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Transcriptomic profiles and diagnostic biomarkers in the Mediterranean seagrasses *Posidonia oceanica* and *Cymodocea nodosa* reveal mechanistic insights of adaptative strategies upon desalination brine stress

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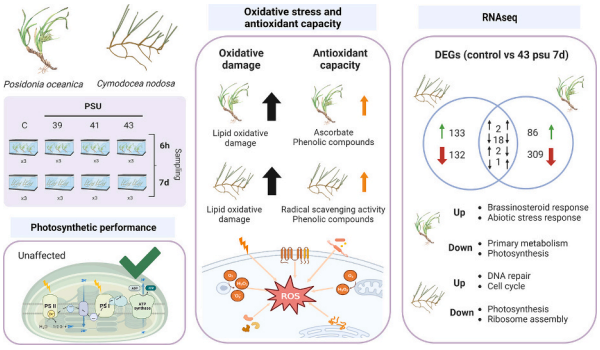
HIGHLIGHTS

- *P. oceanica* and *C. nodosa* suffer oxidative damage at 43 psu.
- Both seagrasses display different antioxidant mechanisms when exposed to brine.
- RNAseq in *P. oceanica* reveal an upregulation of brassinosteroid response genes.
- RNAseq in *C. nodosa* reveal an upregulation of DNA repair and cell cycle genes.
- Genes involved in essential biological processes are severely downregulated.

GRAPHICAL ABSTRACT



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ABSTRACT

Seawater desalination by reverse osmosis is growing exponentially due to water scarcity. Byproducts of this process (e.g. brines), are generally discharged directly into the coastal ecosystem, causing detrimental effects, on benthic organisms. Understanding the cellular stress response of these organisms (biomarkers), could be crucial for establishing appropriate salinity thresholds for discharged brines. Early stress biomarkers can serve as

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valuable tools for monitoring the health status of brine-impacted organisms, enabling the prediction of long-term irreversible damage caused by the desalination industry. In this study, we conducted laboratory-controlled experiments to assess cellular and molecular biomarkers against brine exposure in two salinity-sensitive Mediterranean seagrasses: *Posidonia oceanica* and *Cymodocea nodosa*. Treatments involved exposure to 39, 41, and 43 psu, for 6 h and 7 days. Results indicated that photosynthetic performance remained unaffected across all treatments. However, under 43 psu, *P. oceanica* and *C. nodosa* exhibited lipid oxidative damage, which occurred earlier in *P. oceanica*. Additionally, *P. oceanica* displayed an antioxidant response at higher salinities by accumulating phenolic compounds within 6 h and ascorbate within 7 d; whereas for *C. nodosa* the predominant antioxidant mechanisms were phenolic compounds accumulation and total radical scavenging activity, which was evident after 7 d of brines exposure. Finally, transcriptomic analyses in *P. oceanica* exposed to 43 psu for 7 days revealed a poor up-regulation of genes associated with brassinosteroid response and abiotic stress response, while a high down-regulation of genes related to primary metabolism was detected. In *C. nodosa*, up-regulated genes were involved in DNA repair, cell cycle regulation, and reproduction, while down-regulated genes were mainly associated with photosynthesis and ribosome assembly. Overall, these findings suggest that 43 psu is a critical salinity-damage threshold for both seagrasses; and despite the moderate overexpression of several transcripts that could confer salt tolerance, genes involved in essential biological processes were severely downregulated.

1. Introduction

Seawater (SW) desalination is often considered as the most effective measure to tackle freshwater scarcity, which is seriously hampering sustainable development worldwide (Boretti and Rosa, 2019). This technology can deliver a climate-independent and consistent supply of high-quality water (UN-Water, 2020). Nowadays, desalination plants produce 97.2 million m^3/day (Eke et al., 2020) - mainly by reverse osmosis (RO) – which is projected to increase by two to three times by 2050 (Mayor, 2019; Gao et al., 2017).

Despite the undeniable social benefits of SWRO, it poses negative environmental impacts that must be mitigated. These are mainly attributed to the discharge of reject brine into the sea (Panagopoulos and Haralambous, 2020; Fernández-Torquemada et al., 2019). Brines may contain high salinity levels (65–85 psu), heavy metals, and residual chemicals from the pre- and post-treatment phases (Elsaid et al., 2020). In consequence, direct discharge can negatively affect sensitive marine ecosystems, particularly benthic communities (Fernández-Torquemada et al., 2019). Within the last, an important group of the impacted organisms are seagrasses (Ruíz et al., 2009; Cambridge et al., 2019; Blanco-Murillo et al., 2022).

Seagrasses, as key marine organisms, form meadows that shelter a vast array of fauna and structure one of the most valuable ecosystems on Earth (Unsworth et al., 2019). As such, it is essential to minimize and early detect brine impacts on seagrass meadows, and to understand their tolerance mechanisms for the preservation of these ecosystems (Sandoval-Gil et al., 2022).

One strategy for the early detection of pollutant impacts on organisms involves the use of biomarkers. These represent measurable changes at lower levels of biological organization (cellular, biochemical, and molecular) that pollutants induce. Biomarkers can serve as early warning signals, enabling timely intervention before impacts become visible and irreversible (Macreadie et al., 2014; Lemos, 2021).

Although knowledge regarding the ecophysiological effects of brines and hypersalinity on seagrasses has significantly improved over the past two decades (Sandoval-Gil et al., 2022), the investigation into biomarkers of brine stress and the early cellular/molecular response to salinity is limited. Hypersalinity stress biomarkers were only recently explored in *Posidonia oceanica* within a population chronically exposed to brine discharges (Capó et al., 2020). These individuals demonstrated increased levels of oxidative damage associated biomarkers and antioxidant defense systems (both enzymatic and non-enzymatic). It remains to be unveiled whether oxidative stress biomarkers can be detected at shorter exposure times and consequently serve as early warning signs of brine impacts. The early molecular response of the Mediterranean seagrass *Cymodocea nodosa* to hypersalinity was investigated by Malandrakis et al. (2017) and Tsioli et al. (2022). Both studies examined the transcriptomic response (RNA-seq) exposed to hypersaline (50 psu) and high temperature stress (40 °C) for a short-term duration (24 h), where elongation factors and protein kinases encoding genes were induced. A recently published article that evaluated cellular and molecular biomarkers in the relict south-east Pacific seagrass *Zostera chilensis* against artificial brine, revealed that 37 psu (for 3 days) was sufficient to generate oxidative stress and trigger the up-regulation of genes related to the scavenging of reactive oxygen species (ROS) (Blanco-Murillo et al., 2023).

The purpose of this study was to explore seagrasses cellular and molecular early and mid-term response to hypersalinity exposure. The goal was both, to enhance our understanding of their tolerance mechanisms and to identify potential early-stage biomarkers for desalination brine stress. We selected two species with different salinity tolerances ranges, *P. oceanica* (stenohaline) and *C. nodosa* (more euryhaline), which are key structuring seagrass communities in the Mediterranean. Their response to brine stress was studied using two approaches: a hypothesis-driven approach, examining physiological indicators and biomarkers related to an expected salinity stress response in seagrasses (including oxidative stress and antioxidant defense), and a non-hypothesis driven approach, wherein the overall transcriptomic response was assessed via RNA-seq.

2. Materials and methods

2.1. Sample collection, culture conditions and hypersalinity treatments

A total of 36 individuals of *P. oceanica* and 36 of *C. nodosa* were collected in Autumn (2018) between 20 and 30 m from the shoreline of the "Centro de Investigación Marina" (CIMAR), Alicante University (38°12′35.5"N; 0°30′25.8"W) located in Santa Pola, Spain (natural average salinity ~37 psu). Samples were obtained by scuba divers at 5 m depth, brought to the laboratory and washed twice with filtered (0.22 μm) seawater.

All individuals were acclimated in plastic containers filled with filtered seawater and provided with constant aeration. They were exposed to a photoperiod of 12:12 (day:night), with a photosynthetic active radiation (PAR) intensity of 120 μmol m^{-2} s^{-1}, and a constant temperature of 24 ± 1 °C for 48 h (Rodríguez-Rojas et al., 2020).

For the hypersalinity treatments, the samples were divided into 12 glass aquariums, with 3 aquariums assigned to each condition: 37 psu (control), 39 psu, 41 psu, and 43 psu. Salinity levels were increased by adding artificial seawater salts (Instant Ocean®) and monitored using a multiparameter probe (Hanna HI98494). In all treatments, the water was changed every 48 h to maintain the desired salinity levels. The experimental conditions during the treatments were the same as those during the acclimation period. Subsamples of shoots were collected at 6

h (short-term) and 7 days (mid-term) after the treatments, rapidly frozen using liquid nitrogen, and stored at −80 °C for subsequent biochemical and molecular experiments.

