Plant Physiology and Biochemistry 210 (2024) 108614

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Thermo-priming triggers species-specific physiological and transcriptome responses in Mediterranean seagrasses

Hung Manh Nguyen [a,1], Uyen V.T. Hong [b,c], Miriam Ruocco [a,2,3], Emanuela Dattolo [a,d], Lázaro Marín-Guirao [a,e,4,*], Mathieu Pernice [f,4], Gabriele Procaccini [a,d,4]

[a] Stazione Zoologica Anton Dohrn, Villa Comunale, 80121, Napoli, Italy
[b] La Trobe University, AgriBio Building, Bundoora, 3086, VIC, Australia
[c] Department of Plant Biotechnology & Biotransformation, University of Science, Vietnam National University, 700000, Ho Chi Minh City, Viet Nam
[d] NBFC, National Biodiversity Future Center, Piazza Marina 61, 90133, Palermo, Italy
[e] Oceanographic Center of Murcia, Seagrass Ecology Group, Spanish Institute of Oceanography (IEO-CSIC), C/Varadero, San Pedro del Pinatar, 30740, Murcia, Spain
[f] Faculty of Science, Climate Change Cluster (C3), University of Technology Sydney, Sydney, 2007, NSW, Australia

ARTICLE INFO

Handling Editor: K Kees Venema

Keywords:
Seagrasses
Stress memory
Heat-priming
RNA tag-seq
Epigenetics
Posidonia oceanica
Cymodocea nodosa

ABSTRACT

Heat-priming improves plants' tolerance to a recurring heat stress event. The underlying molecular mechanisms of heat-priming are largely unknown in seagrasses. Here, *ad hoc* mesocosm experiments were conducted with two Mediterranean seagrass species, *Posidonia oceanica* and *Cymodocea nodosa*. Plants were first exposed to heat-priming, followed by a heat-triggering event. A comprehensive assessment of plant stress response across different levels of biological organization was performed at the end of the triggering event. Morphological and physiological results showed an improved response of heat-primed *P. oceanica* plants while in *C. nodosa* both heat- and non-primed plants enhanced their growth rates at the end of the triggering event. As resulting from whole transcriptome sequencing, molecular functions related to several cellular compartments and processes were involved in the response to warming of non-primed plants, while the response of heat-primed plants involved a limited group of processes. Our results suggest that seagrasses acquire a primed state during the priming event, that eventually gives plants the ability to induce a more energy-effective response when the thermal stress event recurs. Different species may differ in their ability to perform an improved heat stress response after priming. This study provides pioneer molecular insights into the emerging topic of seagrass stress priming and may benefit future studies in the field.

1. Introduction

Organismic response to stress involves the activation of different molecular mechanisms that produce phenotypic changes to cope with stressful conditions. Cellular stress response (CSR) is a universal mechanism of cellular response to stress-induced damage (Kültz, 2005). The level of CSR depends on the severity and progression of stress (Evans and Hofmann, 2012). The first level is associated with protein denaturation and is characterized by an increase in the synthesis of molecular chaperones [e.g., HSPs, see also Lindquist (1986)], that ultimately protect proteins from damage and bring unfolded proteins back to their folded and functional conformations. As stress progresses, a second level is activated involving the expression of genes related to ubiquitin-related proteolysis, a biochemical process that removes irreversibly damaged proteins, to achieve protein homeostasis (proteostasis) [e.g., see also Travers et al. (2000)]. When the stress level is too extreme, the third level of CSR is activated, inducing genes that prevent the replication of damaged DNA and/or stimulate programmed cell

* Corresponding author. Oceanographic Center of Murcia, Seagrass Ecology Group, Spanish Institute of Oceanography (IEO-CSIC), C/Varadero, San Pedro del Pinatar, 30740, Murcia, Spain.
E-mail address: lazaro.marin@ieo.csic.es (L. Marín-Guirao).
[1] Present address: French Associates Institute for Agriculture and Biotechnology of Dryland, The Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Sede Boqer Campus, 8499000, Israel.
[2] Present address: Department of Biological, Geological and Environmental Sciences, University of Bologna, Via F. Selmi 3, 40126, Bologna, Italy.
[3] Present address: Fano Marine Center, Viale Adriatico 1/N, 61032, Fano, Italy.
[4] These authors have contributed equally to this work.

https://doi.org/10.1016/j.plaphy.2024.108614
Received 10 December 2023; Received in revised form 3 April 2024; Accepted 5 April 2024
Available online 7 April 2024

death pathways [e.g., see also Logan and Somero (2011)].

Exposure to stress can also enhance tolerance to future stresses. This phenomenon is commonly known as priming, preconditioning or acquired stress response (Hilker and Schmülling, 2019). An enhanced response to stress, as a result of priming, can be earlier, faster, stronger and/or more sensitive, and therefore more likely to succeed (Georgoulis et al., 2021). Priming is linked to epigenetic, cellular, hormonal and phenotypic modifications (D'Urso and Brickner, 2017; Hilker et al., 2016; Lukić et al., 2020) and consists of two phases, i.e *'priming'* (the first exposure of an organism to stress) and *'triggering'* (the enhanced response of that organism when the stress recurs) (Bäurle, 2016). Response can be enhanced also for a stress different from the one acting in the priming phase (cross-stress tolerance) and the priming status can be acquired also through maternal inheritance in plants (inherited stress tolerance) (Hilker et al., 2016). The storage and maintenance of the information acquired from the priming event define the 'stress memory' (Hilker et al., 2016; Hilker and Schmülling, 2019). Stress memory can be long- (e.g., months) or short-lasting (e.g., days or hours), also in accordance to the energetic cost involved (D'Urso and Brickner, 2017).

At the molecular level, priming is commonly linked to epigenetic modifications (see Iwasaki and Paszkowski, 2014; Bäurle, 2016; D'Urso and Brickner, 2017 for some comprehensive reviews and related references) that alter gene expression without changes in the underlying DNA sequence (Bossdorf et al., 2008). Among the epigenetic modifications, histone modifications and DNA methylation can be induced by stress during the priming phase, potentially enhancing gene expression responses when the stress recurs (i.e., during the triggering phase) (Bäurle, 2016; D'Urso and Brickner, 2017; Iwasaki and Paszkowski, 2014).

In plants, several studies have documented the involvement of such molecular mechanisms in the formation and activation of priming under different stress conditions (Bruce et al., 2007; Hilker et al., 2016; Hilker and Schmülling, 2019). For instance, in *Arabidopsis*, an enhanced epigenetic-induced transcriptional response to heat and drought stress has been shown, through the acquisition of a so-called 'transcriptional memory' (Ding et al., 2012; Lämke et al., 2016). Transcription factors, such as HSF A2 (heat shock factor A2) or AtERF7, have been suggested to play a crucial role in priming in terrestrial plants (Bruce et al., 2007; Charng et al., 2007). Heat-priming involves stem cell regulators (Olas et al., 2021), Heat Shock Proteins (Olas et al., 2021), and genes involved in metabolic pathways such as glycolysis and the plastid metalloprotease FtsH6 (Sedaghatmehr et al., 2016).

Seagrasses are a unique group of marine angiosperms that are living all along the shorelines from sub-Artic to tropical regions (Short et al., 2007). Seagrasses provide a wide range of crucial ecosystem services [such as oxygen-production, habitat provision, nutrient recycling, among many others (Orth et al., 2006)] and represent one of the most significant natural carbon sinks on Earth (Fourqurean et al., 2012). Especially, seagrass meadows support the fulfilment of 16 out of 17 Sustainable Development Goals proposed by the United Nations (Unsworth et al., 2022). Seagrass restoration and protection have been recognized as a nature-based solution to help mediate the impacts of global warming (Gattuso et al., 2018).

Recently, the induction of a heat-primed response has been documented in seagrasses at both adult and seedling stages (Nguyen et al., 2020; Pazzaglia et al., 2022a). Nguyen et al. (2020) showed that heat-primed adult plants of two seagrass species from the Australian seas, *Posidonia australis* and *Zostera muelleri*, were able to cope better with further heat stress in respect to non-primed plants. In addition, significant expression of epigenetic-related genes in response to thermal stress from heat-primed plants of both species suggested that also in seagrasses, as seen in terrestrial plants, epigenetic modifications could play an important role in this phenomenon (Nguyen et al., 2020). Pazzaglia et al. (2022a) also showed enhanced photo-physiology and growth rates in heat-primed seedings of the Mediterranean species *P. oceanica*, and suggested the involvement of epigenetic-related genes in priming. However, these pioneer studies were only able to investigate the expressions of a few target genes by RT-qPCR, while the whole molecular mechanism remains largely unexplored.

In this study, we aimed to deepen our understanding of the molecular mechanisms governing heat-priming in seagrasses. To this end, an *ad hoc* mesocosm experiment was conducted using adults of two Mediterranean seagrass species, *P. oceanica* and *Cymodocea nodosa*. Plant responses, including photo-physiology, growth, pigment changes and gene expression, were assessed at the end of the triggering event. The analysis of gene expression was conducted through high-throughput gene-expression profiling (i.e., Tag-Seq approach), while the different levels of the CSR were investigated in the comparison of the whole-transcriptome response to warming between heat-primed and non-primed plants. We hypothesized that (*i*) heat-primed plants would have a better vegetative/morphological/photo-physiological response during the triggering event than non-primed plants and (*ii*) heat-primed plants would show, at the transcriptome level, a lower level of CSR than non-primed plants.

2. Methods

2.1. Sample collection

Plant fragments bearing several shoots were collected on the September 18, 2019 by SCUBA diving at the Island of Ischia, Gulf of Naples, Italy (*P. oceanica*; 40°44.020′N, 13°58.039′E; 5 − 6 m depth) and at Capo Miseno, North of the Gulf of Pozzuoli, Italy (*C. nodosa*; 40°47.021′N, 14°04.404′E; 8 − 10 m depth) (Fig. 1C). The sampled populations of the two species are spatially very close (about 12 km) which means that they experience a similar thermal environment. Furthermore, there are no marked differences in relation to the influence of anthropogenic pressures on them. This ensures, to some extent, that the different responses to experimental treatments between the two species are due to interspecific differences (different thermal tolerance associated with their evolution) rather than to the development of local adaptations promoted by a different environment. To reduce the likelihood of collecting the same genotype twice, plants were sampled at a minimum distance of 10 m from each other (Jahnke et al., 2017; Nguyen et al., 2023; Procaccini et al., 2007; Ruocco et al., 2022; Serra et al., 2010). After collection, plant fragments were kept in dark coolers filled with natural seawater at ambient temperature and transported shortly (<2 h) to a benthic mesocosm facility at Stazione Zoologica Anton Dohrn (SZN), Napoli, Italy. Environmental conditions (i.e., salinity, irradiance, and water temperature) were measured at the sampling time to allow mimicking the natural conditions at the experimental setup.

