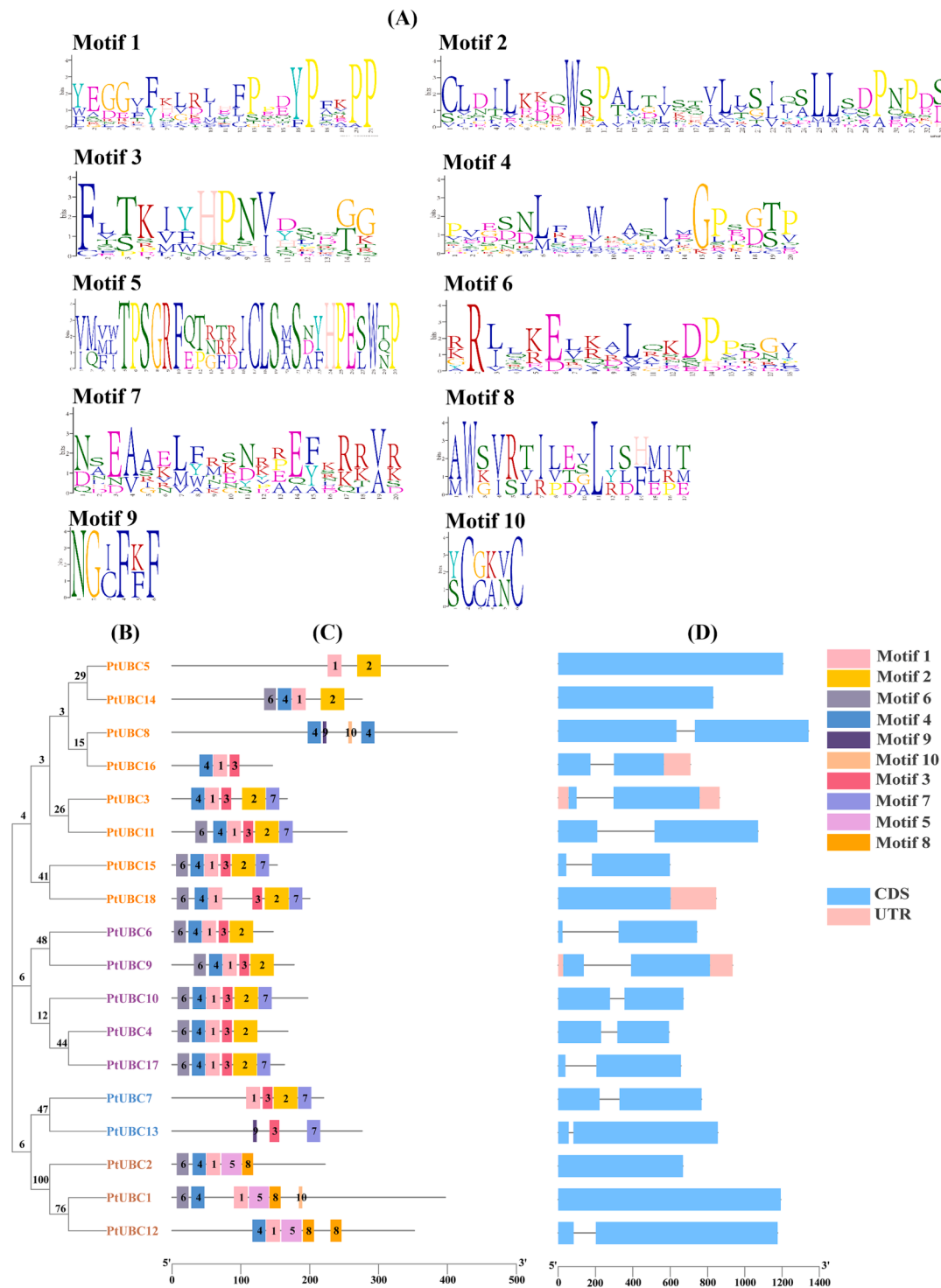


Fig. 2. Phylogenetic relationship, motif and gene structure of PtUBCs in *P. tricornutum*. (A) Ten conserved motifs from 18 PtUBCs. The ten conserved motifs were predicted by the online program Multiple Em for Motif Elicitation (MEME). The parameters were set as following: any number of repetitions, 6–50 amino acids as optimum motif length, and searching for 10 motifs, with all other parameters in default. (B) Phylogenetic tree. The alignment of multiple full-length sequences of PtUBCs were performed using ClustalW. The neighbor-joining (NJ) tree was constructed via MEGA 7.0 with 1,000 bootstrap replicates. Different clades were marked with different colors. (C) Conserved motifs. The conserved motifs of PtUBCs were compared via MEME version 5.4.1. Different motifs were represented by diverse colors. (D) Gene structure. The blue and pink boxes represented CDS (coding sequence) and UTR (untranslated region), respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