2.2. Photosynthetic performance

We determined the in vivo chlorophyll *a* fluorescence associated with Photosystem II using a portable pulse amplitude modulated (PAM) fluorometer (Junior-PAM, Walz GmbH, Germany), as detailed in Celis-Pla et al. (2022). We obtained the maximum quantum yield of PSII (Fv/Fm), maximal electron transport rate (ETRmax), and photosynthetic efficiency (αETR) from the tangent model function, according to Figueroa et al. (2003) and Figueroa et al. (2014)), and Celis-Pla et al. (2022). Additionally, we calculated the irradiance at which ETR is saturated (Ek) from the intercept between ETRmax and αETR. Lastly, we computed non-photochemical quenching (NPQ) and the associated parameter NPQmax following the methodologies of Schreiber et al. (1995) and Eilers and Peeters (1988).

2.3. Oxidative stress parameters

We measured parameters related to ROS production and oxidative damage - quantified through hydrogen peroxide levels (H_2O_2) and membrane lipid peroxidation (MDA) - using spectrophotometric analyses based on the protocols of Sáez et al. (2015), Rodríguez-Rojas et al. (2020), and Celis-Plá et al. (2020).

2.4. Antioxidant capacity and molecules content

We assessed the antioxidant capacity profile by determining phenolic compounds, total radical scavenging activity (RSA), and reduced/oxidized ascorbate (ASC/DHA) levels. We carried out these measurements using spectrophotometric methods as described by Rodríguez-Rojas et al. (2020) and Celis-Pla et al. (2022).

2.5. RNA extraction and sequencing

We chose control salinity and 43 psu at 7 days of exposure as the selected conditions for the transcriptomic analyses, applicable to both species. We purified RNA from 100 mg of frozen tissue (−80 °C), which was obtained from the middle section of green leaves, using the Favor-Prep Plant Total RNA Purification Mini Kit (Favorgen) as per the manufacturer's instructions. We assessed the quality of the RNA in terms of purity and integrity, gauged by the 260/280 ratio and 1 % agarose gel electrophoresis using the "bleach gel" method (Aranda et al., 2012), respectively. We quantified the high-quality RNA samples using the Quant-iT RiboGreen RNA Assay Kit (Invitrogen) and a QFX Fluorometer (DeNovix). We then sent three RNA samples per treatment to BGI Genomics (Shenzhen, China), where library construction and sequencing were conducted on the Illumina Hiseq 2500 150PE platform, according to their protocols.

2.6. Bioinformatic analyses

References transcriptomes were built for both species, followed by a differentially expressed genes (DEGs) analysis. Raw paired-end reads (150 bp) yielded 290,714,986 for *P. oceanica* and 289,983,420 for *C. nodosa*. Reads quality were evaluated using FastQC v.0.1.1.8 for each library showing at least 80 % of reads duplication, a %GC of 52 and no sequencing primers. All samples were trimmed with Prinseq v.0.20.4 using default parameters, excepting minimum length at 50 – Minimum quality mean 30 - trim quality left 30 bp – Trim quality window 5. From Prinseq processed libraries de novo transcriptomes were generated using trinity v.1.9.1 for each species. The following FASTA files were aligned against the *Mus musculus* (GCA_000001635.9) genome to clean contaminating contigs. For this bwa v.0.7.17-r1188 software with the bwa mem option was used, obtaining an alignment "SAM" file. Then subsequent subsamples were generated using Samtools v.1.8 with the view-F4 option including only contigs aligned with *Mus musculus*. From these alignments all the sequences that did not aligned with *M. musculus* were extracted using seqtk v.13-r106 through the subseq algorithm. Final non-aligned reads of each library were assembled for the construction of the *P. oceanica* and *C. nodosa* de novo transcriptomes

We evaluated the assembly quality of both transcriptomes using three methods. First, BUSCO v.4.0.2 using -l embryophyte -m tran parameters that mapped to ultra-conserved genes (transcriptome completeness). Second, Transrate v.1.0.3 using the annotated proteins of *Colocasia esculenta* (taro) (https://www.ncbi.nlm.nih.gov/genome/?term=12429) genome as a reference to evaluate the general quality of the assemblies. Third, the sendsketch.sh script of BBMap v.38.75 for the identification of contaminant sequences.

For the annotation process, complete open reading frames (ORFs) were identified through TransDecoder v.5.5.0 (Haas et al., 2013). Identified proteins were analyzed using the online platforms GhostKoala, EggNog v.5.0.0, and aligned by BLASTp tool against the Swiss-Prot for functional annotation. DEGs were obtained using additional scripts of the Trinity software including appropriate commands as RSEM statistical base for normalization, standardization and genes count, while DESeq2 was used to assess differential gene expression (Love et al., 2014).

Filtration criteria for DEGs that were considered as down-regulated and up-regulated genes were set as those transcripts with a $-1 < \text{Log}(2)\text{FC (fold change)} > 1$, and a p.adj < 0.05, in regard to control salinity samples. All RNA-seq data has been deposited in the NCBI GEO database under the BioProject ID: PRJNA967008. Gene ontology (GO) term annotation was performed firstly by carrying out a local alignment for both species against the annotated genome of *Arabidopsis thaliana* (Cheng et al., 2017) by BLAST tool to generate a TSV file and thus obtaining TAIR gene homologs and ENTREZ codes using the R package "org.At.tair.db" v.3.16.0 (Carlson, 2019). Then, ENTREZ codes of the compared treatments and species were analyzed through the EnrichGO function of the R package "clusterProfiler" v.4.2.6 (Wu et al., 2021).

2.7. Statistical analyses

We performed statistical analyses on phenolic compounds, DPPH, H_2O_2, TBARS, total ascorbate, reduced ascorbate, photosynthetic efficiency, ETRmax, Fv/Fm, and NPQmax. Normality of the data was assessed using Shapiro-Wilk test, and homoscedasticity was evaluated using the Bartlett's test. Once we confirmed normality and homoscedasticity, we conducted a one-way ANOVA to determine significant differences across treatments. To further investigate these differences, we utilized the Tukey's HSD procedure as the method for multiple comparison testing. We employed a significance level of $\alpha = 0.05$ (p-value) for all statistical tests. We conducted all these analyses using Matlab, maintaining a clear focus on identifying potential patterns or differential responses in the investigated variables.

3. Results

3.1. Photosynthetic performance

Salinity treatments had no significant effect ($p < 0.05$) on the photosynthetic parameters measured (Fv/Fm, αETR, ETRmax, NPQmax) in both species (Fig. 1 and Fig. 2). Therefore, photosynthesis parameters were not significantly affected by hypersalinity stress under the experimental conditions studied.

3.2. Quantification of oxidative stress and damage parameters

H_2O_2 concentrations, as a proxy of ROS generation, showed no significant differences between the control and salinity treatments in both

