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Thermo-priming increases heat-stress tolerance in seedlings of the Mediterranean seagrass *P. oceanica*

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ABSTRACT

Seawater warming and increased incidence of marine heatwaves (MHW) are threatening the integrity of coastal marine habitats including seagrasses, which are particularly vulnerable to climate changes. Novel stress tolerance-enhancing strategies, including thermo-priming, have been extensively applied in terrestrial plants for enhancing resilience capacity under the re-occurrence of a stress event. We applied, for the first time in seedlings of the Mediterranean seagrass *Posidonia oceanica*, a thermo-priming treatment through the exposure to a simulated warming event. We analyzed the photo-physiological and growth performance of primed and non-primed seedlings, and the gene expression responses of selected genes (i.e. stress-, photosynthesis- and epigenetic-related genes). Results revealed that during the re-occurring stress event, primed seedlings performed better than unprimed showing unaltered photo-physiology supported by high expression levels of genes related to stress response, photosynthesis, and epigenetic modifications. These findings offer new opportunities to improve conservation and restoration efforts in a future scenario of environmental changes.

1. Introduction

In recent decades, the rates of changes including human pressures and climate change have been rapidly forcing organisms to exceed their resilient capacity and thus the potential to quickly respond and adapt to environmental changes (Doney et al., 2012). In the marine realm, sea warming is increasing at alarming rates inducing severe and significant consequences on ocean physical features as documented in the most recent IPCC (2019) assessment. Sea-surface temperature changes include prolonged anomalous high temperature events that last for five or more days known as marine heatwaves (MHWs; Hobday et al., 2016). The occurrence of these events varies globally and regionally, with high intensity in the western part of the globe (+2–5 °C), followed by the central and eastern equatorial Pacific Ocean (+1–4 °C) and eastern regions (+1–3 °C) considering boundaries of Northern Hemisphere oceans (Oliver et al., 2018). In the Mediterranean Sea, which is considered a hotspot for environmental changes, climatic events represent the main drivers that caused the largest impacts, especially on coastal areas and coastal ecosystems (Micheli et al., 2013). In this framework, it is fundamental to improve new strategies that allow to better estimate and mitigate future impacts on coastal marine environments.

To date, different approaches are being developed to improve conservation and restoration strategies of natural resources by human interventions, generally known as assisted evolution approaches (Filbee-Dexter and Smajdor, 2019). These interventions vary according to the level of organism manipulation, ranging from active-genome editing (e.g. CRISPR, Hsu et al., 2014) to less intrusive methods such as priming treatments (Jisha et al., 2013). In plant stress biology, the term "priming" refers to a stimulus, which prepares an organism for upcoming environmental challenges by improving its response capacity (Conrath et al., 2015). Hence, this priming process modifies the phenotypic state of an organism (i.e. priming stimulus), favoring phenotypic-plastic adjustments to future environmental stress conditions (i.e. triggering stimulus) (Hilker et al., 2016). The maintenance of these phenotypic

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responses constitute the "memory" of the past stress event that may be temporary or persist for several months, depending on the typology of the priming stimulus, its duration and other factors such as the developmental stage of organisms (Pastor et al., 2013; Walter et al., 2013). Thus, the memorization of the past stress event consists in the recognition of the reoccurring event as a stress in order to activate the appropriate response (Friedrich et al., 2019). Terrestrial plants can be primed during young life stages (i.e. seeds and seedlings), improving seed germination, seedling establishment and growth (i.e. *Solanumly copersicum* seeds, González-Grande et al., 2020; *Arabidopsis thaliana* seedlings, Leuendorf et al., 2020). Numerous priming techniques have been applied (chemical, thermal or biotic, Rakshit and Singh, 2018), and all contribute to allow plants to better respond to re-occurring stress, minimizing the investments of resources. Primed plants show faster and stronger activation of defense mechanisms typically involved in stress responses, including expression of key responsive genes, epigenetic mechanisms and signaling pathways such as those involving hormones such as jasmonic acid, salicylic acid and ethylene (Bruce et al., 2007; Kreps et al., 2002).

Molecular mechanisms that regulate the priming process modulate genes transcription during the priming stimulus, producing much higher levels of transcripts during the subsequent stress (triggering stimulus), and resulting in the potential induction for a long-term "transcriptional memory" (e.g. *A. thaliana*, Kotak et al., 2007; Liu et al., 2014). This defense response, which is enhanced by stress-memory, is arbitrated by epigenetic changes and the accumulation of signaling proteins with inactive configuration (Bruce et al., 2007). Epigenetic marks include DNA modifications operated by cytosine methyl-transferases that leave the DNA sequence unchanged, and acetylation, methylation, phosphorylation and ubiquitinylation of the nucleosome core histones (H2A, H2B, H3, H4) (Duncan et al., 2014). These last modifications induce changes in the chromatin structure, regulating the activation or repression of gene expression (Reyes et al., 2002). Thus, the priming of genes may be achieved through chromatin modifications that promote long lasting regulation favoring epigenetic memory (Borg et al., 2020). For instance, vernalization is an epigenetic-regulated process that involves the repression of the gene FLOWERING LOCUS C (FLC) maintained by histone H3 lysine 27 trimethylation (H3K27me3) (Hepworth and Dean, 2015). In plants, histone modifications occur in the presence of different abiotic stress, including heat-stress, which modify the fluidity of the membrane, the interaction of DNA with the nucleosome as well as the folding of chromatin proteins allowing the regulation of stress-responsive genes (Bäurle and Trindade, 2020; Chen et al., 2011; Chinnusamy and Zhu, 2009; Kumar et al., 2020).