2.2. Experimental setup

To standardize experimental results, plant fragments with similar rhizome length and shoot number (∼ 10 vertical shoots connected to an apical shoot) were carefully chosen. Selected *P. oceanica* plants were transplanted onto plastic net cages (i.e., two fragments in each cage) similar to Ruocco et al. (2019), while each *C. nodosa* plant was individually mounted in a plastic container (32 × 23 × 10 cm) filled with natural sediments from the collection site as described in Cardini et al. (2022). After transplantation, 18 plant fragments from each species were randomly allocated into nine 500L-aquaria (i.e., each aquarium contained two *P. oceanica* plants and two *C. nodosa* plants) filled with filtered and UV-treated natural seawater from a nearby area. Plants were arranged inside each aquarium carefully avoiding species shading each other and with a similar light level to that existing at the collection sites (i.e., max. noon irradiance of 250 μmol photons m^{-2} s^{-1} at the middle of *P. oceanica* canopy height) (Fig. 1A and B). Three experimental treatments were selected, including control, non-primed and heat-primed. Each experimental treatment was randomly assigned to three aquaria. Details about the systems for controlling light, water temperature and water quality can be found in a previous study (Ruocco et al., 2019). A

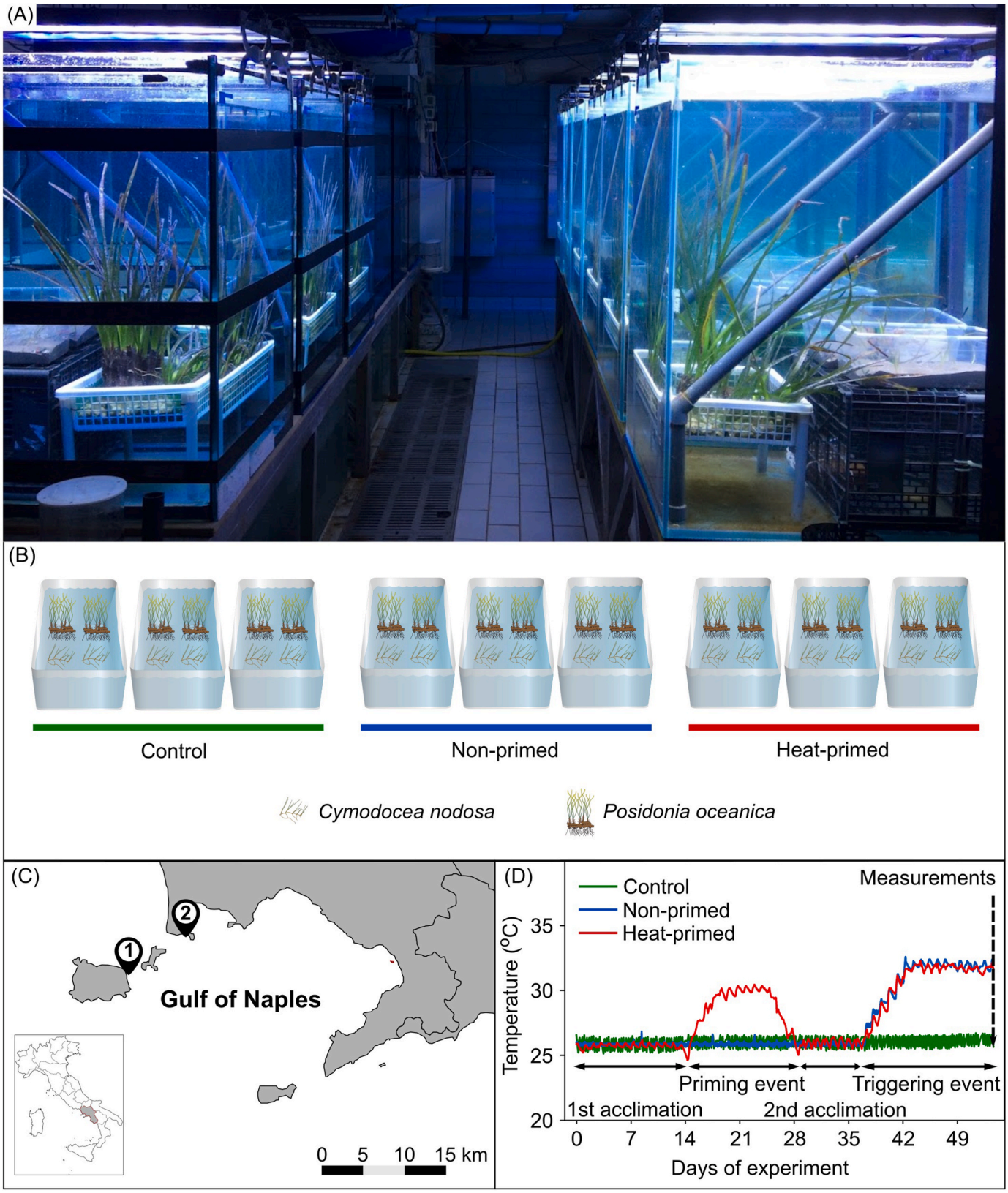

Fig. 1. (**A**) Benthic mesocosm system at Stazione Zoologica Anton Dohrn (Naples-Italy). (**B**) Experimental design. Symbols were taken from the Integration and Application Network, Centre for Environmental Science, University of Maryland (https://ian.umces.edu/media-library/symbols/). (**C**) Collection sites (**1**-*P. oceanica* and **2**-*C. nodosa*) and (**D**) Temperature profile of the experiment.

12h:12h light:dark photoperiod was applied to start from 7:00, where irradiance was progressively increased to a maximum level at 13:00 before a gradual reduction until dark at 19:00. Water temperature was measured automatically every 10 min using HOBO Pendant® Temperature/Light 64K Data Logger (Onset, USA) and manually checked twice a day with WTW Cond 3310 Set 1 (Xylem Analytics, Germany). Throughout the experiment, the salinity level was kept at 37.5 PSU as in natural conditions by adding purified water accordingly to compensate for evaporation. Furthermore, seawater was partially renewed (20%) weekly to sustain water quality.

2.3. Experimental design

Before the beginning of the experimental treatments, all plant fragments were allowed to acclimatize to the mesocosm conditions for 2 weeks at 26 °*C* (similar to the natural seawater temperature at the time of plant collection). After acclimation, seawater temperature in the three heat-primed aquaria was progressively increased up to 29 °*C* at a heating rate of 1 °*C* day^{-1} and maintained for 7 days (Fig. 1D). This temperature was 1 °*C* higher than the maximum year-round temperature the plants experienced in the field and was selected to produce a mild-stress condition to experimental plants, although the degree of stress is strongly dependent on species-specific heat tolerance (Nguyen et al., 2021). Subsequently, the temperature in these aquaria was lowered to 26 °*C* at the same rate of 1 °*C* day^{-1} for a second acclimation period of one week (i.e., lag phase). Seawater temperature in both non-primed and heat-primed aquaria was then gradually increased to 32 °*C* at the same rate (i.e., 1 °*C* day^{-1}) and maintained constant for 11 days (triggering event)(Fig. 1D). This temperature was 4 °*C* higher than the maximum year-round temperature the plants experience in the field in the collection area, which is considered as an extreme temperature increase (Nguyen et al., 2021). Seawater temperature in the three control aquaria was maintained at 26 °*C* during the whole experimental period (Fig. 1D). All plant responses were measured at the end of the triggering event (Fig. 1D). The aquarium was the true experimental replicate for each seagrass species and variable, and measurements performed on plants from the same aquarium (i.e., '*pseudo*' replicates) were averaged to obtain an independent replicated value. Therefore, the number of replicates used in statistical tests was $n = 3$.

2.4. Chlorophyll a fluorescence

A diving-PAM fluorometer (Walz, Germany) was used to assess the photo-physiological responses of *P. oceanica* and *C. nodosa* following the methodology described in Marín-Guirao et al. (2013). Saturation pulses were applied with an intensity of 8000 μmol photons m^{-2} s^{-1} which allows all the PSII reaction centers to close, and a duration of 0.8 s, which reduces the interference of non-photochemical quenching (i.e., it is expected that, with less than 1s duration of the saturation pulse, changes in non-photochemical quenching are too slow to become effective). To secure the same distance between the leaf surface and the fiber optic across measurements, we used an Underwater Adapter DIVING-DA (WALZ) coupled with a Dark Leaf Clip DIVING-LC (WALZ). Due to the difference in the leaf morphology between the two studied species, LIGHT-GAIN was set at 1 and 2 for *P. oceanica* and *C. nodosa*, respectively, to ensure the Fo values ranged between 130 − 300, thus securing the accuracy of the measurements.

Maximum quantum yield (Fv/Fm) of photosystem II (PSII) was measured on whole-night dark-adapted plants (around 6:00 − 7:00 before the light cycle started), while the effective quantum yield of PSII (ΔF/Fm') was determined on light-adapted plants at noon (around 12:30 − 13:30). For each tank, one shoot from each plant fragment (i.e., two shoots per tank for each species) was selected for the measurements. To standardize the procedure, all measurements were performed on the same leaf portion (i.e., 10 cm above the ligule for *P. oceanica* and in the middle of the leaf for *C. nodosa*) of the second youngest leaf from the third youngest shoot from the apical one.

2.5. Plant growth

Plant growth was assessed through the leaf marking method (Zieman, 1974). Shoots selection was done similarly to PAM measurements. Selected leaves were marked with a needle above the ligule in the middle of the second acclimation phase (Fig. 1D). Samples were then collected at the end of the triggering event (Fig. 1D). Newly developed leaf segments were cleaned of epiphytes, dehydrated (70 °*C* for 24 h), and weighted for measuring leaf biomass production (dry weight, mg DW).

2.6. Pigments content

Leaf segments approx. 5 cm long were harvested from the middle portion of the second youngest leaf of *P. oceanica* or the whole second youngest leaf of *C. nodosa* from the fourth youngest shoot of each plant fragment growing in the experimental tanks. Leaf segments were immediately cleaned of epiphytes, covered with aluminum foil, and kept on ice in darkness until further processing on the same day of collection (<4 h). Samples were first weighted before being homogenized in liquid nitrogen by using pestles and mortars. Homogenized samples were then transferred into 1.5 mL tubes filled with 1 mL of 100% methanol. Thenceforward, samples were kept in complete darkness at 4 °*C* for 8 h before centrifugation. An aliquot (200 μ L) of the solution was then used to measure the absorbance at 4 different wavelengths (i.e., 470, 652, 665, and 750 nm) in a microplate reader (TECAN Infinite® M1000PRO, Switzerland). The leaf content in chlorophyll *a*, chlorophyll *b*, and total carotenoids was calculated using equations from Wellburn (1994) after converting microplate readings into 1-cm cuvette readings following Warren (2008). Finally, results were normalized to milligrams of fresh weight.

2.7. Whole transcriptome sequencing and analysis

2.7.1. Sample collection, RNA isolation and sequencing

Samples for RNA extraction were obtained from nine randomly selected plant fragments ($n = 3$ per treatment) per species at the end of the experiment. For each replicate, a 5 cm leaf segment from the middle part of the second youngest leaf or a whole leaf was sampled for *P. oceanica* and *C. nodosa*, respectively. Samples were taken from the second youngest shoot from the apical shoot. Immediately after collection, samples were gently cleaned of epiphytes and preserved in *RNAlater*® (Thermo Fisher Scientific). Samples were first stored at 4 °*C* overnight before being stored at −20 °*C* until RNA extraction.

Total RNA was extracted using Aurum™ Total RNA Mini Kit (BIO-RAD) following the manufacturer's instructions. RNA purity was assessed with a NanoDrop® ND-1000 spectrophotometer (Thermo Fisher Scientific) and its integrity was checked on a 1% agarose gel electrophoresis. RNA was used only when (*i*) Abs260nm/Abs280 nm ratios were >1.8, and (*ii*) 1.8 < Abs260 nm/Abs230 nm ratios <2. RNA integrity was double-checked by measuring the RNA integrity number (RIN) with a 2100 Bioanalyzer (Agilent Technologies). Only high-quality (RIN >6.5) RNA was used for sequencing. RNA quantity was determined by Qubit® RNA BR assay kit using the Qubit 2.0 Fluorometer (Thermo Fisher Scientific). In total, eighteen cDNA libraries (3 replicates × 3 treatments × 2 species) were prepared with QuantSeq 3′ mRNA-Seq Library Prep Kits (Lexogen) and sequenced using Ion Proton™ sequencer at the SZN Molecular Sequencing & Analysis Center (CSAM). The QuantSeq protocol produces one fragment per transcript and generates reads towards the poly (A) tail. This approach is different from the traditional RNA-Seq when directly reversing transcribed cDNAs from the 3′ end of the mRNAs, without a fragmentation step (see Pazzaglia et al., 2022b for a previous application in seagrasses).