expression of PtUBC genes in response to the elevated $p\text{CO}_2$ stress could be found in Supplementary Table 5. The expression of all PtUBC genes were different under the elevated $p\text{CO}_2$ stress, but genes in the same pattern showed similar expression profiles.

4. Discussion

The UBC enzymes play an essential role in ubiquitination mechanism, and they are important for the plant development (Liu et al., 2020). However, previous studies on UBC enzymes focused few on

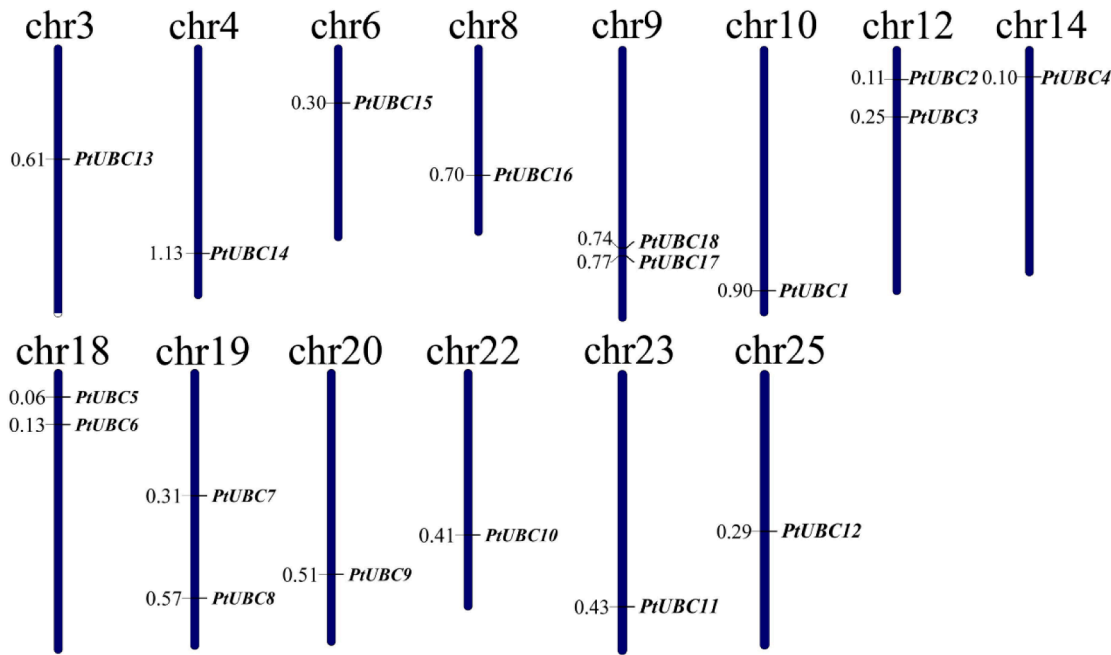


Fig. 3. Chromosome location of PtUBC genes. Blue bars indicate the different chromosomes. The numbers on the top of each chromosome are the numbers of chromosomes. The numbers (Mb) on the left side of each chromosome represent the localization of PtUBC gene. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