This epigenetic regulation can be at the basis of the appearance of different phenotypes resulting from genotype by environment interactions (Abdusalam and Li, 2018). Indeed, the ability of organisms to adjust to environmental changes is related to their degree of phenotypic plasticity which is crucial for the species to withstand and survive the ongoing climate changes (Merilä and Hendry, 2014). This property is particularly relevant in long-lived organisms, such as several seagrass species (i.e. *P. oceanica*), as it modifies individuals' phenotype, through physiological and molecular changes, in order to adjust their performance under changing environmental conditions (Pazzaglia et al., 2021b). Seagrasses are marine flowering plants that form extensive underwater meadows in most coastal areas, representing one of the most valuable ecosystems on earth (Costanza et al., 2014). Despite clonal growth is the most diffuse propagation typology among seagrass species, sexual reproduction through seed fertilization and seedling establishment is crucial for maintaining high genetic diversity, which enhances population resilience to environmental changes (Jahnke et al., 2015; McMahon et al., 2014). Similar to terrestrial forests, seagrasses represent a highly productive system supporting different ecosystem services such as O_2 production and CO_2 sequestration (Champenois and Borges, 2019; Fourqurean et al., 2012). The high degree of phenotypic plasticity that characterizes seagrass species favored their extensive distribution allowing adaptation to different marine environments (Pazzaglia et al., 2021b). However, rapid environmental changes can exceed their tolerance capacity preventing appropriate responses. Seagrasses are declining globally and estimates indicate an increased meadows loss rate of 7% year^{-1} since 1990 (Waycott et al., 2009). Projections estimate the functional extinction of some seagrass species in the next decades, including *Posidonia oceanica* (L.) Delile (Chefaoui et al., 2018), which is endemic to the Mediterranean Sea and one of the largest and long-lived plant species in the world (Arnaud-Haond et al., 2012). Increased temperature trends and MHWs can negatively affect seagrass performances, accelerating respiration rates in a higher proportion than photosynthetic rates, eventually resulting in plant carbon imbalances (Collier and Waycott, 2014; Marín-Guirao et al., 2016; Nguyen et al., 2021). Species responses to heat stress are variable depending on local environmental conditions where plants grow and thus on local (pre-) adaptation (Marín-Guirao et al., 2017; Pazzaglia et al., 2020). MHWs have also the potential to affect flowering, seeds germination, seedlings development and survival thereby compromising the future of natural populations (Ruiz et al., 2018; Salo and Pedersen, 2014; Xu et al., 2016). Seedlings represent one of the most vulnerable life stages of seagrasses (Balestri et al., 2009) and are particularly sensitive to MHWs. The experimental exposure to simulated heat waves induced negative effects on growth and seed germination, increasing mortality and the occurrence of indirect effects such as herbivory (Guerrero-Meseguer et al., 2017; Hernán et al., 2017; Pereda-Briones et al., 2019). Despite these early evidences, there is a lack of research conducted on seedlings and seeds with the aim to explore the effects of warming on these early life stages and more studies are required especially for improving seagrass conservation and management practices. This is particularly relevant in the frame of restoration and reinforcement of natural populations. Using seeds or seedlings as transplant material guarantees high genetic diversity levels, and novel approaches boosting resilience to environmental changes have been proposed in seagrasses (Pazzaglia et al., 2021a). In the present era of environmental changes, seagrass restoration has the potential to slow-down habitat degradation and fragmentation mitigating the negative impacts of the ongoing climate change (Duarte et al., 2020).

In seagrasses, different studies investigated the degree of phenotypic plasticity under different abiotic stressors, including thermal stress (Nguyen et al., 2021). Besides the expression of key stress-related genes under thermal stress conditions (e.g. HSPs), seagrass's responses include also the activation of epigenetics-related genes (i.e. DNA and histone methylation, Marín-Guirao et al., 2017; Marín-Guirao et al., 2019). The methylome assessment of adult *Zostera marina* genets has underlined the existing relation of DNA methylation changes with phenotypic variation of fitness-related traits and heat stress resilience (Jueterbock et al., 2020). Moreover, a recent study has pointed to a relationship between gene body DNA methylation and the transcriptomic responsiveness of Mediterranean seagrasses to warming conditions, together with warming-induced changes in the level of global DNA methylation (Entrambasaguas et al., 2021). These evidences revealed the flexibility of the methylome in response to heat stress and the possibility of marine plants for memorizing heat responses. Only very recently in seagrasses, thermo-priming has been successfully tested in adult plants of two species (*P. australis* and *Z. muelleri*) and the activation of key epigenetic-related genes seems to be involved in the process (Nguyen et al., 2020). Nevertheless, more studies are necessary to assess the potential role of epigenetic mechanisms in seagrass responses and stress-memory.

Here we applied, for the first time in seagrass seedlings, a thermo-priming stimulus to *P. oceanica* through the exposure of seedlings to an anomalous warming event (priming treatment: 30.5 °C). The induction of the priming status was subsequently assessed after two weeks by analyzing the photo-physiological and growth performance of primed and non-primed seedlings, as well as their gene expression responses of a selected set of genes (i.e. stress-, photosynthesis- and epigenetics-related genes) during their exposure to extreme high temperature (triggering treatment: 32 °C). The hypothesis is that young *P. oceanica* seedlings

experiencing a seawater warming event during their first summer (thermo-primed seedlings) are better equipped to respond and resist to a subsequent more intense and longer-lasting warming event than seedlings grown under normal/average summer temperatures (non-primed seedlings).

2. Methods

2.1. Seedlings collection and experimental design

Beach-casted *P. oceanica* seeds were collected in June 2019 along the coasts of Marsala (West Sicily), where one of the largest *P. oceanica* meadow of the western Mediterranean Sea is located. Seeds were germinated and grown at Torretta Granitola/C.N.R. laboratory (N/W Sicily), during early- and mid-summer, in two circular outdoor tanks (2.5 m diameter; 4000 l) with flow-through natural seawater (ca. 22 l min^{-1}) drawn from a well. During this period, seedlings were exposed to irradiance levels ranging from 50 to 80 (μmol m^{-2} s^{-1}) by shading tanks with neutral screens to mimic the irradiance levels existing inside natural *P. oceanica* meadows in the region at 8–10 m depth.