2.7.2. Data filtering and transcriptome analysis

Raw sequencing reads were quality-checked using FASTQC v.0.11.5 (Andrews, 2010) and trimmed for quality using Trimmomatic (Bolger et al., 2014). To avoid potential artifacts caused by sequencing errors, reads with a quality per bases below 15 Phred score and a length <50 bp for *P. oceanica* or below 20 Phred score and a length <25 bp for *C. nodosa* were excluded from the analysis (due to the nature of the sequencing data, different Phred scores were chosen in order to obtain both sufficient number and quality of reads). Thenceforth, a dataset of unique tags was obtained by collapsing all the trimmed reads using Cd-hit [90% identity (Li and Godzik, 2006)]. To re-evaluate the quality of the tags dataset, all the cleaned reads were mapped independently on the published transcriptomes from Ruocco et al. (2020) and Ruocco et al. (2017) for *P. oceanica* and *C. nodosa*, respectively using the Bowtie2 aligner with default settings (Langmead and Salzberg, 2012). For each replicate, read count was computed by the eXpress software (Roberts et al., 2011).

2.7.3. Differential expression analysis and functional annotation

To assess overall similarity across samples and their relationship, a Principal Component Analysis (PCA) was conducted on read count data for each species using an integrated web application iDEP [available online at http://bioinformatics.sdstate.edu/idep/(Ge et al., 2018)]. The analysis of differentially expressed genes (DEGs: non-primed vs. control and heat-primed vs. control) was performed using the edgeR package (Robinson et al., 2010). Transcripts were considered as significantly differentially expressed when FDR-corrected p-value $\leq$0.05. Additionally, a cut-off of $> |1.5|$ was also applied for log2 fold change values (Log2FC). Finally, DEGs were visualized using DiVenn 2.0 [available online at https://divenn.noble.org/index.php (Sun et al., 2019)], while Venn diagrams (available online at http://bioinformatics.psb.ugent.be/webtools/Venn/) were used to identify DEGs shared between non-primed plants and heat-primed plants and DEGs that were unique of each experimental group.

Functional annotation of DEGs was performed through a sequence similarity search against UniProtKB/Swiss-Prot database (downloaded on April 2020) using the BLASTx software (Camacho et al., 2009) with an e-value cutoff of $1e^{-3}$. In addition, DEGs were also annotated against previously published transcriptomes [Ruocco et al. (2020) and Ruocco et al. (2017) for *P. oceanica* and *C. nodosa*, respectively] using the BLASTn software (Camacho et al., 2009) with an e-value cutoff of 0.05 and query cover $\geq$ 90% (only the best hit was selected for each alignment) to maximizing the number of functional-annotated DEGs.

Annotated DEGs were assigned into eight different categories, including low-stress-response, medium-stress-response, high-stress-response, photosynthesis, growth, transcription factor, epigenetic and stress memory. The low-, medium- and high-stress-response categories were adopted from the CSR concept (Kültz, 2005) to represent the three different levels of gene expression responses based on the severity of the stress experienced by plants during the triggering event as previously done in another seagrass study (Traboni et al., 2018). Low-stress-response refers to genes related to protein denaturation and re-folding, medium-stress-response refers to genes related to ubiquitin-related proteolysis and high-stress-response refers to genes related to the inhibition of damaged DNA replication and/or programmed cell death pathways. The photosynthesis category includes genes related to photosynthetic activity overall (Chang et al., 2006). The growth category is associated with genes related to plant growth and development (Cheng et al., 2012). The transcription factor category includes genes related to signal transduction pathways (Kim and Kende, 2004). The epigenetic category includes epigenetic-related genes (Lee et al., 2017). The stress memory category is used for genes that are known to be involved in thermal stress memory (Lämke et al., 2016; Olas et al., 2021). List of associated genes for each category was collected from Google Scholar using the following key words "plant protein protection gene", "protein re-folding" and "protein assembly" for the low-stress-response category; "ubiquitination gene" and "proteolysis gene" for the medium-stress-response category; "DNA repair" and "apoptosis" for the high-stress-response category; "photosynthesis" for the photosynthesis category; "plant growth" and "plant development" for the growth category; "transcription factor" for the transcription factor category; "epigenetic", "DNA methylation" and "histone modification" for the epigenetic category; and "heat stress memory", "heat--priming", "heat preconditioning" for the stress memory category. Each associated gene was selected and confirmed from at least three different publications. In case an associated gene was related to two or more categories, the priority was given to the category with the lowest level (i.e., the most specific GO term) of the related GO hierarchy (Biological Process) for that associated gene.

2.7.4. GO enrichment analysis

Two approaches were applied to maximize the number of Gene Ontology (GO) terms retrieved. First, GO terms associated with tags were retrieved from the corresponding contigs from previously published transcriptomes [Ruocco et al. (2020) and Ruocco et al. (2017) for *P. oceanica* and *C. nodosa*, respectively]. Then all tags were loaded on Blast2GO v.5.2.5 to retrieve GO terms (e-value cutoff $1e^{-6}$) for DEGs with a positive BLAST hit to improve GO-terms assignment. For each species, GO terms retrieved from both methods were combined to build a dataset that was subsequently used for GO enrichment analysis. Enriched GO terms of the biological process of DEGs were tested by Fisher Exact tests using package 'topGO' v. 2.42.0 (Alexa and Rahnenfuhrer, 2010) in R with a threshold FDR of 0.05. We used '*weight01*' method [this is a mixture of '*elim*' and '*weight*' method (Alexa et al., 2006)] that takes the GO hierarchy into account when calculating enrichment. In this way, the 'inheritance problem' (that can lead to false positives) can be avoided (Grossmann et al., 2007). Finally, Venn diagrams were used to identify shared and unique GO terms among different contrasts.

2.8. Statistical analysis

A two-way analysis of variance (Two-way ANOVA) with two fixed factors: Species (2 levels: *P. oceanica* and *C. nodosa*) and Treatment (3 levels: control, non-primed, and heat-primed) was performed to assess significant differences in response to warming at the photophysiological, growth and pigment levels. Before the analysis, homogeneity of variance was checked by using Levene's test. Then, the Shapiro-Wilk test was used to validate data normality. Finally, a Tukey HSD *post-hoc* test was applied to determine significant differences among treatments of each species when applicable. All statistical analyses were conducted using R version 4.1.1 in R-studio v.February 1, 5033 (R Core Team, 2018). Graphs were plotted with R-studio using the package *ggplot2* (Wickham, 2009).

3. Results

3.1. Heat-priming enhanced photochemical tolerance of Posidonia oceanica in response to temperature increase

The triggering event had no significant effects on the photosynthetic performance of heat-primed *P. oceanica* plants, but it significantly affected non-primed *P. oceanica* plants (Table 1, Fig. 2A). In detail, significant differences were detected between non-primed vs. control plants and between non-primed vs. heat-primed plants on maximum quantum yield (Fv/Fm, Turkey HSD *post-hoc* test, $p < 0.05$ & $p < 0.01$, respectively; Fig. 2A). In contrast, in *C. nodosa*, both heated plants (non-primed and heat-primed) maintained their Fv/Fm values unaltered during the triggering event (Fig. 2B). As a result, a significant S × T interaction was detected for Fv/Fm measurements (Two-way ANOVA, $p < 0.01$, Table 1).

On the other hand, the effective quantum yield (Δ F/Fm') of both species showed no significant warming-induced alterations (Fig. 2C and

Table 1
Two-way ANOVA results for photo-physiological, plant growth and pigment responses of *P. oceanica* and *C. nodosa* at the end of the experiment. Significant codes: ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$; ns $p > 0.05$. df: degrees of freedom; MS: Mean Square; F: F-value.

Source of variation	Fv/Fm				ΔF/Fm'				Leaf biomass production			
	df	MS	*F*	*p*	df	MS	*F*	*p*	df	MS	*F*	*p*
Species (S)	1	2.88E-4	1.855	ns	1	2.57E-4	1.230	ns	1	812.300	192.081	***
Treatment (T)	2	1.05E-3	6.772	*	2	2.97E-4	1.421	ns	2	30.800	7.280	**
S × T	2	1.08E-3	6.985	**	2	3.87E-4	1.854	ns	2	72.000	17.044	***
Residual	12	1.55E-4			12	2.09E-4			12	4.200		
Source of variation	Chlorophyll *a*				Chlorophyll *b*				Total carotenoids			
	df	MS	*F*	*p*	df	MS	*F*	*p*	df	MS	*F*	*p*
Species (S)	1	11.387	58.918	***	1	5.274	99.622	***	1	0.561	22.652	***
Treatment (T)	2	0.114	0.588	ns	2	0.138	2.603	ns	2	0.011	0.446	ns
S × T	2	1.039	5.378	*	2	0.420	7.931	**	2	0.071	2.864	ns
Residual	12	0.193			12	0.053			12	0.025		

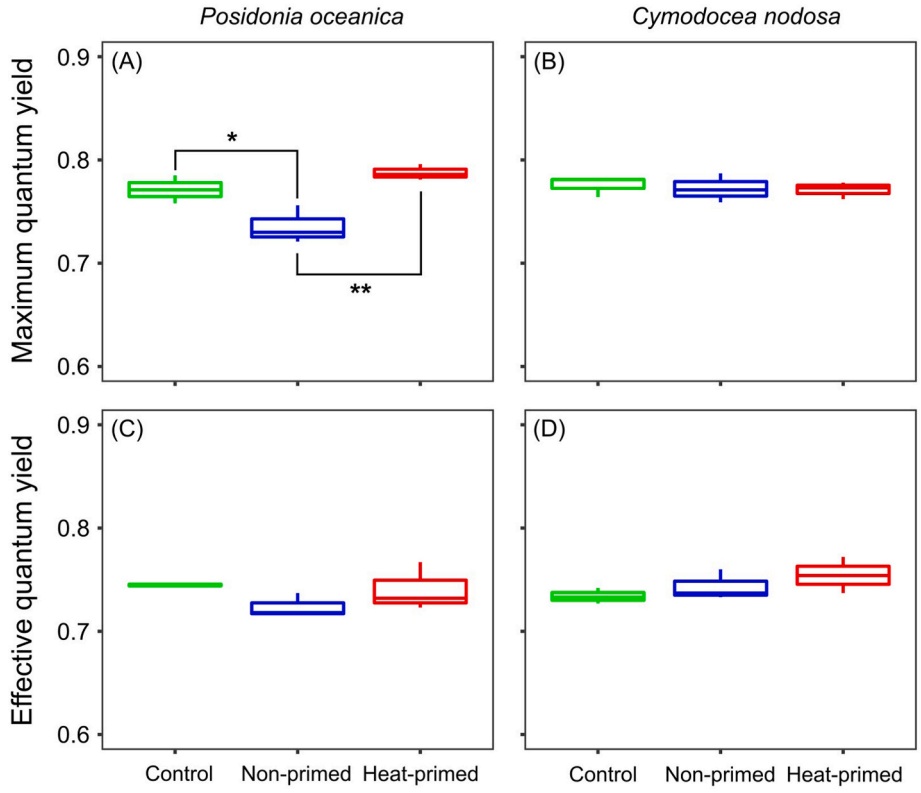

Fig. 2. Photo-physiological responses of *P. oceanica* (**A**,**C**) and *C. nodosa* (**B**,**D**) at the end of the triggering event. Asterisks indicate significant differences as results of Tukey HSD *post-hoc* test (*$p < 0.05$ & **$p < 0.01$), $n = 3$.

D, Table 1).

3.2. Heat-priming facilitated pigment accumulation in Cymodocea nodosa in response to warming

Warming negatively affected the pigment content of *P. oceanica* plants while it enhanced the accumulation of leaf pigments in *C. nodosa* plants, irrespective of whether or not the plants were primed (Fig. 3). Pigment content was significantly higher in *C. nodosa* than in *P. oceanica* (Two-way ANOVA, Species: $p < 0.001$, Table 1).