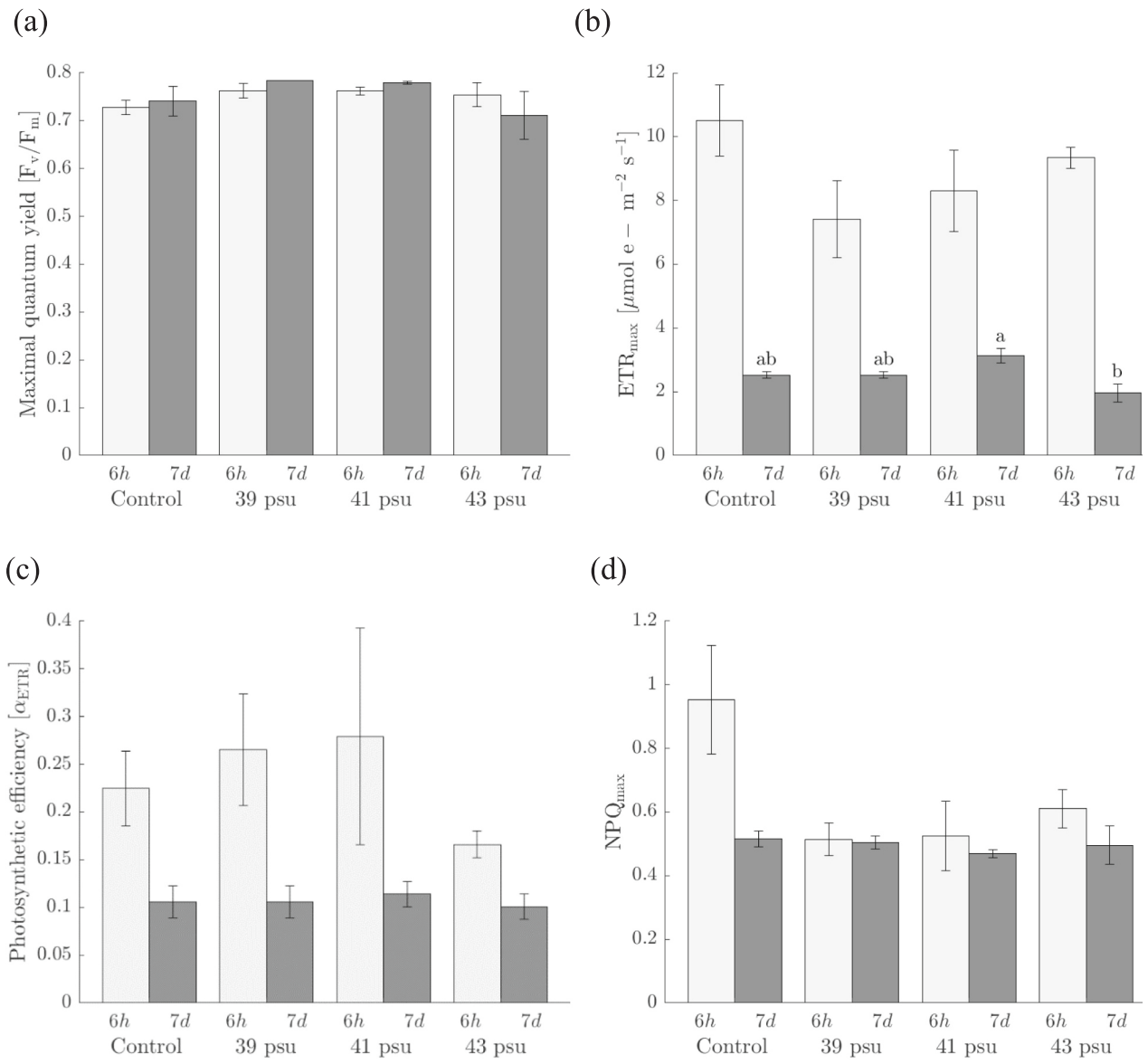

Fig. 1. Effect of salinity on photosynthetic performance: (a) Fv/Fm, (b) ETRmax, (c) αETR, and (d) NPQmax in *P. oceanica* after 6 h (short-term) and 7 d (mid-term) of exposure. Control salinity = 37 psu. Plots are shown as mean ± SE. Uppercase letters represent significant differences ($p < 0.05$) between treatments after 6 h of exposure. Lowercase letters represent significant differences ($p < 0.05$) between treatments after 7 d of exposure.

species (Fig. 3a and Fig. 3c), although certain trends were observed. *P. oceanica* showed comparable trends after 6 h and 7 days of exposure: at 39 psu, the H_2O_2 concentration was lower than the control, increased at 41 psu, reaching a similar level to the control, and surpassed the control at 43 psu. Fig. 3a illustrates that, for both exposure times, H_2O_2 concentrations at 43 psu were significantly higher than those at 39 psu, with increases ranging from 118 % to 703 %. In the case of *C. nodosa*, all treatments exhibited higher H_2O_2 concentrations than the control after 7 days of exposure, with the highest concentration observed at 43 psu, representing a 61 % increase (Fig. 3c).

Oxidative damage in lipid membranes showed that significant differences in TBARS levels were observed between the 43 psu treatment and the control group in both species, while no significant differences were found with the other salinity treatments in both seagrasses (Fig. 3b and Fig. 3d). These differences were evident after both 6 h and 7 days of exposure for *P. oceanica*, with more pronounced differences after 7 days, increasing 298 % (Fig. 3b). Significant differences in TBARS levels in *C. nodosa* were observed between the control and 43 psu treatment groups, but only after 7 days of exposure, with an increase of 106 % (Fig. 3d).

3.3. Quantification of antioxidant molecules

3.3.1. Phenolic compounds

Significant differences in phenolic compounds concentration between the control and salinity treatments were observed during short-term exposure in *P. oceanica* (Fig. 4a). Likewise, H_2O_2 levels, the control group exhibited statistical differences only with the 43 psu treatment, resulting in a 157 % increase. Additionally, plants exposed to 39 psu displayed the lowest concentration of phenolic compounds, significantly lower than those exposed to both 41 psu and 43 psu treatments. However, after 7 days of exposure, no significant differences were observed between the treatments. Nevertheless, the mean concentration of phenolic compounds remained consistently higher than the control for each salinity treatment, ranging from 46 % to 56 % higher.

Significant differences in the concentration of phenolic compounds were observed between the control and salinity treatments for *C. nodosa*, both at short-term and mid-term exposure (Fig. 4d). Specifically, the

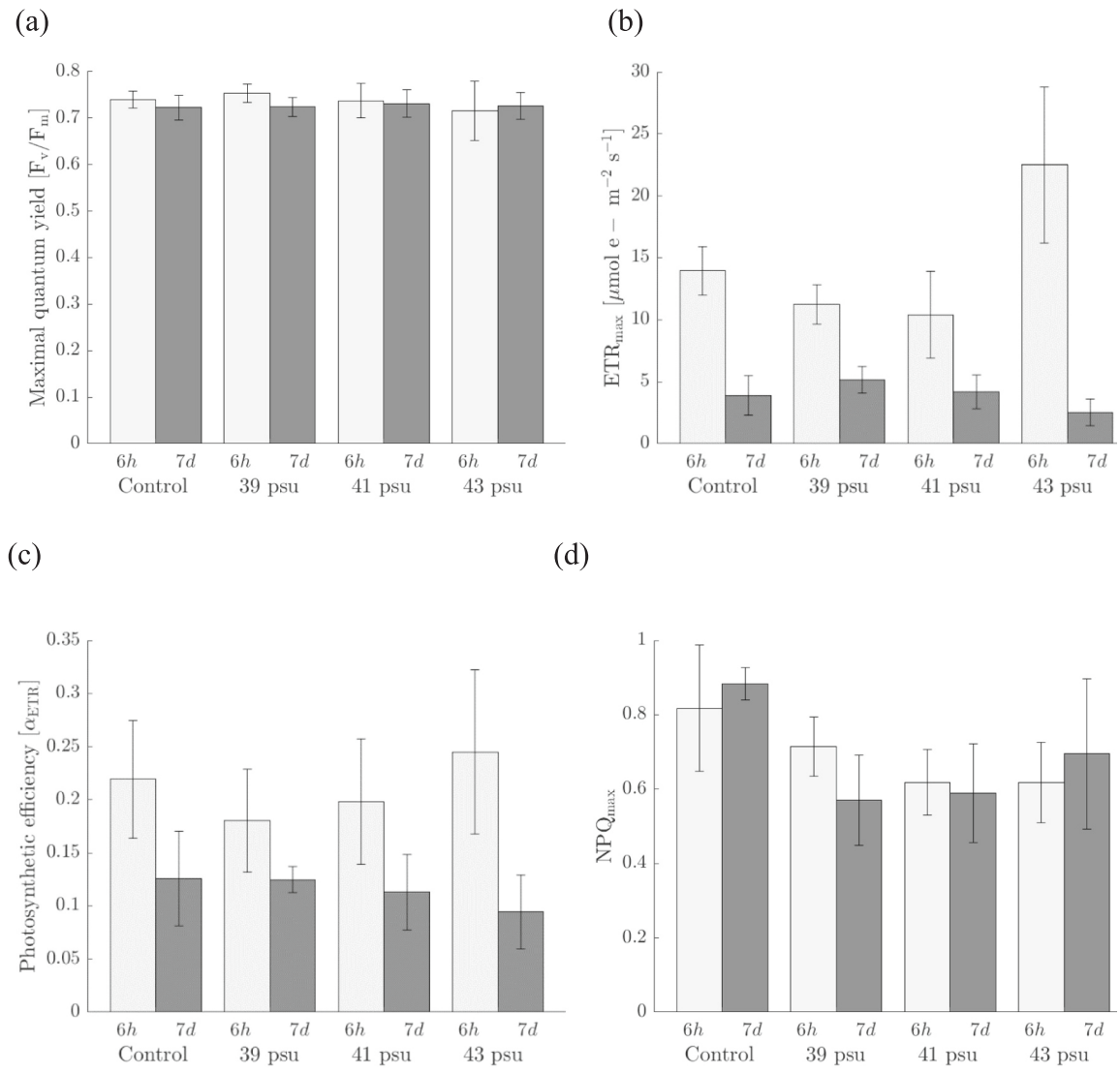

Fig. 2. Effect of salinity on photosynthetic performance: (a) Fv/Fm, (b) ETRmax, (c) αETR, and (d) NPQmax in *C. nodosa* after 6 h (short-term) and 7 d (mid-term) of exposure. Control salinity = 37 psu. Plots are shown as mean ± SE. Uppercase letters represent significant differences ($p < 0.05$) between treatments after 6 h of exposure. Lowercase letters represent significant differences ($p < 0.05$) between treatments after 7 d of exposure.

levels of phenolic compounds in the 39 psu and 41 psu treatment groups were significantly different from the control. However, a contrasting pattern was noted when comparing the effects of the two exposure durations. In the short-term exposure scenario, there was a marked decrease in phenolic compound concentrations at 39 psu and 41 psu compared to the control, by 44 % and 73 % respectively. Conversely, mid-term exposure resulted in a significant increase in phenolic compound levels at the same salinity levels, exhibiting a rise of 61 % and 52 % in comparison to the control.