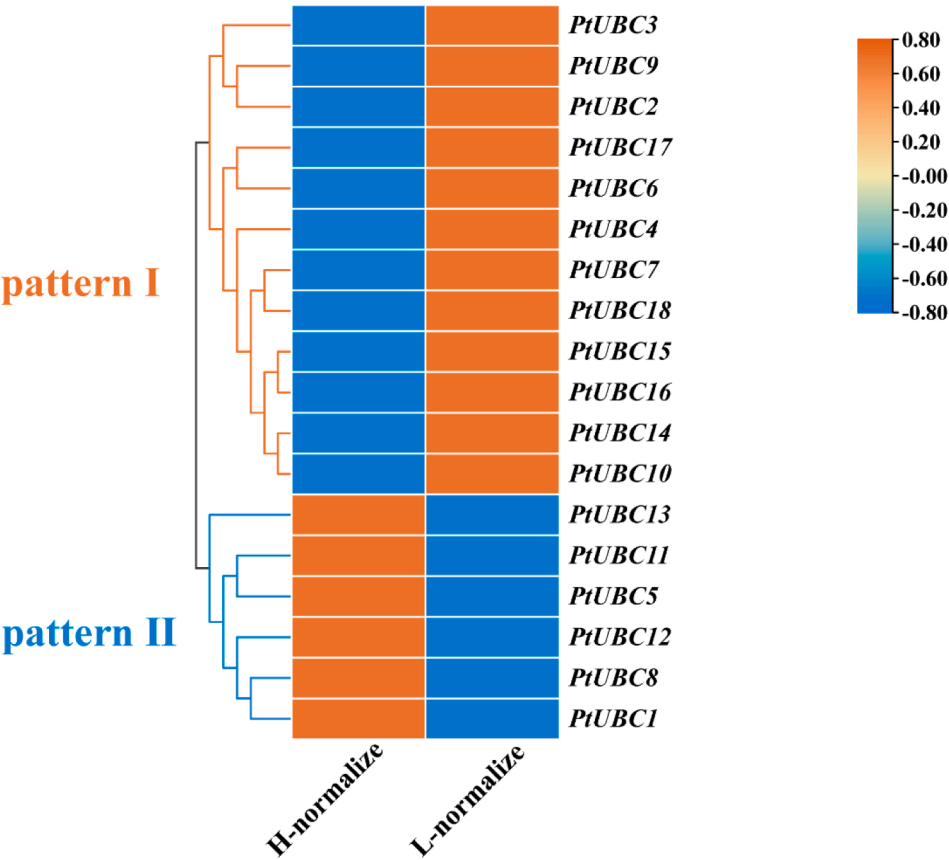


Fig. 4. Expression profile of 18 PtUBC genes in response to the elevated $p\text{CO}_2$ stress. The heat-map was draw via TBtools software. The expression data were gene-wise normalized and hierarchically clustered with complete linkage. The bar on the right of the heat map was the relative expression values. The numbers -0.80 , 0.00 , 0.80 stand for low, intermediate, and high expression, respectively. Different patterns were marked with different colors. Orange and blue lines represent pattern I and pattern II, respectively. H-normalize, the treatment of high CO_2 level; L-normalize, the treatment of low CO_2 level. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

microalgae, especially in diatom. In this work, UBC gene family was systematically studied on diatom *P. tricornutum*. In *P. tricornutum*, a total of 18 genes with UBC family domains were identified. And they were distributed to the 14 chromosomes of total 33 chromosomes. Compared

to the reported UBC members in other organisms, the number of PtUBCs was less than that in plants and green algae. It was previously found that the member of UBC enzymes was 37 in *Arabidopsis thaliana* (Kraft et al., 2005), 75 in *Zea mays* (Jue et al., 2015), 23 in *Chlamydomonas reinhardtii*