In *P. oceanica*, warming reduced pigment content in both non-primed and heat-primed plants. However, pigment content in heat-primed plants was approximately 33% higher than in non-primed plants (Chl *a*, Chl *b* and total carotenoids; Fig. 3), although these differences were not statistically significant.

In *C. nodosa*, the warming exposure increased Chl *a* (44%), Chl *b* (57%) and total carotenoids (44%) in heat-primed plants with respect to control plants. The change was significant for Chl *b* (Turkey HSD *post-hoc* test, $p < 0.05$, Fig. 3D). Likewise, the corresponding percentages for non-primed plants were 31% (Chl *a* - n.s.), 23% (Chl *b* - n.s.) and 29% (total carotenoids - n.s.), respectively.

3.3. Heat-priming slowed leaf growth in P. oceanica and enhanced it in C. nodosa

Warming strongly influenced seagrass growth, as evidenced by the significant differences detected for the Treatment factor (Two-way ANOVA, $p < 0.01$, Table 1). In *P. oceanica*, a substantial decline in growth was detected in heat-primed plants with respect to control and non-primed plants (Turkey HSD *post-hoc* test, $p < 0.01$ and $p < 0.001$, respectively, Fig. 4A). In *C. nodosa*, even if no significant differences were detected among treatments, it is worth to mention that there was a

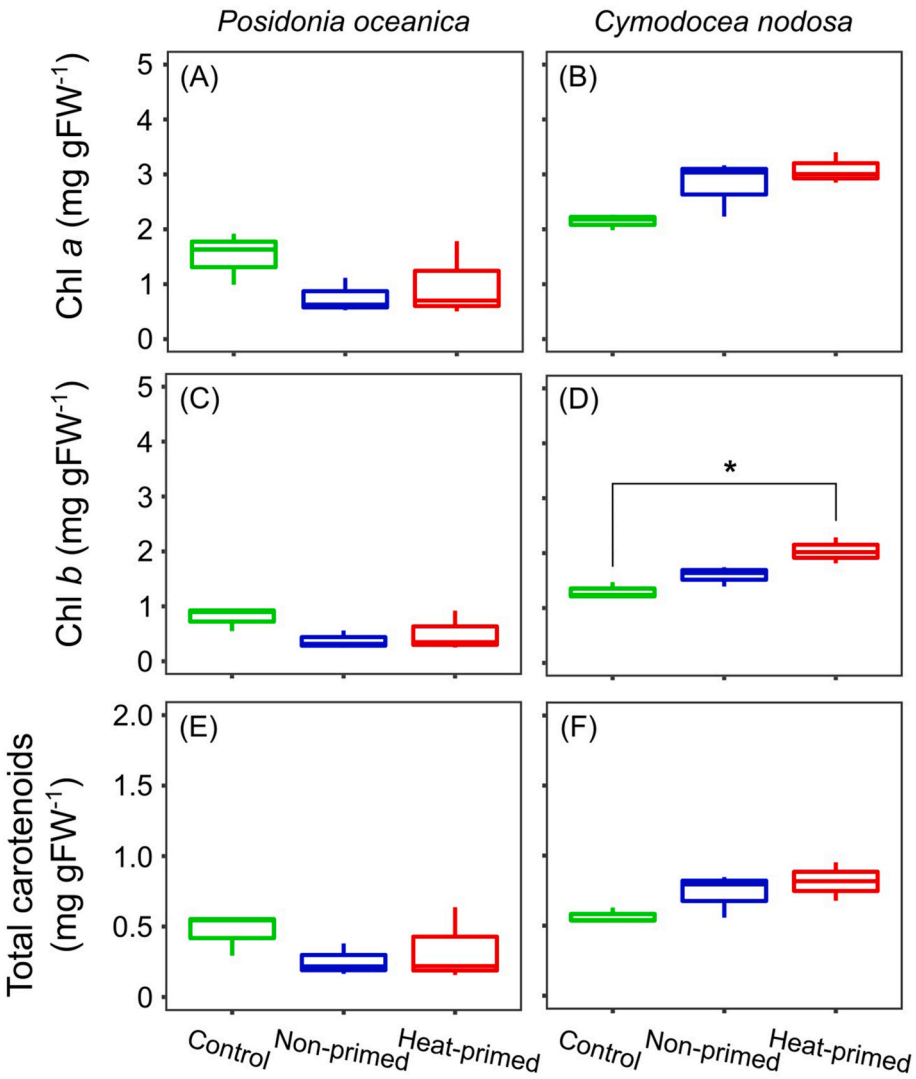

Fig. 3. Leaf pigment content of *P. oceanica* (**A**,**C**,**E**) and *C. nodosa* (**B**,**D**,**F**) at the end of the triggering event. Asterisks indicate significant differences as results of Tukey HSD *post-hoc* test (*$p < 0.05$), $n = 3$.

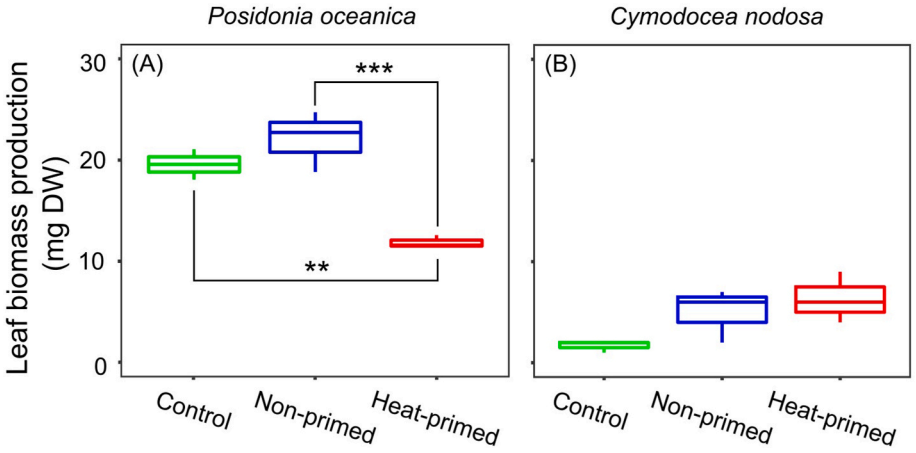

Fig. 4. Leaf growth responses of *P. oceanica* (**A**) and *C. nodosa* (**B**) at the end of the triggering event. Asterisks indicate significant differences as results of Tukey HSD *post-hoc* test (**$p < 0.01$ & ***$p < 0.001$), $n = 3$.

tendency for both non-primed and heat-primed plants to increase their growth rates with respect to control plants. In particular, heat-primed *C. nodosa* plants produced 27% more biomass during the triggering event than non-primed plants (Fig. 4B).

3.4. Overall transcriptome response of non-primed and heat-primed plants

The Tag sequencing generated 69,267,359 and 64,422,853 single-end reads for *P. oceanica* and *C. nodosa*, respectively. Raw reads were quality-trimmed to obtain 53,421,823 (77%) and 52,436,340 (81%) cleaned reads, respectively. The cleaned reads were subsequently used for read collapsing to identify tags. As a result, after collapsing all cleaned reads using Cd-hit (90% identity), 675,032 and 835,468 tags were identified for *P. oceanica* and *C. nodosa*, respectively. The overall profile of expression across different samples was explored through a PCA (Fig. S1). For *P. oceanica*, controls were separated by heated plants (both non-primed and heat-primed) along the PC1 axis (24% of the total variance), while the PC2 axis (16% of the total variance) was responsible for the separation between non-primed and heat-primed plants. Regarding *C. nodosa*, the separation between control and heated plants was driven by both the PC1 (25% of variance) and the PC2 (17% of variance), whereas non-primed and heat-primed plants were mainly separated along the PC1. It is worthy to note that for both species non-primed and heat-primed samples partly overlapped (Fig. S1).

3.5. Differential gene-expression response revealed differences between non-primed and heat-primed plants and between the two seagrass species

The differential expression analysis identified 2524 and 1796 DEGs (FDR-corrected p-value ≤ 0.05 and log2FC $> |1.5|$) in *P. oceanica* and *C. nodosa*, respectively (Fig. 5).

In *P. oceanica*, the response to warming involved 1628 and 1700 DEGs for non-primed and heat-primed plants, respectively, with half of them being unique to each treatment and the other half shared between non-primed and heat-primed plants (Fig. 5). Among 824 DEGs exclusively belonging to non-primed plants, 575 DEGs were up-regulated while only 249 DEGs were down-regulated (Fig. 5). In contrast, among the 896 DEGs uniquely identified in heat-primed plants, the number of down-regulated DEGs was slightly higher than the number of up-regulated DEGs (489 vs. 407, Fig. 5). Finally, 804 DEGs were found in common between non-primed and heat-primed plants in which 612 DEGs were up-regulated and 192 DEGs were down-regulated (Fig. 5).

In *C. nodosa*, 1669 DEGs were detected in non-primed plants while only 321 DEGs were found in heat-primed plants (Fig. 5). Additionally, down-regulated DEGs were found extremely dominant in both non-primed and heat-primed plants (Fig. 5).

3.6. Gene functional categories of the DEGs

Functional annotation identified 903 DEGs with an UniProt ID for *P. oceanica* plants (~ 36% of the total number of DEGs) and 320 DEGs for *C. nodosa* plants (~ 25% of the total number of DEGs). Over the total, 194 annotated DEGs of non-primed and heat-primed *P. oceanica* plants were assigned to the eight functional categories defined in **section 2.8** (Fig. 6A, Table S1). The same was done for 78 annotated DEGs of non-primed and heat-primed *C. nodosa* plants, though none of these DEGs belonged to the red, the growth, or the epigenetic categories (Fig. 6B–Table S2).

In *P. oceanica*, warming induced significant changes in the expression of several low/medium/high stress response DEGs for both non-primed and heat-primed plants. Interestingly, while non-primed *P. oceanica* plants tended to up-regulate genes in response to stress, heat-primed *P. oceanica* plants did the opposite (Fig. 6A–Table S1). For instance, the number of up-regulated low-stress-response-DEGs uniquely found in heat-primed plants was only 20% of the corresponding number in non-primed plants (5 against 24, Fig. 6A). At the same time, while non-primed plants significantly over-expressed a gene from the high-stress-response DEG (the ATM gene, Fig. 6A–Table S1), heat-primed plants down-regulated a gene from this category (the aifA gene, Fig. 6A–Table S1). In both non-primed and heat-primed *P. oceanica* plants, the number of down-regulated photosynthesis DEGs was greater than the number of up-regulated ones (Fig. 6A). In heat-primed plants, where the leaf biomass production was significantly inhibited (Fig. 3A), the number of down-regulated growth DEGs (mostly SAUR50 gene) was 3-fold greater than that of non-primed plants (Fig. 6A–Table S1). Similarly, the number of down-regulated transcription factor DEGs was 4-fold higher in heat-primed plants than in non-primed plants (Fig. 6A). Furthermore, warming modified the level of expression of epigenetic DEGs in *P. oceanica* with the number of up-regulated DEGs being more than double in heat-primed plant than in non-primed plants (Fig. 6A). For example, DEGs representing Probable histone-arginine methyltransferase CARM1 and Protein arginine N-methyltransferase 5 were found exclusively in heat-primed plants (Fig. 6A–Table S1). Several stress memory-related DEGs (e.g., HSP18.2, HSP22, and FBA6; Fig. 6A–Table S1) were found overexpressed in both non-primed and heat-primed plants.