In late summer (mid-September), seedlings were shipped by plane in thermos flasks with clean moist paper to the Oceanographic Center of Murcia (Spain) within about 12 hour time. Upon arrival, seedlings were immediately transplanted in individual small seed pots (5 × 5 × 6 cm) filled with coarse gravel (2.5 cm) (Fig. 1). Subsequently, they were allocated randomly into nine tanks of an indoor mesocosms facility, where temperature was adjusted to 24.5 °C according to the natural summer temperature present in the sampling region (SST was on average 24 °C and 26 °C in July and August, respectively). Tanks were filled with natural seawater from an oligotrophic, unpolluted area. Each tank was equipped with its own circuit of seawater, temperature and irradiance (see Marín-Guirao et al., 2018; Marín-Guirao et al., 2013 for a complete description of the system). The system allows for an accurate control of the water temperature in tanks (±0.2 °C) which was checked daily during the experiment by using a handheld mercury thermometer. Salinity was also checked daily and maintained constant at 37.5 (±0.2) by adding purified fresh water to compensate for evaporation. Seawater quality was maintained throughout the experiment by continuous physical and chemical filtration and weekly partial (30–40%) water renewal. Irradiance in tanks was adjusted to 70 μmol quanta m^{-2} s^{-1} with a 12 h:12 h light:dark photoperiod according to the daily photosynthetic photon flux density measured within natural *P. oceanica* meadows (Marín-Guirao et al., 2015). Temperature in all tanks was progressively increased (0.3 °C/day) from 24.5 °C to 26 °C, and seedlings were allowed to acclimate for ten days. After the acclimation period, temperature in three tanks (n = 3) was progressively increased (0.5 °C/day) up to 30.5 °C to induce a priming stimulus, while the remaining of tanks were kept under control temperature. Previous studies have shown that this temperature level caused heat stress to 3–5 months old *P. oceanica* seedlings from different locations of the western Mediterranean Sea (Guerrero-Meseguer et al., 2017; Hernán et al., 2017; Pereda-Briones et al., 2019). According to previous experiments conducted on seedlings (Guerrero-Meseguer et al., 2020, 2017), this priming treatment lasted a total of 11 days, after which the temperature was progressively lowered to the control level of 26 °C (1 °C/day). Seedlings were kept at control temperature (26 °C) for two weeks. After this period, seedlings from the three priming tanks and from three non-priming tanks were exposed to triggering treatment (i.e. extreme warming event), while the other three non-primed tanks continued growing under control temperature. The triggering treatment was applied by increasing temperature up to 32 °C (0.5 °C/day) and lasted a total of 2 weeks since the beginning of temperature ramping. The response of primed (P), non-primed (NP) and control (C) seedlings, and thus all results showed below, was studied at the end of the triggering treatment, before returning temperature to control conditions (Fig. 1). Measurements performed on seedlings from the same tank (i.e. 'pseudo replicates') were averaged to obtain an independent replicated value since the experimental tank is the true experimental unit in our experiment. Therefore, the number of replicates used in statistical tests was n = 3.

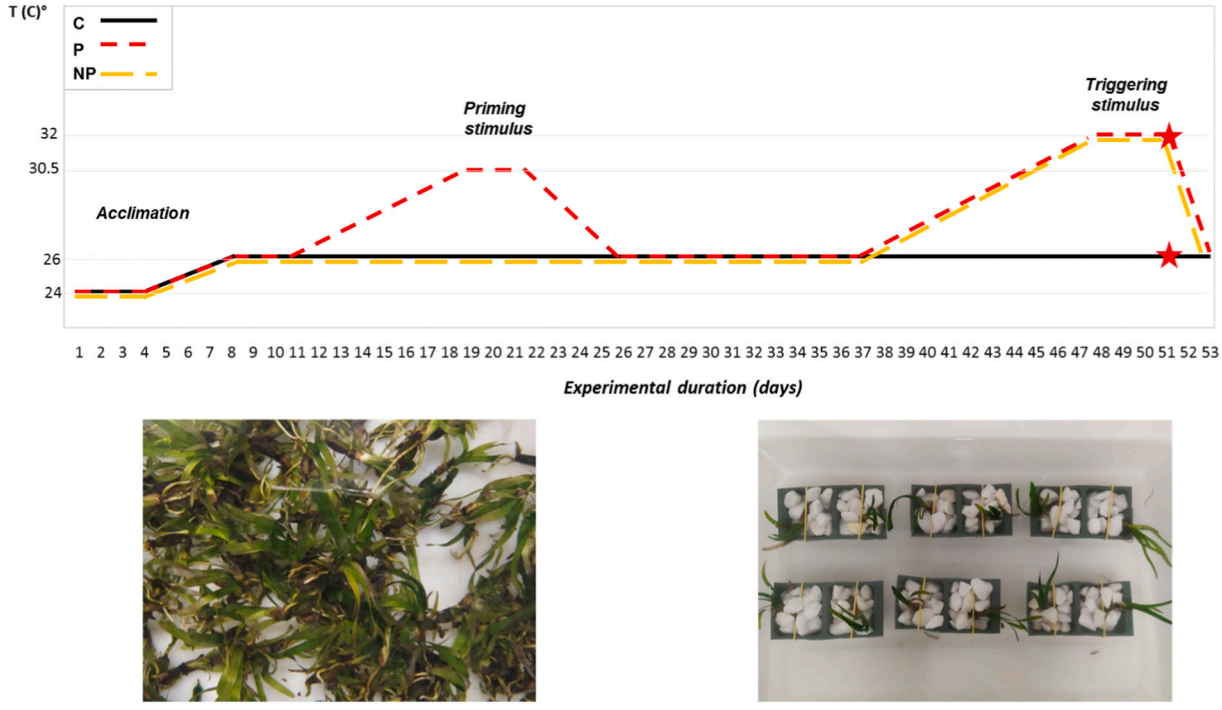

Fig. 1. Experimental design. Seawater temperature during the course of the experiment (above panel). Black line refers to control (C), dashed red line refers to primed seedlings (P), and dashed yellow line refers to non-primed seedlings (NP), while red star refer to sampling point. Pictures of 4-month old *P. oceanica* seedlings upon arriving to the IEO mesocosm facility (lower-left panel) and after their transplantation and allocation in an experimental tank (lower-right panel). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
List of housekeeping genes and genes of interest analyzed in this study.