(Fei et al., 2017), 74 in *Musa acuminata* (Dong et al., 2016), 48 in *Oryza sativa* L. (Bae and Kim, 2014). However, the number of UBC in *P. tricornutum* was more than that in *Saccharomyces cerevisiae*, where 13 UBC genes were identified (Jones et al., 2002). The diverse number of UBC enzymes might be explained by the different evolutionary relationships in different species. Besides, it was proposed that the number of UBC gene family might be related with the genome size of species. In this study, all UBC domains from 18 PtUBCs were constructed into five groups in the phylogenetic tree. All PtUBC proteins were divided into four clusters, and PtUBCs in each independent cluster shared similar structure and conserved motifs via the analyses of phylogenetic relationship and multiple sequence alignment. Although the function of these conserved motifs remains unknown, they might play pivotal roles in the regulation of ubiquitination mechanism. At the same time, it was observed that 72.22% of all PtUBC genes did not have intron, while the other 27.78% harbored one intron. Compared to UBC genes in *Musa acuminata* with 1–11 introns (Dong et al., 2016) and *Carica papaya* with 5 introns (Jue et al., 2017), the PtUBC genes showed great structural integrity. Because introns were not included in the genes of prokaryotes, the analysis of intron gain or loss (IGL) was mainly performed in eukaryotes. Many factors, such as species and selection pressures, would have a great influence on IGL (Kamioka et al., 2016).

A large proportion of PtUBCs were predicted to target to the nucleus (55.56%) and cytoplasm (33.33%). The subcellular localization of UBC proteins were also analyzed in *C. reinhardtii*, where showed that CrUBC proteins were mainly distributed to the nucleus and cytoplasm (Fei et al., 2017). It was already known that nucleus located ZmUBC proteins were primarily function in the nucleus, but not in cytoplasm, chloroplast, mitochondrion, or endoplasmic reticulum (Jue et al., 2015). Therefore, it was speculated that different subcellular PtUBC proteins might play specific roles in cell. A tremendous number of hormone, stress and light response elements were detected in the promoter regions of PtUBCs. According to the research in plants (Sharma et al., 2019), it is reasonable to speculate that PtUBC genes might be activated by lots of hormones and might engage in diverse stress responses. Previous study revealed that gene families usually experience tandem duplication or segmental duplication to maintain the abundant scale of each family during the evolutionary process (Cannon et al., 2004). In this study, tandem duplication, or segmental duplication of PtUBC genes were not observed in the genome of *P. tricornutum*. Therefore, it was proposed that the amplification of PtUBC genes might be the results of rearrangement and transposition events (Jue et al., 2015).

It was already known that some abiotic/biotic stresses (e.g. elevated pCO_2 stress) could disrupt the protein folding and induce ER stress. ER

quality control (ERQC) was firstly activated to maintain ER protein homeostasis. However, when the ER stress exceeded the ability of cell self-regulation, the unfolded protein response was activated to enhance the ER protein folding and the degradation of incorrect/ unfolded proteins via the ERAD (Li et al., 2017). The model of ERAD mechanism was predicted in *P. tricornutum* under the elevated pCO_2 stress (Fig. 5). The ERAD process contained substrate recognition, targeting, retro-translocation, polyubiquitination and degradation by the 26S proteasome (Cui et al., 2012). UBC enzymes participated in the ubiquitination pathway. UBC played key roles in ERAD mechanism, assisting the refolding of incorrect or unfolded proteins under the different abiotic or biotic stresses (Cui et al., 2012).