In *C. nodosa*, high-stress-response DEGs were not detected in either non-primed or heat-primed plants while medium-stress-response DEGs were exclusively found in non-primed plants (Fig. 6B). It is important to note that, among the five medium-stress-response DEGs detected in non-primed plants, four of them were up-regulated (Fig. 6B). Regarding low-stress-response DEGs, the number of up-regulated and down-regulated DEGs was double in non-primed plants in respect to heat-primed plants (5 DEGs & 13 DEGs vs. 2 DEGs & 8 DEGs, respectively; Fig. 6B). On the other hand, photosynthesis-related DEGs were only detected in non-primed plants, being mostly down-regulated (Fig. 6B). Both non-primed and heat-primed *C. nodosa* plants significantly down-regulated several transcription factors, however, up-regulated genes

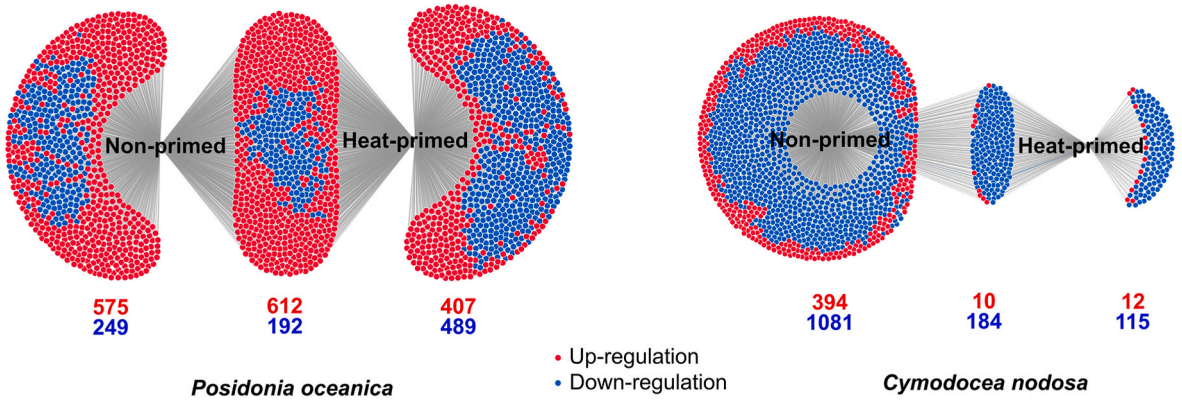

Fig. 5. Global gene expression responses of *P. oceanica* and *C. nodosa* (Red and blue dots/numbers indicate the number of up-regulated and down-regulated DEGs, respectively).

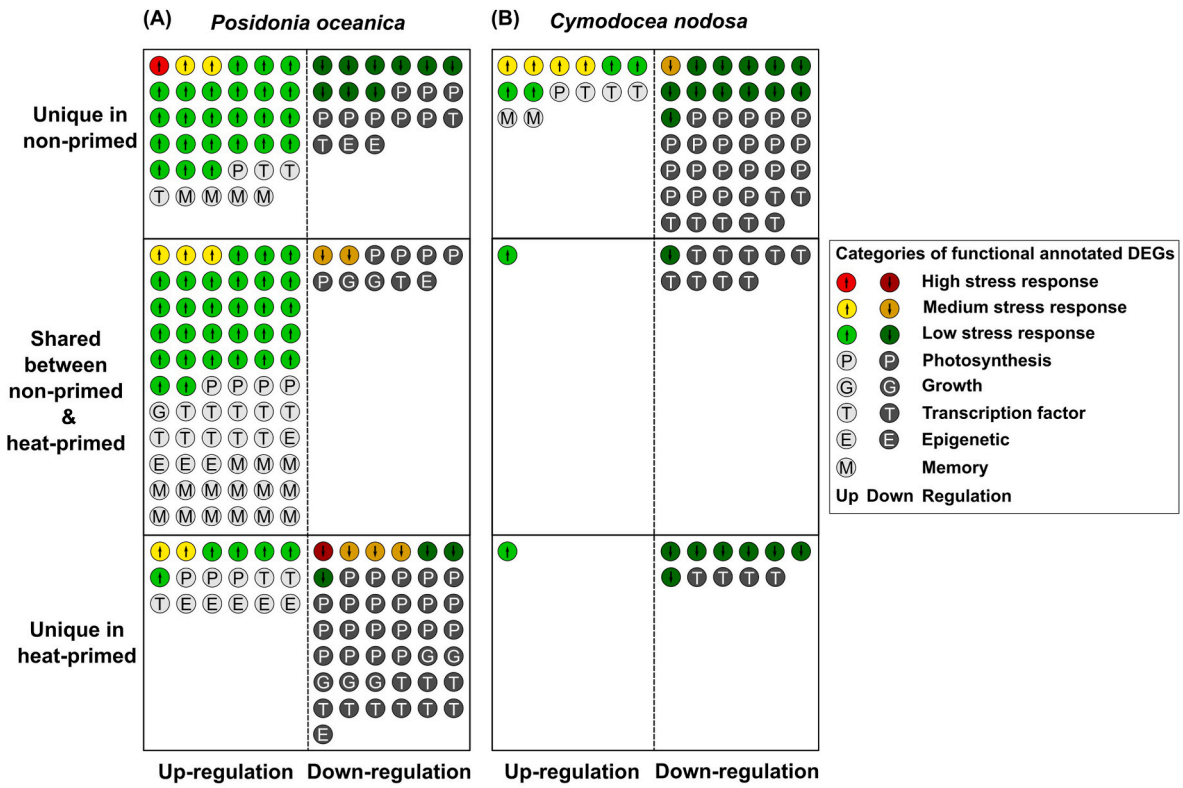

Fig. 6. Differentially expressed genes (DEGs) from the different categories selected in this study detected in *P. oceanica* (A) and *C. nodosa* (B) plants at the end of the triggering event. Each circle represents one DEG.

from this category were only found in non-primed plants (Fig. 6B). Lastly, stress memory DEGs were detected only in non-primed *C. nodosa* plants representing the HSP22 gene family (Fig. 6B–Table S2).

3.7. Gene Ontology (GO) annotation of the DEGs

A total of 167,504 tags in *P. oceanica* (25% of the total tags) and 89,216 tags (11% of the total tags) in *C. nodosa* were associated with at least 1 GO term. These GO-annotated tags were subsequently used for GO enrichment analysis. Results of the enrichment analysis (biological process, GO-BPs) are summarized in Fig. 7 and Table S3– 7. In *P. oceanica*, the number of enriched GO-BPs was 202 in non-primed plants (Fig. 7, Table S3) and 222 in heat-primed plants (Fig. 7, Table S4). Approximately 50% of those GO-BPs were shared between non-primed and heat-primed plants while the other 50% was unique for each group. In contrast, the number of GO-BPs in *C. nodosa* was three-time higher in non-primed plants (Fig. 7, Table S6) than in heat-primed plants, which were enriched in only 17 GO-BPs (Fig. 7, Table S7).

For both species, enriched GO-BPs terms included processes that can be ascribed to plant thermal stress response, such as heat acclimation, response to oxidative stress, regulation of transcription, sucrose starvation, fatty acid biosynthesis, photosynthesis, protein (re)folding, regulation of DNA damage and apoptotic process, signaling pathways involving plant hormones (Wahid et al., 2007) (Fig. 7).

In *P. oceanica*, non-primed and heat-primed plants shared many GO-BPs related to plant heat-stress response (Region 2, 5 and 108, Fig. 7). Notably, positive regulation of apoptotic process was only found in non-primed plants (Region 85, Fig. 7) while negative regulation of intrinsic apoptotic signaling pathway appeared exclusively in heat-primed plants (Region 104, Fig. 7). In contrast, enriched GO-BPs related to epigenetic modifications were only detected in heat-primed *P. oceanica* plants and included histone H3-R26 methylation and histone H3-R17 methylation (Region 104, Fig. 7).

Protein refolding was found in common across different species and treatments (Region 2, Fig. 7). Despite that, only 1 GO-BP related to plant heat-stress response was enriched in heat-primed *C. nodosa* plants (i.e., positive regulation of transcription, DNA; Region 8, Fig. 7) while, in contrast, 11 GO-BPs related to response to oxidative stress, plant hormones, photosynthesis, and transcription were exclusively enriched in non-primed *C. nodosa* plants (Region 5, 3 and 38, Fig. 7).

4. Discussion

This study provides new insights into the functioning of heat-priming in seagrasses. It shows that pre-exposure to a mild increase in temperature makes *Posidonia oceanica* able to cope with a further more intense warming event. Results also indicate clear differences between the two studied seagrass species, *P. oceanica* and *Cymodocea nodosa*, most likely due to the fact that these were subjected to the same priming and triggering treatments, despite having different thermal tolerances. In both species, the response to the triggering event of non-primed plants required a significant activation of many genes while heat-primed plants down-regulated more genes. In general, the reaction of heat-primed plants appears to be more energetically conservative than that of non-heat-primed plants, indicating the activation of a strategy to better cope with elevated temperatures.

4.1. Heat-primed Posidonia oceanica coped better than their non-primed counterparts with the temperature increase at the triggering event

Heat-primed *P. oceanica* plants maintained their photochemical capacity unaltered, while the photosynthetic performance of non-primed plants was negatively affected under the same triggering thermal event. This result concurs with recent findings from other seagrass species [*Posidonia australis* and *Zostera muelleri* (Nguyen et al., 2020)], from *P. oceanica* seedlings (Pazzaglia et al., 2022a)], and from terrestrial plants (Wang et al., 2014) on the positive effect of heat-priming for

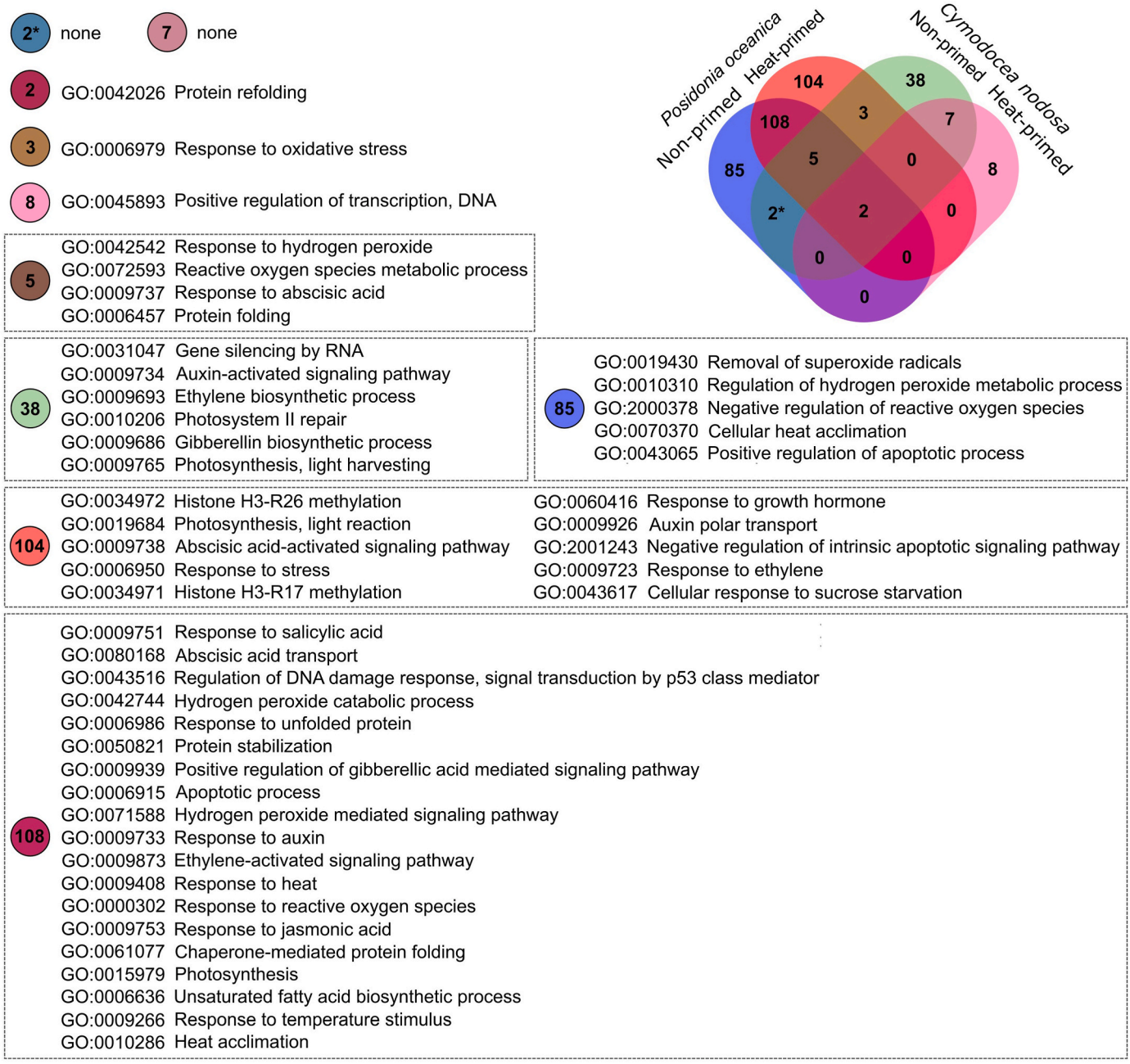

Fig. 7. Venn diagram depicting shared and unique enriched GO terms in the different contrasts (non-primed *P. oceanica*, heat-primed *P. oceanica*, non-primed *C. nodosa* and heat-primed *C. nodosa*) at the end of the triggering event. Only the enriched GO terms (i.e., ID and Biological Processes) related to plant "heat-stress response" and/or "epigenetics" are listed.