3.3.2. *Radical scavenging activity*

RSA showed no significant differences between the control group and salinity treatments in *P. oceanica* (Fig. 4b). In the short-term exposure, however, RSA was slightly higher in plants exposed to salinity, ranging from 3.8 % to 29 % increase compared to the control. In contrast, during the mid-term exposure, the activity was similar to the control in the 39 psu and 41 psu treatments, decreasing 36 % in the 43 psu treatment.

For *C. nodosa,* significant differences in RSA appeared at mid-term exposure (Fig. 4e). In all salinity treatments, RSA levels surpassed the control group by 15 % to 60 %, with statistically significant increments observed at 39 psu and 43 psu. Short-term salinity exposure, in contrast, showed no statistically significant effect on RSA, although this parameter tended to decrease with salinity.

3.3.3. *Total, reduced and oxidized ascorbate*

During short-term exposure in *P. oceanica*, significant differences were observed in total and reduced ascorbate concentration between the control and salinity treatments (Fig. 4c). Likewise, H_2O_2 levels, the control group exhibited statistical differences only with the 43 psu treatment, with decreases of 47 % and 63 % in total and reduced ascorbate levels, respectively. Also, *P. oceanica* exhibited higher total and reduced ascorbate levels at 39 psu compared to the control (29 % and 19 %, respectively). Regarding ASC/DHA ratio, a clear declining trend with salinity was observed (Fig. 4c). During mid-term exposure, both total and reduced ascorbate concentrations also increased at 39 psu relative to the control group, with the total concentration showing a significant surge by 37 %. However, in contrast to short-term experiments, the total and reduced ascorbate levels in the 41 psu and 43 psu surpassed the control, although without statistical differences. For the ASC/DHA ratio, unlike short-term observations, all treatments revealed higher ratios than the control group (Fig. 4c).

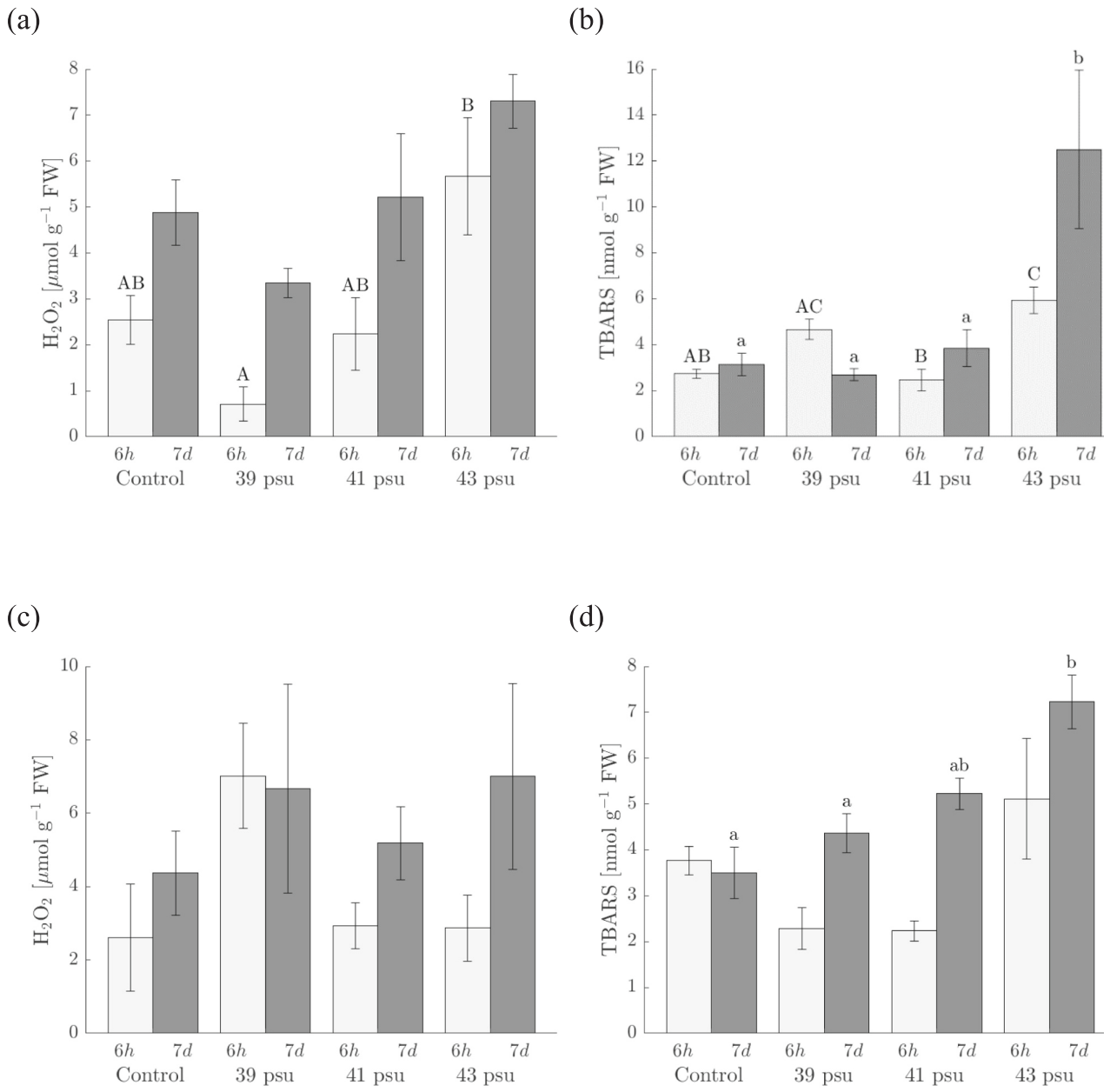

Fig. 3. Salinity effects on oxidative stress and damage parameters: (a) H_2O_2 and (b) TBARS concentrations in *P. oceanica*; and (c) H_2O_2 and (d) TBARS concentrations in *C. nodosa*. Measurements were taken after 6 h (short-term) and 7 days (mid-term) of exposure. Control salinity = 37 psu. Plots are shown as mean ± SE. Uppercase letters represent significant differences ($p < 0.05$) between treatments after 6 h of exposure. Lowercase letters represent significant differences ($p < 0.05$) between treatments after 7 d of exposure.

For *C. nodosa*, during short-term salinity exposure, total ascorbate remained consistent among treatments, demonstrating no salinity-dependent trend. However, reduced ascorbate exhibited a tendency to decrease at salinities above 41 psu, with a significant 18 % decline compared to the control at the 41 psu treatment. Moreover, for 41 psu and 43 psu treatments, the ASC/DHA ratio decreased compared to the control (Fig. 4f). During mid-term salinity exposure in *C. nodosa*, both total and reduced ascorbate levels declined. However, only total ascorbate showed significant decreases of 36 % and 37 % at 41 psu and 43 psu treatments respectively, compared to the control. The ASC/DHA ratio didn't show any evident trend with salinity.

3.4. Differentially expressed genes (DEGs) analysis

C. nodosa exposed to hypersalinity (7 d of exposure to 43 psu) differentially expressed 288 transcripts compared to the control (7 d of exposure to 37 psu) (Supplementary Tables 1 and 2). Of these transcripts, 137 were up-regulated and 151 were down-regulated. Similarly, *P. oceanica* exposed to hypersalinity differentially expressed 418 transcripts compared to the control, with 89 up-regulated and 329 down-regulated genes (Supplementary Tables 3 and 4). *C. nodosa* and *P. oceanica* shared 23 differentially expressed transcripts, based on the same best hit during annotation assignment, with 2 up-regulated in both species, 18 down-regulated in both species, and 3 up-regulated in one species and one down-regulated in the other (Fig. 5).

Gene ontology analysis showed that *P. oceanica* most up-regulated biological processes were related with response to brassinosteroids (BR) and steroid hormones (Fig. 6a). Conversely, the most down-regulated ones, were related to the primary metabolism, such as the generation of precursor metabolites and energy, amino acid biosynthesis