Gene category	Gene	Protein	Forward sequence (5 → 3)	Reverse sequence (3 → 5)	S	E (%)	R2	Reference
Housekeeping genes	GADPH	Glyceraldehyde-3-phosphate dehydrogenase	AGGTTCTTCCTGCTTTGAATG	CTTCCTTGATTGCTGCCTTG	138	93	0.99	Serra et al., 2012
	18S	Ribosomal RNA 18S	AACGAGACCTCAGCCTGCTA	AAGATTACCCAAGCCTGTCG	200	100	0.99	Serra et al., 2012
	elF4A	Eukaryotic initiation factor 4A	TTCTGCAAGGGTCTTGACGT	TCACACCCAAGTAGTCACCAAG	192	85	0.99	Lauritano et al., 2015
Stress-related genes	HSP90	Heat shock protein 90	CTCCATCTTGCTTCCCTCAG	TCAGTTTGGAGGAACCGAAC	146	100	0.99	Lauritano et al., 2015
	SHSP	Small heat shock protein	ACCGGAGGATGTGAAGATTG	AGCTTGCTGGACAAGGTGAT	125	99	0.99	Lauritano et al., 2015
	AOX	Alternative oxidase 1a	TGCTGCATTGCAAGTCTCTAC	GTTGTGACACCTCCATGAAGGTC	116	100	0.99	Procaccini et., 2017
	MSD	Manganese superoxide dismutase	GGCGGAGGTCATATAAACCA	ATAAGCAAGCCACACCCATC	192	0.93	0.99	Lauritano et al., 2015
	DDB	Damaged DNA binding protein	TCTCAGGTCCGGCACTAATC	GAAAGGCTTGCTCGTATTGC	224	100	0.99	Lauritano et al., 2015
Photosynthesys-related genes	CAB-151	Chlorophyll a-b binding protein 151, chloroplastic	AAGCCCATTAGCACAACCTG	GGGCAATGCTTGGTACTCTC	199	93	0.99	Dattolo et al., 2014
	POR	Protochlorophyllide reductase	AGTTCCACAGACGGTTCCAC	AATCACCACCTGAGCGAGTC	194	98	0.99	Ruocco et al., 2018
	FD	Ferredoxin-1, chloroplastic	TCAGACTGGGGGTAAGCAAC	TCTACATCCTCGACCACTGC	187	100	0.98	Dattolo et al., 2014
	psbA	Photosystem II protein D1	GACTGCAATTTTAGAGAGACGC	CAGAAGTTGCAGTCAATAAGGTAG	137	92	0.99	Dattolo et al., 2014
	PSBS	Photosystem II 22 kDa protein, chloroplastic	CCGCTCCTGTTGTTCTTCAT	GGACCTCCTTCCTTGAGACC	158	100	0.99	Dattolo et al., 2014
Epigenetic-related genes	ATX2	Histone-lysine *N*-methyltransferase ATX2	CCAGATACAAAGCTGCACCA	GCATTGTCATCCCCTTGAGT	170	94	0.99	This study
	ATRX7	Histone-lysine *N*-methyltransferase ATXR7 isoform X1	CGAGTAGGGTCGAATGTGGT	ATCCATCCAGTCACACACGA	149	95	0.98	This study
	ASH2L	Ash2 histone methyl transferase complex subunit ash-2	CTATCCTGCTGCCTCCATGT	TCAACTGCACCTTCAACTCG	170	94	0.99	This study
	SETD3	Histone-lysine *N*-methyltransferase setd3	TGGGCTTGRGAACTGTGGTA	CGAATGATTGAGTCGTCCAG	200	99	0.99	This study
	DME	Transcriptional activator DEMETER	CAACTGTTCCCCTCACTGGT	CCACAGGTTCAGGTTCTGGT	162	94	0.99	This study

Table 2
Output of the PERMANOVA analysis carried out on photo-physiological and gene expression data obtained from different treatments (C = control, P = primed, NP = non-primed). Df = degrees of freedom; MS = mean square; Pseudo-F=F statistic; P (MC) = probability levels obtained from Monte Carlo asymptotic distributions.

PERMANOVA				
Source of variation	Df	MS	Pseudo-*F*	P (MC)
Treatment	2	202,550	13.351	**0.005**
Residuals	6	15,171		
Total	8			

Pairwise test	*T*	P (MC)
C, P	2.6887	**0.048**
C, NP	4.7513	**0.008**
P, NP	2.9555	**0.040**

Values in bold indicate significant differences (P (MC) < 0.05).

2.2. Chlorophyll a fluorescence

Measurements of chlorophyll-fluorescence emissions were performed on four seedlings per tank using a pulse amplitude modulation portable fluorometer (diving-PAM; Walz, Germany). The PAM measurements were performed at the beginning of the experimental treatments and at the end of the triggering stimulus to determine the physiological status of seedlings. The light saturation pulse method was used to characterize the performance of the photosynthetic apparatus at the level of photosystem II (PSII). Measurements of basal (F_0) and maximum (F_m) fluorescence were conducted on whole-night dark-adapted seedlings to calculate the maximum photochemical efficiency of PSII ($F_v/F_m = F_m - F_0 / F_m$). The method was applied again in the same seedlings after 5 h of illumination in aquaria to determine the basal (F) and maximum (F_m') fluorescence of light-adapted leaves in order to calculate the effective photochemical efficiency of PSII ($\Delta F/F_m' = F_m' - F / F_m'$).