enhancing photo-physiological tolerance of plants to repeated warming events. Maintaining photosynthetic performance under stressful conditions reflects a more successful response (higher tolerance) as it allows the provision of resources to cope with the cost associated with high temperatures (Wahid et al., 2007). Additionally, the heat-primed *P. oceanica* down-regulated four times the number of DEGs related to photosynthesis (e.g., genes related to Photosystem I & II and oxygen-evolving enhancer proteins) than the non-primed plants. Though we did not measure respiration in our experiment, we can assume that keeping the photosynthetic capacity unaltered together with slowing down plant growth, allows the maintenance of a positive carbon balance, to overcome the stress events. This 'energy-saving' strategy has been documented for *P. oceanica* in response to several abiotic stress factors, including heat stress (Marín-Guirao et al., 2018) as well as for terrestrial plants (Holcik and Sonenberg, 2005).

By arresting growth, *P. oceanica* can satisfy the carbon demand for basal metabolism and ensure survival under stressful environmental conditions. In this case, *P. oceanica* can avoid drawing on the carbon resources stored in the rhizome what is needed for overwintering (Marín-Guirao et al., 2018; Pazzaglia et al., 2022b). This strategy increases rates of survival and recovery after the cessation of stress. The molecular analysis further supports this hypothesis. Biological processes related to carbon resources (GO:0043617, cellular response to sucrose starvation) and growth (GO:0060416, response to growth hormone) were uniquely enriched in heat-primed plants, with a down-regulation of several growth-related genes (e.g., SAUR50). SAUR50 belongs to the largest family of early auxin response genes, which have been suggested to promote plant growth in response to high temperature in

Arabidopsis through controlling cell expansion or division, contributing to auxin-regulated leaf growth and development (Spartz et al., 2014). The downsizing response of heat-primed *P. oceanica* plants observed in this study is different to what reported in *P. australis*, *Z. muelleri* (Nguyen et al., 2020), and *P. oceanica* seedlings (Pazzaglia et al., 2022a) which could indicate that the effect of heat-priming on growth in seagrasses is rather complex and can vary among different species and among different life stages of the same species involving different types of ecological developmental responses, as well as depending on the level of stress experienced by plants.

It is worth noting that at the end of the triggering event, both non-primed and heat-primed *P. oceanica* plants clearly reduced their pigment content (Chl *a*, Chl *b* and carotenoids). It is also interesting to see many DEGs related to Chlorophyll binding proteins were also down-regulated in these samples. This strengthens the link between the response of the plants across different levels of organization from molecular to morphology and physiology.

According to the cellular stress response concept, the triggering event induced the highest level of stress response in non-primed *P. oceanica* plants. A clear example is the high expression of the ATM gene only in these plants. The ATM gene plays a crucial role in protective mechanisms for DNA damage repair in response to adverse environmental stress (Su et al., 2017). This is further supported by GO enrichment analysis showing a positive regulation of apoptotic processes (i.e., an indication of serious DNA damage) in non-primed plants, whereas, in contrast, heat-primed plants were enriched in the negative regulation of apoptotic processes. Similar evidence was also found at the second level of cellular stress response. In detail, gene L-ascorbate peroxidase 1 (APX1 - involved in the defences against oxidative stress) was more up-regulated in non-primed *P. oceanica* plants than in heat-primed plants (Marín-Guirao et al., 2017). In addition, it is important to note that non-primed *P. oceanica* plants showed a more intense activation of genes encoding for HSPs when compared to heat-primed plants. HSPs are among the most common and rapid responsive mechanisms of plants to cope with adverse conditions (Kiang and Tsokos, 1998). Nonetheless, the production of HSPs itself is an energy-costly process (Nover et al., 2001). This HSPs-related response often occurs within minutes/hours of the start of stress in terrestrial model plants (Oberkofler et al., 2021). In the intertidal seagrass *Z. noltii* the HSPs response is also very fast and tends to stop just few hours after the beginning of a heat shock (Massa et al., 2011). In this study, the transcriptomic response was studied many days after the onset of the triggering event, so it might have not captured the possible early and fleeting HSP response. In any case, the fact that the expression level was higher in non-primed plants could suggest that non-primed *P. oceanica* had to 'pay a higher cost' to minimize the negative impacts of warming than its heat-primed counterpart.

4.2. Both non-primed and heat-primed Cymodocea nodosa plants grew better at the triggering event

In *C. nodosa*, the heat triggering event did not induce significant changes in physiological performances, growth and pigment contents (except for Chlorophyll *b* content) between non-vs. heat-primed plants. Nevertheless, the high temperature of the triggering treatment favoured a significant accumulation of chlorophylls in the leaves of heat-primed plants. Heat-primed *C. nodosa* also showed higher photochemical efficiency as seen in the effective quantum yield results. They also grew faster than the controls and the non-heat-primed plants, although not significantly, possibly because of the short duration of the heating period and/or the small sample size used for these plant variables (i.e., $n = 3$).

The elevated capability of the heat-primed *C. nodosa* to take advantages from the temperature at the triggering event was confirmed at the molecular level. Only non-primed *C. nodosa* plants exhibited the second level of cellular stress response (i.e., ubiquitination and proteolysis) by activating FTSH8 and At1g55760 genes, whose functions are related to the removal of irreversibly damaged proteins (Zaltsman et al., 2005). Possibly, heat-primed *C. nodosa* may had already modified their proteins in the priming event to make them functional under increased temperatures and thus more prepared for the second heating event. As a result, heat-primed *C. nodosa* did not need to activate these pathways involved in protein homeostasis. Similarly, a lower number of differentially expressed genes, belonging to fewer biological processes, were found in heat-primed plants as response to the triggering event, in comparison with non-primed ones. It is interesting to note that only molecular data provided indications of stress in non-primed *C. nodosa* at the triggering event, that would otherwise be hardly detected from other measurements (e.g., morphology and physiology). Indeed, plants response at molecular and biochemical levels under stressful conditions can anticipate signs in physiological and morphological traits (Macreadie et al., 2014).

4.3. Interspecific differences in thermal priming performance between the two seagrass species

The priming induction in organisms can be considered as an hormetic response, characterized by a nonlinear dose-response relationship to environmental stressors (Costantini et al., 2010), and depending on the dose-response relationship of single organisms. Therefore, the level of the stress experienced by plants seems to be determinant for the acquisition of a primed status that allows them to activate an improved response to subsequently more-severe stress situations. Mild stress can induce damage-repair and background-adaptive responses (Villagómez-Aranda et al., 2022). The priming treatment applied (29 °*C*) can be considered stressful for the temperate species *P. oceanica* and less so for *C. nodosa,* whose genus is of tropical origin and has a higher tolerance to warming (Marín-Guirao et al., 2018; Savva et al., 2018). This thermal treatment (29 °*C*) seems to induce a primed state in *P. oceanica*, as evidenced by the lower level of molecular stress experienced by heat-primed plants with respect to non-primed plants when exposed to the triggering treatment (32 °*C*), as discussed above. In *C. nodosa*, although the priming treatment can be considered rather less stressful, or even non-stressful, this pre-heating also seems to induce a certain level of priming or preconditioning that allows plants to benefit better from the higher temperature of the triggering treatment. In fact, the performance of heat-primed *C. nodosa* plants at the end of the triggering treatment was improved in respect to non-primed plants, as pointed out by the significant increment of Chl *b* concentration.

4.4. Heat-priming induced changes in the expression of epigenetics- and thermomemory-related genes

Epigenetic modifications have been suggested to play an important role in the response to warming (Pazzaglia et al., 2023) and in heat-priming mechanism of seagrasses (Nguyen et al., 2020; Pazzaglia et al., 2022a). In this study, molecular analysis revealed significant changes in the expression of some well-known epigenetic-related genes [e.g., ATXR2, HTR4 (Lee et al., 2017)] in both non- and heat-primed *P. oceanica* plants, thus confirming the potential role of epigenetic modifications not only in thermal response but also in heat-priming in seagrasses.

In *Arabidopsis thaliana*, HSP18.2 (Shahnejat-Bushehri et al., 2012) and HSP22 (Lämke et al., 2016) have been shown to maintain remarkably high expressions at 28 − 48 h in the re-acclimation phase after the priming event (i.e., the first exposure to thermal stress). Also in *A. thaliana*, FRUCTOSE-BISPHOSPHATE ALDOLASE 6 (FBA6) has been demonstrated to be an essential component of the establishment of the shoot apical meristem's heat stress transcriptional memory (Olas et al., 2021). FBA6 was highly expressed in both the priming and the triggering phase, though the expression was significantly higher in the second (Olas et al., 2021). Here, we also identified significantly higher expressions of HSP18.2, HSP22, and FBA6 for non- and heat-primed

P. oceanica plants and HSP22 for non-primed *C. nodosa* plants. The identification of these thermo-memory genes suggests the potential involvement of HSP18.2, HSP22, and FBA6 in both the formation (during the priming phase) and activation (during the triggering phase) of thermal stress memory in seagrasses.

4.5. Contributions and limitations of the study

Our study gives new insights into the functioning of stress priming in seagrasses (Nguyen et al., 2020; Pazzaglia et al., 2022a), especially at the molecular level, and in the differences existing among seagrass species with different thermal tolerances. The duration of the so-called stress memory still remains to be assessed. A recent study by Provera et al. (2024) showed that a long lag phase does not allow an enhanced heat and salinity stress response in *P. oceanica* seedlings undergoing a second stress events, suggesting that stress transcriptional memory was not maintained over several weeks. This could only be confirmed looking at the expression of genes involved in the first stress response and in the dynamics of expression potentially underlying a stress memory. Our study confirms the need for future research, across different approaches, to have a complete understanding of the mechanisms. Natural stress priming can ultimately affect the way seagrasses interact with their surrounding environments as well as their future distribution in an era of ocean changes. This study widens up the spectrum of seagrass ecology by opening questions concerning the contribution of stress memory in the existence and evolution of seagrasses [see also Hilker et al. (2016) for evolutionary ecology aspects of priming of stress responses]. This is of particular relevance in case of mixed seagrass meadows that consist of several seagrass species with different stress tolerance or in the interaction among native and introduced species (Beca-Carretero et al., 2023). Future changes of seagrass landscape can be driven not only by environmental variations or by the tolerance level of each seagrass species and populations, but also by their capability to acquire stress memory. We acknowledge some limitations in this study due to (*i*) the use of two seagrass species with different thermal tolerance and a single level of heat-priming, which was insufficient to discriminate whether the differences in priming effects observed were simply because the two species had different thermal tolerance or different capacity to acquire stress memory, (*ii*) the use of only one population per species, (*iii*) the relatively small number of annotated genes, which prevented us from exploring specific patterns of thermal stress memory (such as DNA methylation or histone modifications) and (*iv*) the small sample size ($n = 3$) for morpho-physiological measurements.