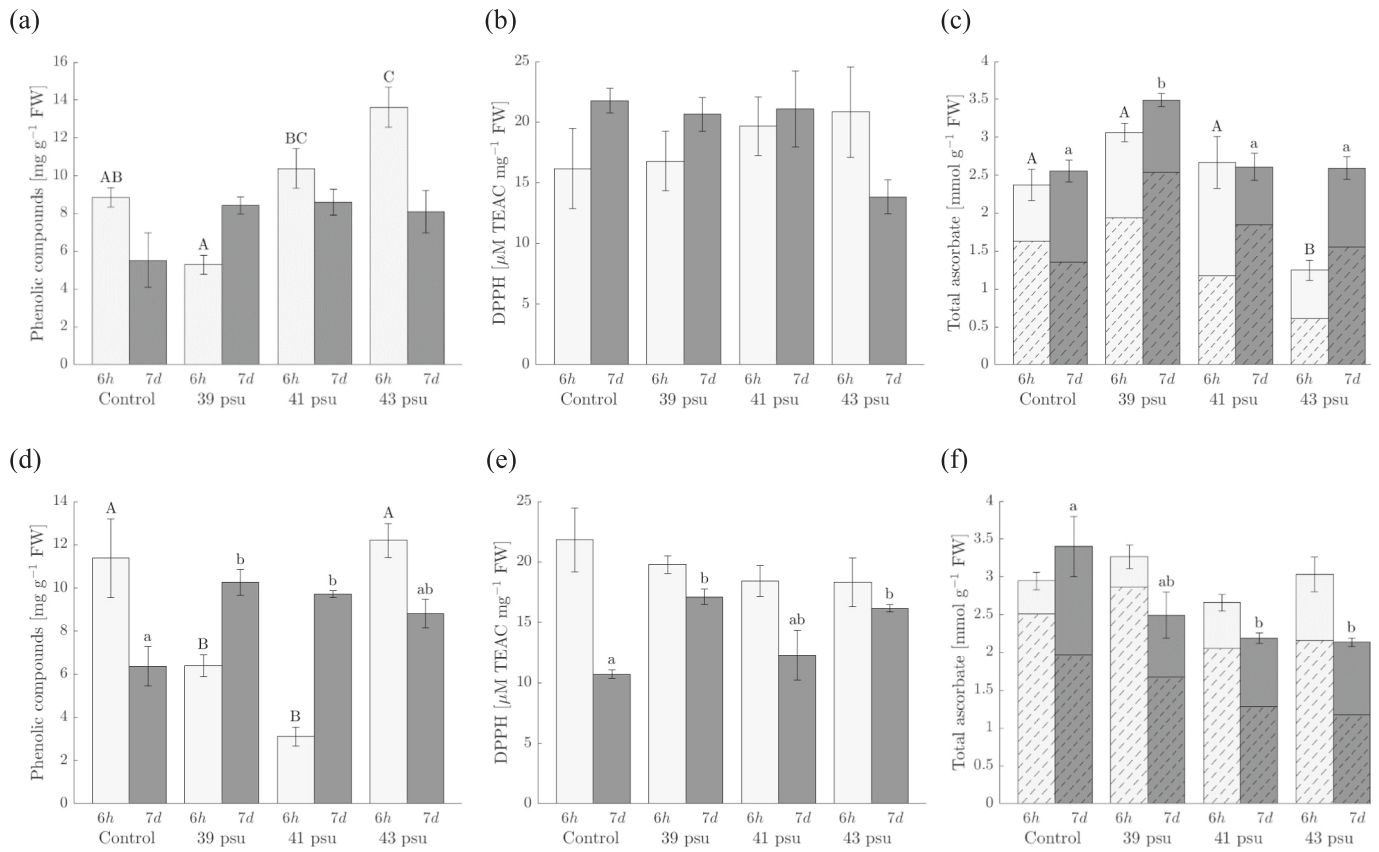

Fig. 4. Effect of salinity on antioxidant molecules: (a) phenolic compounds concentration, (b) Radical Scavenging Activity (DPPH concentration) and (c) ascorbate in its reduced (ASC - hatched) and oxidized (DHA) forms in *P. oceanica*; and (d) phenolic compounds concentration, (e) Radical Scavenging Activity (DPPH concentration) and (f) ascorbate in its reduced (ASC - hatched) and oxidized (DHA) forms in *C. nodosa.* Measurements were taken after 6 h (short-term) and 7 days (mid-term) of exposure. Control salinity = 37 psu. Plots are shown as mean ± SE. Uppercase letters represent significant differences ($p < 0.05$) between treatments after 6 h of exposure. Lowercase letters represent significant differences ($p < 0.05$) between treatments after 7 d of exposure.

and glucose metabolic process, among others (Fig. 6c). In the case of *C. nodosa*, the up-regulated biological processes were related to growth and reproduction, such as DNA replication, mitotic and regulation of cell cycle, and pollen development (Fig. 6b). The down-regulated processes in this case were related to essential biological processes, such as photosynthesis and ribosome assembly (Fig. 6d). Complete detailed GO enrichment tables are provided in Supplementary Table 5.

Table 1 and Table 2 present selected DEGs with a potential relevant role against hypersalinity stress in *P. oceanica* and *C. nodosa*, respectively. Up-regulated genes encoded for protein/enzymes involved in protein folding, biotic and abiotic response, cell signaling, hormone metabolism, and nucleic acids stability. On the other hand, down-regulated genes were mostly related to primary metabolism, photosynthesis, and ROS scavenging. The most up-regulated transcript in *P. oceanica* was peptidyl-prolyl cis-trans isomerase (PIPase) and the most down-regulated one was light-harvesting complex stress-related protein 1 (Table 1). In the case of *C. nodosa*, dynein light chain 1 was the most up-regulated transcript, together with genes associates with stress response and jasmonate biosynthesis, while ROS scavenging, photosynthesis and 40S ribosomal protein were the prevailing down-regulated ones (Table 2). Regarding shared transcripts, half of the down-regulated genes (9/18) were related to photosynthesis process (Table 3). The two genes that were up-regulated in both species encoded for lysine histidine transporter-like 8 and nuclear pore complex protein NUP155. All putative biological function/role were obtained from the UniProt database (www.uniprot.org).

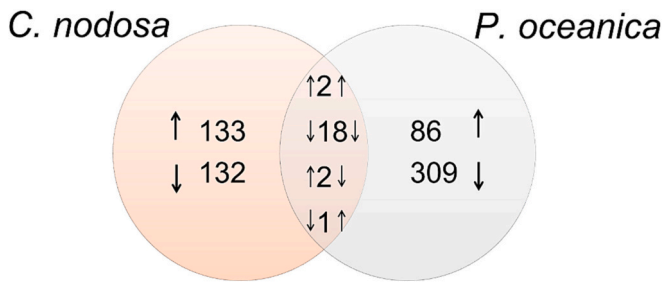

Fig. 5. Venn diagram showing up-regulated and down-regulated transcripts in *C. nodosa* and *P. oceanica* exposed to 43 psu for 7 days. Pairs of arrows in the intersection refer to the direction of fold change in the comparisons on the left- and right-hand sides, respectively.

4. Discussion

4.1. Photosynthetic performance was not affected by hypersalinity stress

The inhibition of photosynthesis is a well-documented response to chronic hypersaline stress in seagrasses (Sandoval-Gil et al., 2022). However, the sensitivity of photosynthetic processes to such stress varies across different species, and even within the same species (Sandoval-Gil et al., 2014). Furthermore, the duration of exposure drastically affects the degree of this sensitivity (Marín-Guirao et al., 2013).

In our study, photosynthesis was not affected by hypersalinity which aligns with previous research on the reactions of *P. oceanica* and *C. nodosa* to hypersaline stress levels reaching up to 43 psu. These studies reveal that substantial effects on photosynthesis typically emerge after longer exposure periods than those examined in this study. For

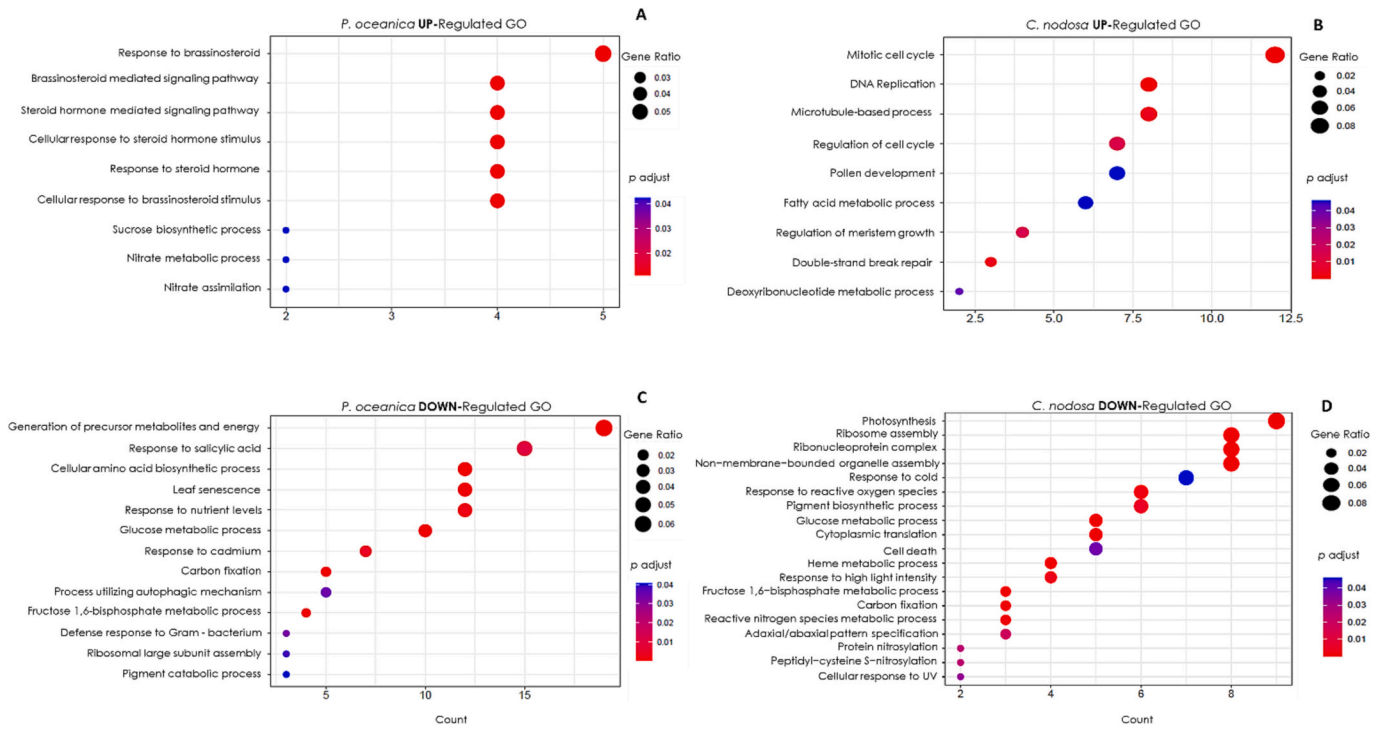

Fig. 6. Biological processes up- and down-regulated in *P. oceanica* and *C. nodosa* exposed to 43 psu for 7 days, according to GO analysis ($p < 0.05$).