2.3. Photosynthetic and respiratory rates

Determination of the maximum photosynthetic and respiratory rates was carried out on two seedlings from each tank using an incubation chamber with a Clark-type O_2 electrode (Hansatech, UK) connected to a controlled temperature-circulating bath. From each seedling, a 2 cm long leaf segment, taken from the middle part of the first mature leaf, was used in incubations. Leaf segments were first incubated in darkness for 10 min to determine dark respiration rates (R_d) and then exposed to six increasing irradiances (from 10 to 500 μmol quanta m^{-2} s^{-1}) to determine maximum photosynthetic rates (net-P_{max}). Gross photosynthesis (gross-P_{max}) was then calculated as the sum of net-P_{max} and R_d, and the ratio of gross-P_{max}:R_d was used as a proxy of the leaf metabolic carbon balance. The gross photosynthetic rates were also calculated using the irradiance in the tanks (70 μmol quanta m^{-2} s^{-1}; results are provided in Supplementary Fig. S1).

2.4. Leaf pigments content

Leaf pigment content (chlorophyll *a*, chlorophyll *b* and total carotenoids) was analyzed in the same leaf segments used in photosynthetic

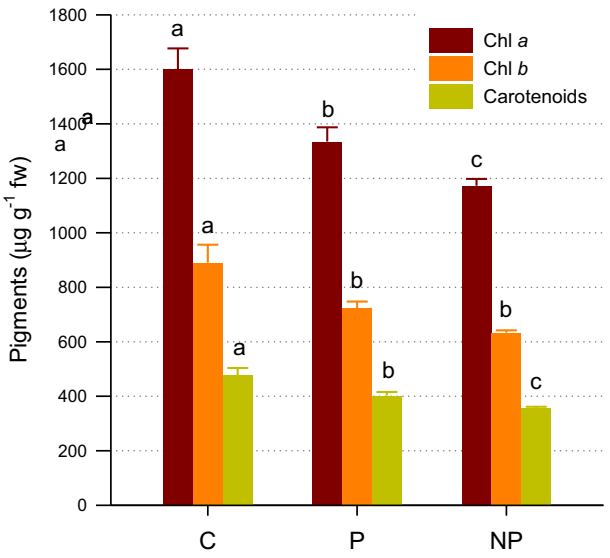

Fig. 2. Pigment responses. Pigments content in leaves of control (C), primed (P) and non-primed (NP) *P. oceanica* seedlings at the end of the triggering treatment. Letters denote significant differences with respect to controls as derived from the Newman-Keuls post-hoc test.

and respiratory measurements. Leaf pigments were extracted from each leaf segment in 10 ml of 80% acetone and examined spectrophotometrically at 470, 646, and 663 nm. The concentration of chlorophyll *a* and *b* along with total carotenoids was calculated from the readings using the equations of Lichtenthaler and Wellburn (1983).

2.5. Seedlings growth and morphology

Seedlings growth was determined by using the punch-hole technique (Zieman, 1974). The leaves of three seedlings from each tank were marked with a needle at the height of the ligule before the beginning of the triggering treatment. Marked seedlings were collected two weeks later at the end of the warming period (triggering treatment) and the surface area of newly formed leaf tissues (i.e. those below the mark) was measured to estimate leaf growth (cm^2 day^{-1}). Seedlings size (i.e. leaf surface area; cm^2) was also characterized by measuring the length and width of all leaves form each marked seedling.

2.6. Gene expression analysis

Total leaf tissue of seedlings was collected per each condition in triplicates to perform gene expression analysis. Samples were cleaned from epiphytes and entirely submerged in RNAlater© (Ambion, life technologies), stored overnight at 4 °C to let the solution penetrate into the tissue and finally stowed at −20 °C until RNA extraction. Total RNA was extracted with Aurum™ Total RNA Mini Kit (BIO-RAD) following manufacturer's protocol. Purity and concentration of RNA (absence of DNA and protein contaminations) were checked using the NanoDrop® ND-1000 Spectrophotometer (Thermo Fisher Scientific) and 1.5% agarose gel electrophoresis. RNA concentration used in this experiment ranged from 100 to 250 ng/µl with a ratio of the absorbance at 260 and 280 nm (A260/280) ~ 2. Subsequently, 500 ng of RNA from each sample and condition was retro-transcribed into cDNA with the iScript™ cDNA synthesis kit (BIO-RAD), according to manufacturer's instructions.

2.7. Quantitative real-time PCR (RT-qPCR) on genes of interest (GOIs)

Primers for 15 genes of interest (GOIs) were selected from previous studies or designed based on *P. oceanica* transcriptomes (D'Esposito et al., 2017; Marín-Guirao et al., 2017) with the primer analysis software Primer3 v. 0.4.0 (Koressaar and Remm, 2007; Untergasser et al., 2012). In detail, 10 GOIs were selected from previous studies according to specific functional categories including stress-related and photosynthesis-related genes. General-stress response was assessed targeting heat shock genes (HSP90 and SHSP), key gene involved in mitochondrial energy dissipation mechanisms (AOX), antioxidant response (MSD) and DNA repair response (DDB) (Table 1). A number of genes involved in light reaction functions of photosynthesis (psbA, PSBS, FD), chlorophyll *a-b* binding proteins (CAB-151), and a key enzyme involved in the chlorophyll biosynthetic pathways (POR) were also targeted. Epigenetics-related genes with specific methylation activities (ATX2, ATRX7, ASHL2, SETD3) and the Transcriptional activator-DEMETER, which catalyzes the release of 5-methylcytosine (5-meC) from DNA, were designed setting the primer length to 18-20 bp, product size to 100–200 bp and Tm = 59–61 °C. Three reference genes (18S, elf4A and GADPH) were selected and used to normalize gene expression of target genes according to previous related works based on plant response to thermal stress conditions or abiotic stresses (Serra et al., 2012; Dattolo et al., 2014; Lauritano et al., 2015). The best reference genes showing the higher stability values for our experimental conditions were identify by using the web-based tool RefFinder integrating major computational programs (geNorm, Normfinder, BestKeeper, and