5. Conclusions

Our results provide important contributions in the understanding of thermal stress memory in seagrasses. This study can represent a starting point for future studies to deepening into the molecular mechanism underlying the formation and activation of stress priming. Here, we worked with two species with different thermal tolerance, and the potential different ability to be primed should be further tested in species with similar thermal tolerance. In addition, we used only one stress temperature and analyzed samples at the end of the triggering event. Future studies are strongly encouraged to fill the gap in knowledge related to the different variables of the priming induction mechanism, including stress level (intensity and duration) needed to induce a primed status and its persistence in time (see Provera et al., 2024). Only a deep understanding of the whole process can provide a valuable tool for assisting seagrass restoration and an additional perspective in the interpretation of seagrass evolution in time and seagrass resilience against environmental change.

Contributions

HMN, LMG, MP and GP conceived the idea and designed the experiment; HMN and GP participated in sample collection; HMN, ED and GP maintained the mesocosm system. HMN performed physiological, growth, pigment and molecular measurements with support from ED and MR. HMN analyzed the data with support from ED, MR and UVTH. HMN wrote the first draft of the manuscript. All authors reviewed the manuscript and gave final approval for publication.

CRediT authorship contribution statement

Hung Manh Nguyen: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Uyen V.T. Hong:** Writing – review & editing, Data curation. **Miriam Ruocco:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Emanuela Dattolo:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Lázaro Marín-Guirao:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization. **Mathieu Pernice:** Writing – original draft, Supervision, Methodology, Investigation, Conceptualization. **Gabriele Procaccini:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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

RNA-Seq reads from this study are deposited and made publicly available at the Sequence Read Archive (SRA) at the National Centre for Biotechnology Information (NCBI) with accession number PRJNA1099457. The lists of differentially expressed genes (DEGs) and enriched GO-BP terms included in this study can be found in the supporting information. Commands and scripts, as well as other data supporting the conclusions of this study, will be available upon request.

Acknowledgments

HMN was supported by an SZN Ph.D. fellowship via the Open University. This work was funded by EPIC-SEA Project, Extra-Eu Scientific Research and Cooperation Grant of the SZN, by the project Marine Hazard, PON03PE_00203_1, Italian Ministry of Education, University and Research (MUR), by the project Assemble Plus EU-FP7 and partially by the European Union – NextGenerationEU. Project code CN_00000033, Concession Decree No. 1034 of June 17, 2022 adopted by the Italian Ministry of University and Research, CUP C63C22000520001, Project title “National Biodiversity Future Center - NBFC”. The work of LMG was partially supported by the project GRASSREC, PID2020-118144RB-C31 (Spanish Ministry of Science and Innovation). Authors are grateful for the precious support from Alex Santillán-Sarmiento, Ludovica Pedicini and Jessica Pazzaglia (SZN) for the mesocosm experiment.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2024.108614.

References

Alexa, A., Rahnenfuhrer, J., 2010. topGO: Enrichment Analysis for Gene Ontology. R Packag. version 2, 2010.

Alexa, A., Rahnenführer, J., Lengauer, T., 2006. Improved scoring of functional groups from gene expression data by decorrelating GO graph structure. Bioinformatics 22, 1600–1607. https://doi.org/10.1093/bioinformatics/btl140.

Andrews, S., 2010. FastQC: a Quality Control Tool for High Throughput Sequence Data.

Bäurle, I., 2016. Plant heat adaptation: priming in response to heat stress. F1000Research 5. https://doi.org/10.12688/f1000research.7526.1.

Beca-Carretero, P., Winters, G., Teichberg, M., Procaccini, G., Schneekloth, F., Zambrano, R.H., Chiquillo, K., Reuters, H., 2023. Climate change and the presence of invasive species will threaten the persistence of the Mediterranean seagrass community. Sci. Total Environ. 168675 https://doi.org/10.1016/j.scitotenv.2023.168675.

Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30, 2114–2120.

Bossdorf, O., Richards, C.L., Pigliucci, M., 2008. Epigenetics for ecologists. Ecol. Lett. https://doi.org/10.1111/j.1461-0248.2007.01130.x.

Bruce, T.J.A., Matthes, M.C., Napier, J.A., Pickett, J.A., 2007. Stressful "memories" of plants: evidence and possible mechanisms. Plant Sci. https://doi.org/10.1016/j.plantsci.2007.09.002.

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., Madden, T.L., 2009. BLAST+: architecture and applications. BMC Bioinf. 10, 421.

Cardini, U., Marín-Guirao, L., Montilla, L.M., Marzocchi, U., Chiavarini, S., Rimauro, J., Quero, G.M., Petersen, J.M., Procaccini, G., 2022. Nested interactions between chemosynthetic lucinid bivalves and seagrass promote ecosystem functioning in contaminated sediments. Front. Plant Sci. 13 https://doi.org/10.3389/fpls.2022.918675.

Chang, Ching-Chun, Lin, H.-C., Lin, I.-P., Chow, T.-Y., Chen, H.-H., Chen, W.-H., Cheng, C.-H., Lin, C.-Y., Liu, S.-M., Chang, Chien-Chang, 2006. The chloroplast genome of *Phalaenopsis aphrodite* (Orchidaceae): comparative analysis of evolutionary rate with that of grasses and its phylogenetic implications. Mol. Biol. Evol. 23, 279–291.

Charng, Y., Liu, H., Liu, N., Chi, W., Wang, C., Chang, S., Wang, T., 2007. A heat-inducible transcription factor, HsfA2, is required for extension of acquired thermotolerance in Arabidopsis. Plant Physiol. 143, 251–262.

Cheng, Y., Zhou, Y., Yang, Y., Chi, Y.-J., Zhou, J., Chen, J.-Y., Wang, F., Fan, B., Shi, K., Zhou, Y.-H., 2012. Structural and functional analysis of VQ motif-containing proteins in *Arabidopsis* as interacting proteins of WRKY transcription factors. Plant Physiol. 159, 810–825.

Costantini, D., Metcalfe, N.B., Monaghan, P., 2010. Ecological processes in a hormetic framework. Ecol. Lett. 13, 1435–1447. https://doi.org/10.1111/j.1461-0248.2010.01531.x.

D'Urso, A., Brickner, J.H., 2017. Epigenetic transcriptional memory. Curr. Genet. https://doi.org/10.1007/s00294-016-0661-8.

Ding, Y., Fromm, M., Avramova, Z., 2012. Multiple exposures to drought "train" transcriptional responses in *Arabidopsis*. Nat. Commun. 3, 740. https://doi.org/10.1038/ncomms1732.

Evans, T.G., Hofmann, G.E., 2012. Defining the limits of physiological plasticity: how gene expression can assess and predict the consequences of ocean change. Philos. Trans. R. Soc. B Biol. Sci. 367, 1733–1745. https://doi.org/10.1098/rstb.2012.0019.

Fourqurean, J.W., Duarte, C.M., Kennedy, H., Marbà, N., Holmer, M., Mateo, M.A., Apostolaki, E.T., Kendrick, G.A., Krause-Jensen, D., McGlathery, K.J., 2012. Seagrass ecosystems as a globally significant carbon stock. Nat. Geosci. 5, 505–509.

Gattuso, J.-P., Magnan, A.K., Bopp, L., Cheung, W.W.L., Duarte, C.M., Hinkel, J., Mcleod, E., Micheli, F., Oschlies, A., Williamson, P., Billé, R., Chalastani, V.I., Gates, R.D., Irisson, J.-O., Middelburg, J.J., Pörtner, H.-O., Rau, G.H., 2018. Ocean solutions to address climate change and its effects on marine ecosystems. Front. Mar. Sci. 5, 337. https://doi.org/10.3389/fmars.2018.00337.

Ge, S.X., Son, E.W., Yao, R., 2018. iDEP: an integrated web application for differential expression and pathway analysis of RNA-Seq data. BMC Bioinf. 19, 534. https://doi.org/10.1186/s12859-018-2486-6.

Georgoulis, I., Feidantsis, K., Giantsis, I.A., Kakale, A., Bock, C., Pörtner, H.O., Sokolova, I.M., Michaelidis, B., 2021. Heat hardening enhances mitochondrial potential for respiration and oxidative defence capacity in the mantle of thermally stressed *Mytilus galloprovincialis*. Sci. Rep. 11, 1–18.

Grossmann, S., Bauer, S., Robinson, P.N., Vingron, M., 2007. Improved detection of overrepresentation of Gene-Ontology annotations with parent–child analysis. Bioinformatics 23, 3024–3031. https://doi.org/10.1093/bioinformatics/btm440.

Hilker, M., Schmülling, T., 2019. Stress priming, memory, and signalling in plants. Plant Cell Environ.

Hilker, M., Schwachtje, J., Baier, M., Balazadeh, S., Bäurle, I., Geiselhardt, S., Hincha, D. K., Kunze, R., Mueller-Roeber, B., Rillig, M.C., 2016. Priming and memory of stress responses in organisms lacking a nervous system. Biol. Rev. 91, 1118–1133.

Holcik, M., Sonenberg, N., 2005. Translational control in stress and apoptosis. Nat. Rev. Mol. Cell Biol. 6, 318–327. https://doi.org/10.1038/nrm1618.

Iwasaki, M., Paszkowski, J., 2014. Epigenetic memory in plants. EMBO J. 33, 1987–1998. https://doi.org/10.15252/embj.201488883.

Jahnke, M., Casagrandi, R., Melià, P., Schiavina, M., Schultz, S.T., Zane, L., Procaccini, G., 2017. Potential and realized connectivity of the seagrass *Posidonia oceanica* and their implication for conservation. Divers. Distrib. 23, 1423–1434.

Kiang, J.G., Tsokos, G.C., 1998. Heat shock protein 70 kDa: molecular biology, biochemistry, and physiology. Pharmacol. Ther. 80, 183–201.

Kim, J.H., Kende, H., 2004. A transcriptional coactivator, AtGIF1, is involved in regulating leaf growth and morphology in *Arabidopsis*. Proc. Natl. Acad. Sci. USA 101, 13374–13379.

Kültz, D., 2005. Molecular and evolutionary basis of the cellular stress response. Annu. Rev. Physiol. 67, 225–257. https://doi.org/10.1146/annurev.physiol.67.040403.103635.

Lämke, J., Brzezinka, K., Altmann, S., Bäurle, I., 2016. A hit-and-run heat shock factor governs sustained histone methylation and transcriptional stress memory. EMBO J. 35, 162–175.

Langmead, B., Salzberg, S.L., 2012. Fast gapped-read alignment with Bowtie 2. Nat. Methods 9, 357.

Lee, K., Park, O.-S., Seo, P.J., 2017. *Arabidopsis* ATXR2 deposits H3K36me3 at the promoters of LBD genes to facilitate cellular dedifferentiation. Sci. Signal. 10.

Li, W., Godzik, A., 2006. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. Bioinformatics 22, 1658–1659.

Lindquist, S., 1986. The heat-shock response. Annu. Rev. Biochem. 55, 1151–1191.

Logan, C.A., Somero, G.N., 2011. Effects of thermal acclimation on transcriptional responses to acute heat stress in the eurythermal fish *Gillichthys mirabilis* (Cooper). Am. J. Physiol. Integr. Comp. Physiol. 300, R1373–R1383.

Lukić, N., Kukavica, B., Davidović-Plavšić, B., Hasanagić, D., Walter, J., 2020. Plant stress memory is linked to high levels of anti-oxidative enzymes over several weeks. Environ. Exp. Bot. 178, 104166.

Macreadie, P.I., Schliep, M.T., Rasheed, M.A., Chartrand, K.M., Ralph, P.J., 2014. Molecular indicators of chronic seagrass stress: a new era in the management of seagrass ecosystems? Ecol. Indicat. 38, 279–281.