example, *P. oceanica* plants exposed to 41.5 psu for seven days did not display significant changes in ETRmax, NPQ, Fv/Fm, or αETR when compared to the control (37 psu) group (Garrote-Moreno et al., 2015). Notably, Fv/Fm remained unaltered by hypersalinity, even after exposure periods extending beyond 30 days (Marín-Guirao et al., 2013; Marín-Guirao et al., 2011). The adverse effects on this parameter became prominent only after prolonged exposure periods (over two months) (Sandoval-Gil et al., 2014; Marín-Guirao et al., 2013). However, both net and gross photosynthesis rates significantly decreased after 47 days of exposure to 39 psu (Marín-Guirao et al., 2011; Sandoval-Gil et al., 2014; Marín-Guirao et al., 2013). As for *C. nodosa*, its photosynthetic response to hypersaline stress is similarly influenced by the duration of exposure. A mid-term exposure (7 days) to 41.5 psu had no substantial effects on ETRmax and NPQ, but led to an increase in Fv/Fm and αETR (Garrote-Moreno et al., 2015). However, numerous studies indicate that Fv/Fm remains unaffected by hypersalinity (up to 43 psu) across a range of exposure durations, from 15 to 62 days (Piro et al., 2015; Sandoval-Gil et al., 2012; Sandoval-Gil et al., 2014). Similar to *P. oceanica*, net and gross photosynthesis rates in *C. nodosa* also significantly decrease after 47 and 62 days of exposure at higher salinity levels (43 psu; Sandoval-Gil et al., 2012; Sandoval-Gil et al., 2014).

It is worth noting that the Fv/Fm values observed in our study (0.71–0.78) are within the healthy range for seagrasses (0.7–0.8; Marín-Guirao et al., 2011).

4.2. Hypersalinity stress induced antioxidant response but produced oxidative damage

4.2.1. Oxidative stress and damage in P. oceanica and C. nodosa

Salinity is known to induce oxidative stress in plants due to osmotic stress and ion toxicity (Liang et al., 2018). Salinity stress disrupts the delicate equilibrium between ROS generation and scavenging, leading to an overaccumulation of ROS. At high concentrations, these species can react and damage proteins, lipids, and DNA (Singh, 2022). Both *P. oceanica* and *C. nodosa* exhibited signs of oxidative damage (lipid peroxidation) at 43 psu. For *P. oceanica*, this damage occurred rapidly, with significantly higher TBARS levels observed during both short-term and mid-term exposure. In contrast, *C. nodosa* showed significant differences only during mid-term exposure, suggesting a lower sensitivity to salinity, as expected (Sandoval-Gil et al., 2014). Prior studies have reported increased levels of oxidative stress markers in lipids in *P. oceanica* chronically exposed to hypersalinity (Capó et al., 2020), in brown algae *Ectocarpus* (Rodríguez-Rojas et al., 2020), and in green algae *Ulva prolifera* (Luo and Liu, 2011) after three and six days of salinity stress, respectively. In terms of salinity's impact on ROS accumulation, our findings showed no statistically significant overaccumulation in the treatments compared to the control. Nonetheless, H_2O_2 concentration in the 43 psu treatment was higher than the control during short-term and mid-term exposures in *P. oceanica* and during mid-term exposure in *C. nodosa*, indicating a positive correlation with TBARS content in both cases.

4.2.2. Antioxidant response

Plants counteract the effects of oxidative stress through its antioxidant defense system (enzymatic and non-enzymatic). Non-enzymatic antioxidants include ascorbate (the most significant antioxidant in plant tissue), glutathione, phenols, and lipophilic (e.g., carotenoids and tocopherols). These antioxidants mitigate ROS both directly and indirectly. The indirect method involves the chelation of transition metals, which prevents them from participating in the Haber-Weiss or Fenton reactions. The direct method, on the other hand, involves the donation or acceptance of electrons, allowing these antioxidants to scavenge radicals preventing their reaction with biological molecules (Dumanović et al., 2021). We observed different patterns of response for the different antioxidant molecules, species and salinity exposure periods. The behavior of antioxidant defense system is discussed separately for each species, in the following sections.

4.2.2.1. Antioxidant response in P. oceanica. First, it is interesting to note that although *P. oceanica* experienced oxidative damage when exposed to a salinity of 43 psu, an examination of the relationship between ROS, TBARS levels and salinity suggests that the seagrass's antioxidant defense mechanism functioned effectively at lower salinity levels. At a salinity of 39 psu, the antioxidant defense system would be

Table 1
Selected DEGs in *P. oceanica* exposed to 43 psu for 7 days ($p < 0.05$).

Transcript_ID	Putative encoded protein	Putative biological function/role	Log (2) FC
TRINITY_DN9725_c0_g2	Peptidyl-prolyl cis-trans isomerase	Protein folding	6,78
TRINITY_DN8385_c0_g1	Nucleobase-ascorbate transporter 6	Transmembrane transporting of ascorbate, pyrimidines and purines	5,72
TRINITY_DN4_c0_g4	Probable receptor-like protein kinase	Receptor-like protein kinase	4,93
TRINITY_DN8409_c0_g1	Wound-induced protein WIN2	Defense response to bacterium and fungus	2,45
TRINITY_DN507_c0_g1	Abscisic acid-insensitive 5	Abscisic acid-activated signaling pathway	1,81
TRINITY_DN12320_c0_g1	Altered phosphate starvation response 1	Cell differentiation and cell elongation in the root tip	1,67
TRINITY_DN1827_c0_g1	Enhanced disease resistance 2-like	Regulator of the salicylic acid-mediated resistance to pathogens	−1,20
TRINITY_DN2086_c1_g1	Stay-green homolog, chloroplastic	Required to trigger chlorophyll degradation during leaf senescence and fruit ripening	-1,98
TRINITY_DN220_c0_g4	Detoxification 49	Xenobiotic detoxification by transmembrane export	−3,12
TRINITY_DN194305_c0_g1	Carbonic anhydrase	Reversible hydration of carbon dioxide	−4,02
TRINITY_DN71036_c0_g1	Peroxidase 20	H_2O_2 scavenger	−5,37
TRINITY_DN8583_c0_g2	Peroxiredoxin-2D	Hydrogen peroxide and organic hydroperoxides detoxification	−6,38
TRINITY_DN40924_c0_g2	Sodium/potassium-transporting ATPase subunit alpha	Ion transporter	−6,47
TRINITY_DN104227_c0_g1	Thylakoid formation 1	Thylakoid membrane biogenesis	−6,55
TRINITY_DN10845_c0_g2	Photosystem I chlorophyll *a*/b-binding protein 5, chloroplastic	Binds to chlorophyll in photosystem I	−6,64
TRINITY_DN41100_c0_g2	Phycobilisome 27.9 kDa linker polypeptide	Rod linker protein, associated with phycoerythrin	−6,85
TRINITY_DN43093_c1_g1	Photosystem II manganese-stabilizing polypeptide	Photosystem II assembly	−7,23
TRINITY_DN48823_c0_g1	Fucoxanthin-chlorophyll *a*-c binding protein A, chloroplastic	Photosynthesis pigments binding protein	−7,25
TRINITY_DN19005_c0_g2	Heat shock cognate 70 kDa	Required for the activation of heat-shock factor 1	−7,27
TRINITY_DN4580_c1_g1	Light-harvesting complex stress-related protein 1	Response to photooxidative stress	−8,50

Table 2
Selected DEGs in *C. nodosa* exposed to 43 psu for 7 days ($p < 0.05$).