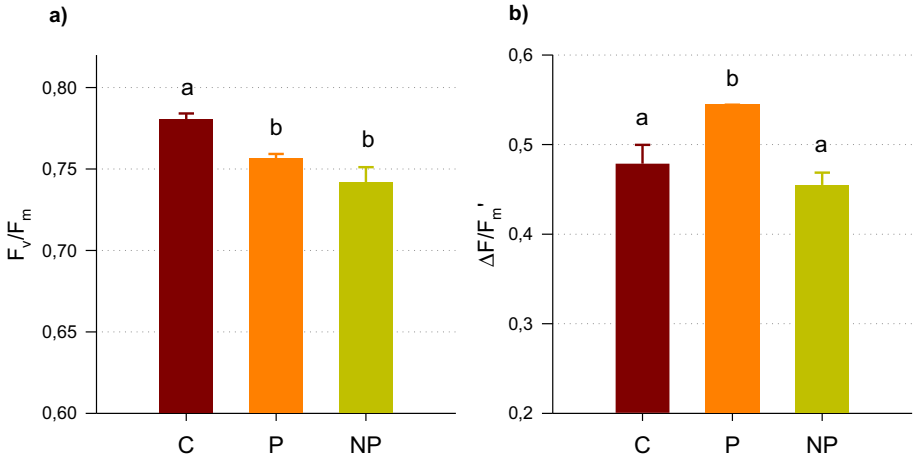

Fig. 3. Photochemical responses. Maximum (F_v/F_m; a) and effective quantum yield ($\Delta F/F_m'$; b) of control (C) primed (P) and non-primed (NP) *P. oceanica* seedlings at the end of the triggering treatment. Different letters indicate significant differences obtained in the post hoc Newman-Keuls test ($P < 0.05$).

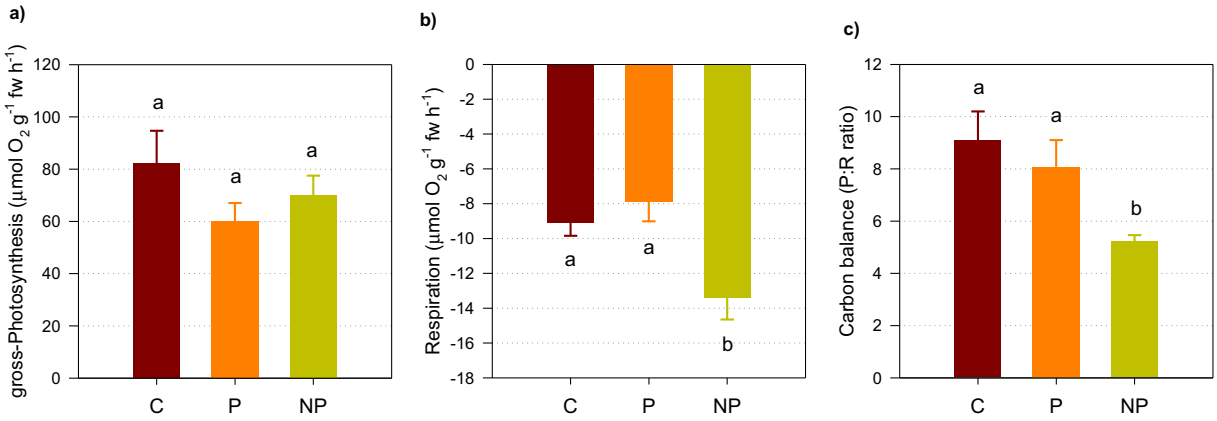

Fig. 4. Photosynthetic and respiratory responses. Gross-Photosynthesis (a), respiration (b) and carbon balance (c) of control (C), primed (P) and non-primed (NP) *P. oceanica* seedlings at the end of the triggering treatment. Different letters indicate significant differences obtained in the post hoc analysis once significant effects were detected in the ANOVA analysis.

the comparative Delta-Ct method; Xie et al., 2012). Primers efficiency was assessed with different cDNA dilutions and using a linear regression model to calculate the percentage of efficiency as follows: E (%) = (10^{-1} / slope^{-1}) × 100 (Radonić et al., 2004; Table 1). Primers with efficiencies (E) within the range 90–110% and correlation coefficient > 0.95 were used in the study (Table 1). RT-qPCR reactions were performed in triplicates in a Viia7 Real Time PCR System (Applied Biosystems) using Fast SYBR®Green MasterMix (Applied Biosystems) as fluorescent detection chemistry and MicroAmp Optical 384-well reaction plates (Applied Biosystems). Reactions were carried out in a 10 µl final volume with 5 µl MM SYBR ® Green, 2 µl of 1.4 pmol µl^{-1} primers and 1 µl of 1:50 cDNA template. The thermal profile of the reactions was as follows: 95 °C for 20 s, 40 times 95 °C for 1 s and 60 °C for 20 s. Relative quantification of gene expression was obtained using the following equations.

$$\Delta CT = CT\text{reference gene} - CTGOIs$$

to evaluate the negative differences in cycles to cross the threshold value between the reference and the target GOI (-ΔCT). Subsequently, ΔΔCT were calculated on ΔCT means for each GOI by comparing ΔCT of treatments (2HW and 1HW) with the control (C). The Fold change expression was assessed according the following equation:

$$\text{Fold expression change} = +2(|(\Delta\Delta CT\text{treatment}) - (\Delta\Delta CT\text{control})|)$$

2.8. Statistical analysis

One-way analysis of variance (ANOVA) was conducted to detect significant differences in the response to the simulated MHW among experimental *P. oceanica* seedlings (i.e. primed, non-primed and control seedlings). Before carrying out ANOVA analyses, Shapiro–Wilk and Levene's tests were applied to assess the normality and homoscedasticity of the data and transformed where necessary. Subsequently, Student-Newman-Keuls (SNK) post hoc test was used whenever significant differences ($P < 0.05$) among treatments were detected using the statistical package STATISTICA (StatSoft, Inc., v. 10). Photo-physiological and gene expression results of GOIs were also analyzed using Permutational Multivariate Analyses of Variance (PERMANOVA) that were carried out on Euclidean distances of data, using 9999 permutations of the residuals under a reduced model. Significant differences were investigated using a posteriori pair-wise test. P values in the PERMANOVA and pairwise tests were obtained from Monte Carlo asymptotic distributions, because of the restricted number of unique permutations. The analysis was performed using Primer 6 v.6.1.16 and PERMANOVA + v.1.0.6 software package (PRIMER-E Ltd) (Anderson et al., 2008). Data is presented as average values ± standard error (n = 3).