Most of PtUBC genes were not differentially expressed under the treatment of high CO_2 level, indicating that these PtUBC genes expressed stably in response to the elevated pCO_2 stress and might not be important under the high CO_2 level. Interestingly, it was observed that 3 PtUBC genes (*PtUBC15*, *PtUBC16* and *PtUBC7*) were significantly downregulated under the exposure to the high CO_2 level. Previous study suggested that *A. thaliana* UBC13 played an important role in the ubiquitination during the pathway of ERAD (Feng et al., 2020). Owing to the high homology between *PtUBC15*, *PtUBC16* and *A. thaliana* UBC13, it was reasonable to infer that *PtUBC15* and *PtUBC16* might play roles in ERAD pathway in *P. tricornutum*. The model of ERAD mechanism in *P. tricornutum* demonstrated how misfold/ unfolded proteins were degraded under the elevated pCO_2 treatment. ER stress was activated under the elevated pCO_2 treatment. When the ER stress was higher than the capacity of self-regulation in cell. The misfolded/unfolded proteins were accumulated in the cell and will be finally degraded by the ERAD mechanism. Therefore, it was proposed that the downregulated *PtUBC15*, *PtUBC16* and *PtUBC7* genes might participate in the protein ubiquitination, assisted the degradation of misfolded/ unfolded protein, and finally maintain the protein and cell homeostasis under the stress of elevated pCO_2 treatment.

Additionally, PtUBCs might also have important functions in response to other stresses. It was known that ubiquitination of proteins participated in carbon metabolism and induced degradation of proteins in premature senescence leaf (Yu et al., 2022). Therefore, it was speculated that PtUBCs as the key components of ubiquitination might also involve in carbon metabolism responding to the elevated pCO_2 stress. The differentially expressed PtUBC genes (*PtUBC15*, *PtUBC16* and *PtUBC7*) might be able to regulate the level of ubiquitination and then effect carbon metabolism. Moreover, it was previously reported that in vitro conjugation of di-Ub formation at K48 via E2-25 K protein was obviously reduced when the CO_2 concentration arose from the normal

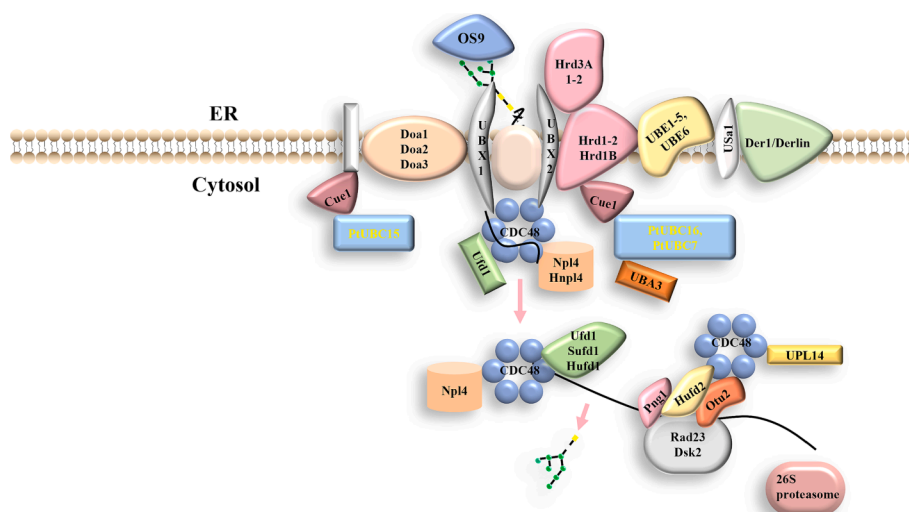


Fig. 5. The model of ERAD mechanism in *P. tricornutum* under the elevated pCO_2 stress. Down-regulated PtUBC genes were marked by yellow under the elevated pCO_2 stress. Note: OS9, osteosarcoma 9; Doa2, putative degradation of alpha-2 protein; UBA: ubiquitin-associated (UBA) domain; UBC, ubiquitin-conjugating enzyme E2; UBE, ubiquitin-protein ligase E3; UBCc, UBC core domain-containing protein; CDC48, cell division control protein 48; Ufd, ubiquitin fusion degradation protein; Npl, substrate-recruiting factor Npl; Otu, ubiquitin thioesterase Otu; Png, peptide N-glycanase; Rad23, ubiquitin receptor Rad23; Dsk2, ubiquitin receptor Dsk2; Cue1: an ER anchor protein; Usa1: U1-Snp1 associating-1; UBx, ubiquitin regulatory X domain-containing protein; UPL, ubiquitin-protein ligase. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