Transcript_ID	Putative encoded protein	Putative biological function/role	Log (2) FC
TRINITY_DN6448_c0_g1	Dynein light chain 1	Unknown function in plants	6,98
TRINITY_DN49649_c0_g1	Galactolipase DONGLE	Jasmonate biosynthesis	6,55
TRINITY_DN88818_c0_g1	Transposon Ty3-I Gag-Pol polyprotein	Stress response	6,24
TRINITY_DN73940_c0_g1	Breast cancer susceptibility 1 (BRCA1) homolog	DNA double-strand breaks	4,46
TRINITY_DN4306_c1_g1	LRR receptor-like serine/threonine-protein kinase HSL2	Controls floral abscission	3,74
TRINITY_DN5570_c0_g1	Pentatricopeptide repeat-containing protein	RNA stability and expression control	2,55
TRINITY_DN2600_c0_g2	Germin-like protein 8–12	Role in broad-spectrum disease resistance	2,13
TRINITY_DN10516_c0_g1	Dehydration-responsive element-binding protein 1 A	Transcriptional activator that mediates cold-inducible transcription	1,62
TRINITY_DN4539_c0_g1	Putative chloride channel-like protein (CLC)	Chloride expulsion	1,49
TRINITY_DN4692_c0_g1	G-type lectin S-receptor-like serine/threonine-protein kinase	Response to abiotic stress	1,37
TRINITY_DN29174_c0_g1	Chaperone protein clpb3	Chloroplast thermotolerance	1,30
TRINITY_DN8038_c0_g1	Brassinazole insensitive pale green 2	Post-transcriptional and translational regulation in the chloroplast	1,12
TRINITY_DN3847_c0_g1	Stress enhanced protein 2	Protection against photo-oxidative damage	−1,12
TRINITY_DN8969_c0_g1	Zeaxanthin epoxidase	ABA biosynthesis and xanthophyll cycle	−1,51
TRINITY_DN22383_c0_g1	L-ascorbate peroxidase 2	ROS scavenger	−2,02
TRINITY_DN19290_c0_g1	Anthocyanidin reductase	Biosynthesis of condensed tannins	−2,47
TRINITY_DN8676_c0_g1	Catalase	H_2O_2 scavenger	−3,08
TRINITY_DN24700_c0_g1	Cold shock domain-containing protein	RNA chaperone	−3,52
TRINITY_DN7122_c0_g1	Photosystem II manganese-stabilizing polypeptide	Oxygen evolving activity in photosystem II	−3,75
TRINITY_DN26385_c0_g1	40S ribosomal protein	Translation	−7,12

activated, facilitating the elimination of surplus H_2O_2. This led to a decrease in H_2O_2 levels, even when compared to the control group, under both short-term and mid-term exposure scenarios. However, when salinity exceeded a certain limit - between 41 psu and 43 psu - the defense system proved inadequate in reverting the H_2O_2 levels back to normal, producing oxidative damage (as shown by TBARS levels). This could be consequence of an overly high production of ROS or a deactivation of the defense system, potentially due to damage to the proteins involved.

Responses to salinity stress varied among antioxidant compounds. In the short term, both phenolic compounds and RSA increased in response to salinity. However, during mid-term exposure, while phenolic compounds continued to rise (though not significantly), RSA levels decreased. These findings suggest that salinity initially stimulates the non-enzymatic antioxidant response, which may become less active over mid-term exposures. Capó et al. (2020) examined the total antioxidant capacity and polyphenols levels in *P. oceanica* chronically exposed to hypersalinity. Contrary to our findings, they observed an increase in

Table 3
Common DEGs in *C. nodosa* and *P. oceanica* exposed to 43 psu for 7 days.

Transcript_ID *C. nodosa* /*P. oceanica*	Putative encoded protein	Log(2) FC *C. nodosa*	Log(2) FC *P. oceanica*
TRINITY_DN24368_c0_g1 /TRINITY_DN5486_c0_g1	60S ribosomal protein L4–1	−3,79	−2,07
TRINITY_DN31302_c0_g1 /TRINITY_DN2591_c0_g3	60S ribosomal protein L6	−4,56	−6,98
TRINITY_DN48062_c0_g1 /TRINITY_DN91517_c0_g6	Adenosylhomocysteinase	−6,64	−7,04
TRINITY_DN12594_c0_g1 /TRINITY_DN22722_c1_g1	ADP,ATP carrier protein	−1,34	−2,52
TRINITY_DN4372_c1_g1 /TRINITY_DN9024_c0_g3	ADP-ribosylation factor 2-B	−1,46	−4,50
TRINITY_DN4802_c0_g1 /TRINITY_DN10778_c0_g1	Agamous-like MADS-box protein AP1	−1,29	3,95
TRINITY_DN22112_c0_g3 /TRINITY_DN82437_c0_g1	Ammonium transporter 1 member 2	−1,90	−7,16
TRINITY_DN37116_c0_g1 /TRINITY_DN193163_c0_g1	Ankyrin repeat, bromo and BTB domain-containing protein	−3,85	−6,74
TRINITY_DN19176_c0_g1 /TRINITY_DN48460_c0_g3	Fructose-1,6-bisphosphatase, cytosolic	−3,32	−6,99
TRINITY_DN8328_c0_g1 /TRINITY_DN48823_c0_g1	Fucoxanthin-chlorophyll *a*-c binding protein A, chloroplastic	−1,30	−7,25
TRINITY_DN7117_c0_g2 /TRINITY_DN34904_c0_g1	G-type lectin S-receptor-like serine/threonine-protein kinase	1,31	−3,00
TRINITY_DN7738_c0_g1 /TRINITY_DN4580_c1_g1	Light-harvesting complex stress-related protein 1, chloroplastic	−2,32	−8,50
TRINITY_DN7242_c1_g1 /TRINITY_DN31703_c0_g4	Light-harvesting complex stress-related protein 3.2, chloroplastic	−2,18	−3,03
TRINITY_DN4130_c0_g2 /TRINITY_DN80427_c0_g1	Lysine histidine transporter-like 8	1,39	2,21
TRINITY_DN7976_c0_g1 /TRINITY_DN15500_c0_g1	Nuclear pore complex protein NUP155	1,18	1,24
TRINITY_DN7990_c0_g1 /TRINITY_DN45366_c1_g2	Phosphoglycerate kinase, chloroplastic	−3,98	−4,13
TRINITY_DN7122_c0_g1 /TRINITY_DN43093_c1_g1	Photosystem II manganese-stabilizing polypeptide	−3,75	−7,23
TRINITY_DN14273_c0_g1 /TRINITY_DN54203_c0_g1	Phycobilisome 31.8 kDa linker polypeptide, phycoerythrin-associated, rod	−1,87	−4,55
TRINITY_DN22472_c0_g1 /TRINITY_DN43166_c0_g1	Protein cbbX homolog, chloroplastic	−1,44	−3,98
TRINITY_DN607_c4_g1 /TRINITY_DN30647_c0_g1	R-phycoerythrin gamma chain, chloroplastic	−2,71	−6,38

both parameters at 40.8 psu (although not at salinities up to 39.5 psu). The discrepancies with Capó et al. (2020) could stem from the fact that their study only involved plants naturally living under brine discharges, and which likely possessed a more robust antioxidant system to cope with ROS. In our case, it seems probable that plants could not survive at salinities exceeding 39 psu. Therefore, signs of oxidative damage combined with basal antioxidant levels might signal high risk for *P. oceanica*.

Ascorbate behavior, on the other hand, may indicate that ASC would be synthesized at 39 psu, at both short-term and mid-term exposures. This supports the hypothesis presented in the previous paragraph, which is that *P. oceanica* responds with tolerance mechanisms at moderate salinities. This was also observed in the Chilean seagrass *Z. chilensis* when exposed to a similar increased salinity (37 psu) using artificial brines at 1 and 6 d of exposure (control salinity = 34 psu), suggesting a common moderate stress response among these seagrasses (Blanco-Murillo et al., 2023).

4.2.2.2. Antioxidant response in C. nodosa. Responses to salinity stress varied among antioxidant compounds. RSA decreased with salinity (though not significantly) in the short term, but significantly increased during mid-term exposure. Phenolic compounds also showed a significant increase during mid-term exposure, suggesting that the antioxidant defense in *C. nodosa* was active during this period. As for total ascorbate, it exhibited a significant decrease with salinity at mid-term exposure, although no effects were observed during short-term exposure. ASC also declined during both short-term and mid-term exposures, though this effect was only significant in the short term. This indicates that ascorbate would not be playing a significant role at mid-term exposures. Ascorbate usage against oxidative stress and damage could be highly species and/or ecotype specific. For instance, *Z. chilensis* and the brown macroalgae *Ectocarpus* respond rapidly adjusting their ascorbate levels at increased salinities in the short and mid-term (Blanco-Murillo et al., 2023; Rodríguez-Rojas et al., 2020). Conversely, different ecotypes of the green macroalga *Ulva compressa* responded in a dissimilar manner regarding ascorbate production at higher salinities (42 psu) after 6 d (Muñoz et al., 2020).