3. Results

The multivariate analysis (PERMANOVA) showed that the overall photo-physiological and gene expression responses of non-primed seedlings (NP) significantly differed from the response of control (P (MC) = 0.008) and thermo-primed seedlings (P (MC) = 0.040) (Table 2). The overall response of the latter was also significantly different from C (P (MC) = 0.048), though with the weakest significance value.

Effects of heat priming on leaf pigment responses and photochemical responses to warming.

The triggering treatment significantly affected chlorophylls (Chl *a* and Chl *b*) and total carotenoids content of *P. oceanica* seedlings (Table 2). All analyzed pigments showed a generalized reduction after the warming exposure, being stronger and more significant in non-primed seedlings (NP) with respect to primed (P) seedlings (Fig. 2; Table 2). In fact, the percentage of reduction of Chl *a*, Chl *b* and total carotenoids against controls was respectively of 16.6, 18.6 and 15.5% in P seedlings, while the corresponding values for NP seedlings were of 26.7, 28.9 and 25.1%, respectively.

The physiological status of plants determined via chlorophyll *a* fluorescence before the priming stimulus revealed that seedlings were acclimated to the experimental system (Supplementary Table S1). At the end of the triggering treatment, the maximum photochemical efficiency of PSII (Fv/Fm) of P and NP seedlings was significantly lower with respect to controls (Fig. 3a; Table 2). However, primed seedlings showed 14% and 20% greater effective photochemical efficiency (light-adapted $\Delta F/F_m'$) than controls (P = 0.019) and non-primed seedlings (P = 0.011), respectively (Fig. 3b).

3.1. Effect of heat priming on photosynthetic and respiratory responses to warming

Photosynthesis-irradiance curves exhibited by treatments (C, P and NP) are showed in the Supplementary Fig. S2. The exposure to an extreme warming event, the triggering treatment, did not significantly affect the maximum gross-photosynthetic rates of *P. oceanica* seedlings, although the average rates of primed (P) and non-primed (NP) seedlings were respectively 27% and 15% lower than controls (gross-P_{max}; Fig. 4a; Table 3). This triggering treatment significantly increased the respiratory rates of NP seedlings by 48% ($P < 0.001$) while, on the contrary, the respiration of P seedlings was reduced by 13%, although not significantly different from control (R_d; Fig. 4b; Table 3). As a consequence of the above responses, C and P seedlings showed a similar leaf carbon balance, whereas the carbon balance of NP seedlings was 43% and 35% lower than C and P seedlings, respectively (P:R_d ratio; Fig. 4c; Table 3).

Table 3
Results of one-way ANOVA analysis for factor "Treatment" (T) for leaf growth rate, leaf surface area, maximum quantum yield (Fv/Fm), effective quantum yield ($\Delta F/F_m$), chlorophyll *a* (Chl a), chlorophyll *b* (Chl b), carotenoids, dark respiration rate (Rd), gross maximal photosynthesis (Gross-Pmax) and carbon balance (P:R).

One-way ANOVA						
Variable	Factor	df	MS	F	P	SNK pairwise tests
Relative growth (biomass)	Treatment (T)	2	0.00	3.87	0.083	
	Error	6	0.00			
Leaf surface area	Treatment (T)	2	15.33	2.83	0.137	
	Error	6	5.42			
F0	**Treatment (T)**	2	2211.47	9.96	**0.012**	**P, NP ≠ C (P < 0.05)**
	Error	6	222.06			P = NP (P > 0.05)
Fm	Treatment (T)	2	4400.06	3.81	0.086	
	Error	6	1155.00			
Fv/Fm	**Treatment (T)**	2	0.00	10.65	**0.011**	**P, NP ≠ C (P < 0.05)**
	Error	6	0.00			P = NP (P > 0.05)
DF/Fm′	**Treatment (T)**	2	0.01	9800.00	**0.013**	**P ≠ C, NP (P < 0.05)**
	Error	6	0.00			
ETRmax	Treatment (T)	2	4.45	3.65	0.092	
	Error	6	1.22			
NPQ	Treatment (T)	2	0.21	4.47	0.065	
	Error	6	0.05			
Chl *a*	**Treatment (T)**	2	140,159.62	15.41	**0.004**	**P, NP ≠ C (P < 0.05)**
	Error	6	9094.68			P = NP (P > 0.05)
Chl *b*	**Treatment (T)**	2	50,991.87	10.27	**0.012**	**P, NP ≠ C (P < 0.05)**
	Error	6	4963.11			P = NP (P > 0.05)
Carotenoids	**Treatment (T)**	2	10,915.02	11.43	**0.009**	**P, NP ≠ C (P < 0.05)**
	Error	6	954.93			P = NP (P > 0.05)
Rd	**Treatment (T)**	2	25.48	15.62	**0.004**	**NP ≠ C, P (P < 0.01)**
	Error	6	1.63			
Gross-Pmax	Treatment (T)	2	374.42	2.64	0.151	
	Error	6	141.93			
P:R	**Treatment (T)**	2	12.05	11.25	**0.009**	**P, NP ≠ C (P < 0.05)**
	Error	6	1.07			P = NP (P > 0.05)

Values in bold indicate significant differences (P < 0.05).

3.2. Effect of heat priming on seedlings growth and seedlings size

The warming exposure significantly affected leaf growth rates of *P. oceanica* seedlings (Fig. 5a; Table 3). Primed seedlings (P) showed 27% higher growth rates than control seedlings (P = 0.034), whereas the rates of non-primed seedlings (NP) were similar to controls (P = 0.545). At the end of the simulated MHW the leaf surface area of P seedlings was 21% and 24% higher than C and NP, although the differences were not statistically significant (Fig. 5b; Table 3).