physiological level to the hypercapnic range (Linthwaite et al., 2021). Hence, it was presumed that PtUBC15, PtUBC16 and PtUBC7 might participate in the process of di-Ub formation at K48 under the high CO₂ level via similar pathway as E2-25 K. In addition, *A. thaliana* UBC13 genes were reported for DNA damage and other error-free damage tolerances (Wen et al., 2006). Because PtUBC15 and PtUBC16 had homologies with *A. thaliana* UBC13, it was inferred that PtUBC15 and PtUBC16 might have the ability to repair DNA damage and sustain cellular homeostasis. At the same time, *A. thaliana* UBC13 was also involved in the response to low-temperature stress (Wang et al., 2019). PtUBC15 and PtUBC16 might have the similar function as AtUBC13 under the cold stress. PtUBC15 also displayed homology with UBC2 in *Oryza sativa*. OsUBC2 was obviously upregulated by hormone stress at 24 h and strongly down-regulated by drought and/or salt stresses (E et al., 2015). It was possible that PtUBC15 participated in the regulation of such abiotic stresses. PtUBC7 had a homology with UBC2 in *Sorghum bicolor*, it was speculated that PtUBC7 might response to abiotic stresses, including cold, NaCl and PEG 6000 treatments as SbUBC2 (Jia et al., 2019). Therefore, we speculated that the 3 PtUBC genes (*PtUBC15*, *PtUBC16* and *PtUBC7*) in *P. tricornutum* were able to respond to different abiotic and/or biotic stresses, including elevated pCO₂ stress, cold treatment and drought stress and served essential functions to maintain cellular homeostasis. However, further studies are still needed to clarify the accurate function of these PtUBCs. In a word, our results of these differentially expressed PtUBC genes laid a solid foundation for further study of the biological functions in the PtUBC gene family under different stresses, especially the elevated pCO₂ stress.

5. Conclusion

Totally, 18 PtUBC genes were identified and divided into 5 clades. Different *cis*-acting elements related to stress responses were characterized from PtUBCs, indicating their importance in the elevated pCO₂ stress. Based on the transcriptomic analysis, *PtUBC15*, *PtUBC16* and *PtUBC7* genes were downregulated under the elevated pCO₂ stress. Moreover, the ERAD mechanism model of ERAD in *P. tricornutum* displayed that misfolded/ unfolded proteins might be degraded by the ERAD to maintain the cellular homeostasis under the elevated pCO₂ stress. Overall, this study provided a foundation for comprehensive understanding of distribution, evolutionary relationship and probable function of PtUBC genes and created a base paradigm for further research on ERAD mechanism in microalgae, especially in diatom.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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CRedit authorship contribution statement

Wanying Ma: Investigation, Methodology, Writing – original draft, Writing – review & editing. **Hua Du:** Investigation, Methodology, Writing – original draft, Writing – review & editing. **Syed Shabi Ul Hassan Kazmi:** Investigation, Methodology, Writing – review & editing. **Jichen Chen:** Investigation, Methodology, Writing – review & editing. **Weizhou Chen:** Investigation, Methodology, Writing – review & editing. **Yanmei Fan:** Investigation, Methodology, Writing – review & editing. **Zidong Liu:** Investigation, Methodology, Writing – review & editing. **Haodong Luo:** Investigation, Methodology, Writing – review & editing. **Hao Fang:** Investigation, Methodology, Writing – review & editing. **Zhen Wang:** Conceptualization, Project administration, Supervision, Funding acquisition, Writing – review & editing. **Xiaojuan Liu:** Conceptualization, Project administration, Supervision, Funding acquisition, Writing – review & editing.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecolind.2023.110106>.

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