4.3. Hypersalinity effect on gene expression profiles of seagrasses

4.3.1. P. oceanica transcriptomic response to hypersalinity

P. oceanica most enhanced biological processes were related to the response to BR and steroid hormones. BR are steroid hormones essential to plant growth and play a vital role in alleviating various stresses in terrestrial plants, including salinity (Manghwar et al., 2022). These increase photosynthesis and biomass, strengthen antioxidant enzymes and the potential of detoxification, as well as stimulate the expression of related genes (Manghwar et al., 2022). In fact, BR application protects crops against salinity stress (Furio et al., 2022; Serna et al., 2015). BR effect on seagrasses has not been investigated so far. Nevertheless, it was recently reported that *P. oceanica* exposed to warming for 6 weeks overexpressed genes related to BR (e.g., CYP85A; Marín-Guirao et al., 2019). All these suggests that BR would have a key role in seagrasses abiotic stress response, as it has in other plants.

Regarding transcripts putative functions, *P. oceanica* exposed to hypersalinity showed an overexpression of transcripts related to abiotic stress response in plants. The most over-expressed transcripts were a PPIase, a nucleobase-ascorbate transporter 6 and a probable receptor-like protein kinase (RLK). PPIase catalyzes cis-trans isomerization of the peptidyl-prolyl bond, which is a rate-limiting step in protein folding. The overexpression of PPIase occurs in several seagrasses responding to abiotic stress, such as ocean acidification in *Cymodocea nodosa* (Ruocco et al., 2017), temperature in *Zostera noltii* (Massa et al., 2011), light-limited conditions in *Zostera muelleri* (Kumar et al., 2017), and desiccation in the seaweed *P. orbicularis* (López-Cristoffanini et al., 2015). PPIase overexpression can suggest an increase in protein biosynthesis, which could be related to the production of stress-response molecules, or a protective role under stress conditions. As for the nucleobase-ascorbate transporter family, the seagrass *Zostera marina* also overexpressed it under saline stress conditions (Kong et al., 2013). Moreover, its over-expression confers salt tolerance to apple (Sun et al., 2021) and cadmium tolerance to barley, through enhancing the expression of genes for ROS scavenging and antioxidant enzyme activity (Wang et al., 2023). Finally, RLKs are involved in salt stress signal transduction, and their over-expression enhances plants tolerance to salinity (Zhang et al., 2022a,Zhang et al., 2022b). RLKs alleviate salt-stress in plants by, for example, activating ROS-scavenging enzymes and water channels (Ye et al., 2017). It is interesting to note that an RLK (RLK1) also showed a marked overexpression in *C. nodosa* exposed to 50 psu for 24 h (Malandrakis et al., 2017 and Tsioli et al., 2022).

Although *P. oceanica* over-expressed several transcripts that confer tolerance to salt stress, transcripts involved in primary metabolism biological processes were severely down-regulated. Also, several transcripts involved in photosynthesis as well as some involved in ROS scavenging and detoxification were down-regulated. This suggests that primary metabolism and photosynthesis would be affected by hypersalinity and that salinity could seriously affect *P. oceanica*, despite the up-

regulation of several genes involved in hypersalinity tolerance.

4.3.2. *C. nodosa* transcriptomic response to hypersalinity

C. nodosa exposed to hypersalinity up-regulated biological processes involved in growth and reproduction. This was not observed by Malandrakis et al. (2017) or Tsioli et al. (2022) who studied the short-term transcriptomic response to salt stress of this species (50 psu for 24 h). Discrepancy may be explained by the differences in the salinity magnitude and period of exposure studied, where certain biological processes require specific threshold of salinity-dependent activation (Yang and Guo, 2018). *C. nodosa* exposed to hypersalinity overexpressed transcripts related to abiotic stress response in plants. The most over-expressed DEGs encoded for: dynein light chain 1, galactolipase DONGLE and transposon Ty3-I Gag-Pol polyprotein (Table 2). Dynein light chain 1 function in plants is unknown; although dynein light chain family genes can be over-expressed or down-regulated in plants exposed to salt stress (Cao et al., 2017). Galactolipase DONGLE catalyzes the initial step of jasmonic (JA) acid biosynthesis. JA is a plant-signaling molecule that enhances plant salt stress tolerance. This occurs through, for example, increasing the concentrations of antioxidative compounds and antioxidant enzyme activity, increasing photosynthetic rates, proline contents or ABA levels, or reducing Na^+ accumulation rates in shoots (Wang et al., 2020). Finally, transposon Ty3-I Gag-Pol polyprotein was also highly expressed in the hypersalinity treatment. Transposons and retrotransposons are major components of plant genomes, and genes encoded within these mobile elements can be activated in response to heat, drought and salt stress representing a potential mutagenic threat to the host genome (Alzohairy et al., 2014; Ito, 2022). Other over-expressed DEGs included genes related with double strand breaks repairing (BRCA1; Trapp et al., 2011), chloride expulsion (CLC) and several RLKs (e.g. LRR receptor-like serine/threonine-protein kinase HSL2 and G-type lectin S-receptor-like serine/threonine-protein kinase). As discussed above, RLKs alleviate salt-stress in plants and a member of this family (RLK1) showed a marked overexpression in *C. nodosa* exposed to 50 psu for 24 h (Malandrakis et al., 2017; Tsioli et al., 2022).

The most downregulated biological processes in *C. nodosa* were related to photosynthesis, ribosome assembly and response to ROS. The most downregulated transcript encoded for 40S ribosomal protein S16 and several other down-regulated transcripts encoded for 40S or 60S ribosomal proteins (data not shown). All this suggests that fundamental primary metabolic biological processes could be severely impaired and that *C. nodosa* could be affected by hypersalinity stress.

4.3.3. *P. oceanica and C. nodosa common DEGs*

P. oceanica and *C. nodosa* common DEGs were mostly down-regulated transcripts associated with photosynthesis. The down-regulation of genes related to photosynthesis occurs in other plant species exposed to hypersalinity (e.g. Lin et al., 2018, Zhang et al., 2022a, Zhang et al., 2022b), and in the seagrass *Z. marina* exposed to high temperature (Jüterbock et al., 2016). Moreover, hypersaline stress down-regulated genes encoding for structural proteins and enzymes of PSII and PSI in *C. nodosa*, although Fv/Fm values were not affected (Piro et al., 2015).

Only two DEGs were up-regulated in both species. From these, lysine histidine transporter like 8 is known to be induced under salt stress (Çakır Aydemir et al., 2020), and could suggest an induction of amino acid transport, as an osmoprotectant, to maintain cell turgor in response to stress.

5. Conclusions

Our results demonstrate that hypersalinity induced lipid membranes oxidative damage in *P. oceanica* and *C. nodosa*. Damage occurred rapidly in *P. oceanica*, while it developed more slowly in *C. nodosa*, specifically at 6 h and 7 days of exposure to 43 psu, respectively. This oxidative damage took place even though salinity triggered the antioxidant defense system (RSA and phenolic compounds) in *P. oceanica* in the short-term, and in *C. nodosa* in the mid-term. As for photosynthetic performance, no hypersalinity effects were evident in any of the treatments, suggesting that these symptoms require longer periods to develop. Among the biomarkers studied, TBARS emerged as the most promising candidate for early detection of brine impact in both species.

RNA-seq results suggest that *P. oceanica* activated mechanisms to cope with salinity stress at 43 psu. Potential biomarkers for hypersalinity stress include BR, PPIase nucleobase-ascorbate transporter 6, and a RLK. *C. nodosa* also overexpressed transcripts related to plant salinity stress response, such as dynein light chain 1, galactolipase DONGLE, and transposon Ty3-I Gag-Pol polyprotein. Notably, several RLKs were also overexpressed, underscoring the potential role of these proteins as salinity stress biomarkers in seagrasses. Lysine histidine transporter like-8 was also overexpressed in both species, suggesting its potential as a biomarker.

Finally, although *P. oceanica* and *C. nodosa* overexpressed several transcripts that confer salt tolerance, transcripts involved in primary metabolic biological processes (in the case of *P. oceanica*) and in photosynthesis, ribosome assembly, and response to ROS (in the case of *C. nodosa*) were downregulated. This highlights the deleterious effect of hypersalinity on both species.

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CRediT authorship contribution statement

Fernanda Rodríguez-Rojas: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing. **Camilo Navarrete:** Investigation, Methodology. **Consuelo Rámila:** Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Patricio Tapia-Reyes:** Data curation, Formal analysis, Methodology. **Paula S.M. Celis-Plá:** Investigation, Methodology, Writing – review & editing. **Christian González:** Data curation, Formal analysis, Visualization. **Jeniffer Pereira-Rojas:** Visualization, Writing – review & editing. **Fabio Blanco-Murillo:** Formal analysis, Investigation, Visualization, Writing – review & editing. **Pablo Moreno:** Formal analysis, Methodology, Writing – review & editing. **Catalina Gutiérrez-Campos:** Formal analysis, Methodology, Writing – review & editing. **José Luis Sánchez-Lizaso:** Conceptualization, Investigation, Supervision, Writing – review & editing. **Claudio A. Sáez:** Conceptualization, Investigation, Methodology, Supervision, Writing – review & editing.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT4 to improve readability and language of the text. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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

I have shared the data through an accession number that can be found in NCBI website.

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