Marín-Guirao, L., Bernardeau-Esteller, J., García-Muñoz, R., Ramos, A., Ontoria, Y., Romero, J., Pérez, M., Ruiz, J.M., Procaccini, G., 2018. Carbon economy of Mediterranean seagrasses in response to thermal stress. Mar. Pollut. Bull. 135, 617–629.

Marín-Guirao, L., Entrambasaguas, L., Dattolo, E., Ruiz, J.M., Procaccini, G., 2017. Molecular mechanisms behind the physiological resistance to intense transient warming in an iconic marine plant. Front. Plant Sci. 8, 1142.

Marín-Guirao, L., Ruiz, J.M., Sandoval-Gil, J.M., Bernardeau-Esteller, J., Stinco, C.M., Meléndez-Martínez, A., 2013. Xanthophyll cycle-related photoprotective mechanism in the Mediterranean seagrasses *Posidonia oceanica* and *Cymodocea nodosa* under normal and stressful hypersaline conditions. Aquat. Bot. 109, 14–24.

Massa, S.I., Pearson, G.A., Aires, T., Kube, M., Olsen, J.L., Reinhardt, R., Serrão, E.A., Arnaud-Haond, S., 2011. Expressed sequence tags from heat-shocked seagrass *Zostera noltii* (Hornemann) from its southern distribution range. Mar. Genomics 4, 181–188.

Nguyen, H.M., Bulleri, F., Marín-Guirao, L., Pernice, M., Procaccini, G., 2021. Photophysiology and morphology reveal divergent warming responses in northern and southern hemisphere seagrasses. Mar. Biol. 168, 129. https://doi.org/10.1007/s00227-021-03940-w.

Nguyen, H.M., Kim, M., Ralph, P.J., Marín-Guirao, L., Pernice, M., Procaccini, G., 2020. Stress memory in seagrasses: first insight into the effects of thermal priming and the role of epigenetic modifications. Front. Plant Sci. 11, 494. https://doi.org/10.3389/FPLS.2020.00494.

Nguyen, H.M., Ruocco, M., Dattolo, E., Cassetti, F.P., Calvo, S., Tomasello, A., Marín-Guirao, L., Pernice, M., Procaccini, G., 2023. Signs of local adaptation by genetic selection and isolation promoted by extreme temperature and salinity in the Mediterranean seagrass *Posidonia oceanica*. Mol. Ecol. 32, 4313–4328. https://doi.org/10.1111/mec.17032.

Nover, L., Bharti, K., Döring, P., Mishra, S.K., Ganguli, A., Scharf, K.-D., 2001. *Arabidopsis* and the heat stress transcription factor world: how many heat stress transcription factors do we need? Cell Stress Chaperones 6, 177.

Oberkofler, V., Pratx, L., Bäurle, I., 2021. Epigenetic regulation of abiotic stress memory: maintaining the good things while they last. Curr. Opin. Plant Biol. 61, 102007 https://doi.org/10.1016/j.pbi.2021.102007.

Olas, J.J., Apelt, F., Annunziata, M.G., John, S., Richard, S.I., Gupta, S., Kragler, F., Balazadeh, S., Mueller-Roeber, B., 2021. Primary carbohydrate metabolism genes participate in heat-stress memory at the shoot apical meristem of *Arabidopsis thaliana*. Mol. Plant. https://doi.org/10.1016/j.molp.2021.05.024.

Orth, R.J., Carruthers, T.J.B., Dennison, W.C., Duarte, C.M., Fourqurean, J.W., Heck, K. L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Olyarnik, S., 2006. A global crisis for seagrass ecosystems. Bioscience 56, 987–996.

Pazzaglia, J., Badalamenti, F., Bernardeau-Esteller, J., Ruiz, J.M., Giacalone, V.M., Procaccini, G., Marín-Guirao, L., 2022a. Thermo-priming increases heat-stress tolerance in seedlings of the Mediterranean seagrass *P. oceanica*. Mar. Pollut. Bull. 174, 113164 https://doi.org/10.1016/j.marpolbul.2021.113164.

Pazzaglia, J., Dattolo, E., Ruocco, M., Santillán-Sarmiento, A., Marin-Guirao, L., Procaccini, G., 2023. DNA methylation dynamics in a coastal foundation seagrass species under abiotic stressors. Proc. R. Soc. B Biol. Sci. 290, 20222197 https://doi.org/10.1098/rspb.2022.2197.

Pazzaglia, J., Santillán-Sarmiento, A., Ruocco, M., Dattolo, E., Ambrosino, L., Marín-Guirao, L., Procaccini, G., 2022b. Local environment modulates whole-transcriptome expression in the seagrass *Posidonia oceanica* under warming and nutrients excess. Environ. Pollut. 303, 119077.

Procaccini, G., Olsen, J.L., Reusch, T.B.H., 2007. Contribution of genetics and genomics to seagrass biology and conservation. J. Exp. Mar. Biol. Ecol. 350, 234–259. https://doi.org/10.1016/j.jembe.2007.05.035.

Provera, I., Martinez, M., Zenone, A., Giacalone, V.M., D'Anna, G., Badalamenti, F., Marín-Guirao, L., Procaccini, G., 2024. Exploring priming strategies to improve stress resilience of *Posidonia oceanica* seedlings. Mar. Pollut. Bull. 200, 116057 https://doi.org/10.1016/j.marpolbul.2024.116057.

R Core Team, 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Austria, 2015.
Roberts, A., Trapnell, C., Donaghey, J., Rinn, J.L., Pachter, L., 2011. Improving RNA-Seq expression estimates by correcting for fragment bias. Genome Biol. 12, 1–14.
Robinson, M.D., McCarthy, D.J., Smyth, G.K., 2010. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26, 139–140.
Ruocco, M., Entrambasaguas, L., Dattolo, E., Milito, A., Marín-Guirao, L., Procaccini, G., 2020. A king and vassals' tale: molecular signatures of clonal integration in *Posidonia oceanica* under chronic light shortage. J. Ecol. 109, 294–312. https://doi.org/10.1111/1365-2745.13479.
Ruocco, M., Jahnke, M., Silva, J., Procaccini, G., Dattolo, E., 2022. 2b-RAD genotyping of the seagrass *Cymodocea nodosa* along a latitudinal cline identifies candidate genes for environmental adaptation. Front. Genet.
Ruocco, M., Marín-Guirao, L., Procaccini, G., 2019. Within- and among-leaf variations in photo-physiological functions, gene expression and DNA methylation patterns in the large-sized seagrass *Posidonia oceanica*. Mar. Biol. 166, 24. https://doi.org/10.1007/s00227-019-3482-8.
Ruocco, M., Musacchia, F., Olivé, I., Costa, M.M., Barrote, I., Santos, R., Sanges, R., Procaccini, G., Silva, J., 2017. Genomewide transcriptional reprogramming in the seagrass *Cymodocea nodosa* under experimental ocean acidification. Mol. Ecol. 26, 4241–4259.
Savva, I., Bennett, S., Roca, G., Jordà, G., Marbà, N., 2018. Thermal tolerance of Mediterranean marine macrophytes: vulnerability to global warming. Ecol. Evol. 8, 12032–12043.
Sedaghatmehr, M., Mueller-Roeber, B., Balazadeh, S., 2016. The plastid metalloprotease FtsH6 and small heat shock protein HSP21 jointly regulate thermomemory in *Arabidopsis*. Nat. Commun. 7, 12439 https://doi.org/10.1038/ncomms12439.
Serra, I.A., Innocenti, A.M., Di Maida, G., Calvo, S., Migliaccio, M., Zambianchi, E., Pizzigalli, C., Arnaud-Haond, S., Duarte, C.M., Serrao, E.A., 2010. Genetic structure in the Mediterranean seagrass *Posidonia oceanica*: disentangling past vicariance events from contemporary patterns of gene flow. Mol. Ecol. 19, 557–568.
Shahnejat-Bushehri, S., Mueller-Roeber, B., Balazadeh, S., 2012. Arabidopsis NAC transcription factor JUNGBRUNNEN1 affects thermomemory-associated genes and enhances heat stress tolerance in primed and unprimed conditions. Plant Signal. \& Behav. 7, 1518–1521. https://doi.org/10.4161/psb.22092.
Short, F.T., Carruthers, T., Dennison, W., Waycott, M., 2007. Global seagrass distribution and diversity: a bioregional model. J. Exp. Mar. Bio. Ecol. 350, 3–20.
Spartz, A.K., Ren, H., Park, M.Y., Grandt, K.N., Lee, S.H., Murphy, A.S., Sussman, M.R., Overvoorde, P.J., Gray, W.M., 2014. SAUR inhibition of PP2C-D phosphatases activates plasma membrane H+-ATPases to promote cell expansion in *Arabidopsis*. Plant Cell 26, 2129–2142.
Su, C., Zhao, H., Zhao, Y., Ji, H., Wang, Y., Zhi, L., Li, X., 2017. RUG3 and ATM synergistically regulate the alternative splicing of mitochondrial nad2 and the DNA damage response in *Arabidopsis thaliana*. Sci. Rep. 7, 43897 https://doi.org/10.1038/srep43897.
Sun, L., Dong, S., Ge, Y., Fonseca, J.P., Robinson, Z.T., Mysore, K.S., Mehta, P., 2019. DiVenn: an interactive and integrated web-based visualization tool for comparing gene lists. Front. Genet. 10, 421. https://doi.org/10.3389/fgene.2019.00421.
Traboni, C., Mammola, S.D., Ruocco, M., Ontoria, Y., Ruiz, J.M., Procaccini, G., Marín-Guirao, L., 2018. Investigating cellular stress response to heat stress in the seagrass *Posidonia oceanica* in a global change scenario. Mar. Environ. Res. 141, 12–23. https://doi.org/10.1016/j.marenvres.2018.07.007.
Travers, K.J., Patil, C.K., Wodicka, L., Lockhart, D.J., Weissman, J.S., Walter, P., 2000. Functional and genomic analyses reveal an essential coordination between the unfolded protein response and ER-associated degradation. Cell 101, 249–258.
Unsworth, R.K.F., Cullen-Unsworth, L.C., Jones, B.L.H., Lilley, R.J., 2022. The planetary role of seagrass conservation. Science 377, 609–613. https://doi.org/10.1126/science.abq6923 (80-).
Villagómez-Aranda, A.L., Feregrino-Pérez, A.A., García-Ortega, L.F., González-Chavira, M.M., Torres-Pacheco, I., Guevara-González, R.G., 2022. Activating stress memory: eustressors as potential tools for plant breeding. Plant Cell Rep 41, 1481–1498. https://doi.org/10.1007/s00299-022-02858-x.
Wahid, A., Gelani, S., Ashraf, M., Foolad, M.R., 2007. Heat tolerance in plants: an overview. Environ. Exp. Bot. 61, 199–223.
Wang, X., Cai, J., Liu, F., Dai, T., Cao, W., Wollenweber, B., Jiang, D., 2014. Multiple heat priming enhances thermo-tolerance to a later high temperature stress via improving subcellular antioxidant activities in wheat seedlings. Plant Physiol. Biochem. 74, 185–192. https://doi.org/10.1016/J.PLAPHY.2013.11.014.
Warren, C.R., 2008. Rapid measurement of chlorophylls with a microplate reader. J. Plant Nutr. 31, 1321–1332.
Wellburn, A.R., 1994. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J. Plant Physiol. 144, 307–313.
Wickham, H., 2009. Elegant Graphics for Data Analysis (Ggplot2).
Zaltsman, A., Ori, N., Adam, Z., 2005. Two types of FtsH protease subunits are required for chloroplast biogenesis and photosystem II repair in *Arabidopsis*. Plant Cell 17, 2782–2790.
Zieman, J.C., 1974. Methods for the study of the growth and production of turtle grass, *Thalassia testudinum* König. Aquaculture 4, 139–143.