3.3. Gene expression responses

Among 15 selected GOIs, nine showed significant fold expression changes, especially those included in the stress-related and epigenetics categories. Expression level of stress-related genes was significantly affected by the triggering treatment. Specifically, heat shock proteins (HSP90 and SHSP) were significantly over-expressed in both P and NP (Table 4; Fig. 6) in respect to control conditions. Despite no statistical differences were observed between P and NP, the former showed twice the expression level of the latter. The Alternative oxidase 1a (AOX) increased its level of expression up to 10-fold in both P and NP, with particular relevance in NP. Photosynthesis-related genes were over-expressed in P and NP, but significant differences were observed only for Ferredoxin-1 (FD) in P. Interestingly, photosynthetic pigment-related genes (CAB-151 and POR) followed an opposite regulation in P respect to NP, even if this was not supported by a statistical significance.

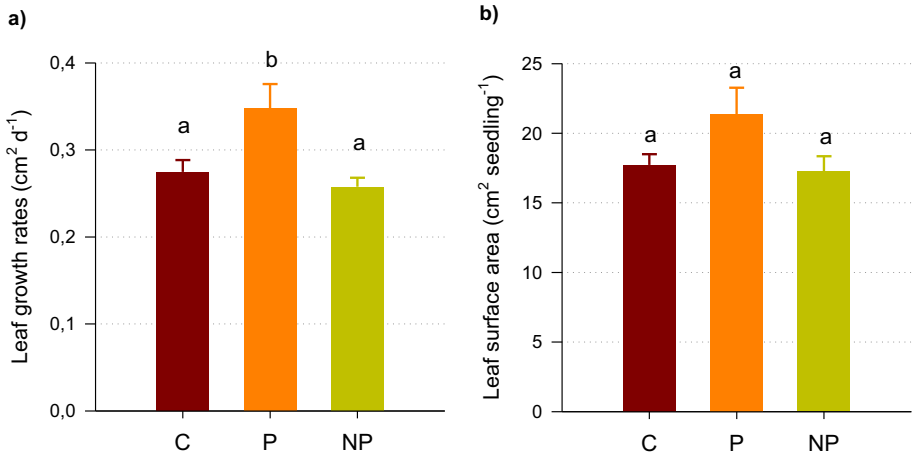

Fig. 5. Morphological responses. Leaf growth rates (a) and leaf surface area (b) of control (C), primed (P) and non-primed (NP) *P. oceanica* seedlings at the end of the triggering treatment. Different letters indicate significant differences (P < 0.05) from Newman-Keuls post hoc test once significant effects were detected in the ANOVA analysis.

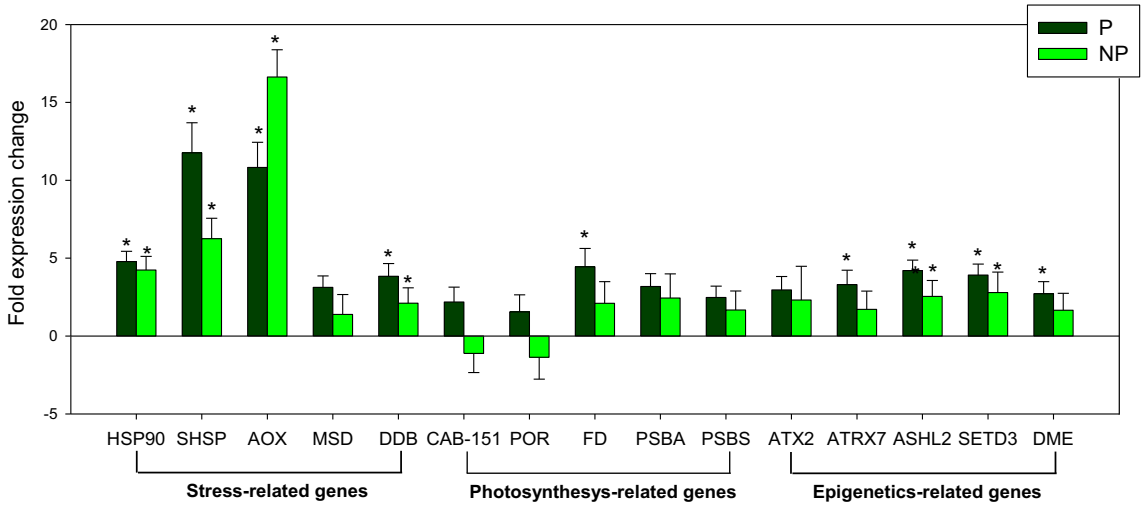

Fig. 6. Gene expression. Relative expression of GOIs selected for stress-related category (HSP90, SHSP, AOX, MSD, DDB), photosynthesis category (CAB-151, POR, FD, psbA, PSBS) and epigenetic category (ATX2, ATRX7, ASHL2, SETD3, DME) in primed (P) and not-primed (NP) seedlings vs. control conditions (x-axis) at the end of the triggering treatment. Asterisks indicate post hoc significant differences of the treatment respect to the control.

Epigenetics-related genes were all up-regulated in both P and NP, where ASHL2 and SETD3 genes were commonly over expressed among treatments, contrary to ATRX7 and DME genes that showed significantly expression levels only for P seedlings. Overall, epigenetics-related genes showed a higher activation in P seedlings without significant differences among treatments.

4. Discussion

Findings from this study provide experimental evidences about the potential of *P. oceanica* seedlings to acquire a thermo-primed status that eventually confers an enhanced tolerance and resistance to an extreme warming event. Thermo-primed seedlings performed better during the

Table 4
Results of one-way ANOVA analysis conducted on −ΔCT values for primed (P) and not-primed (NP) seedlings. Significant factors and values are in bold.

One-way ANOVA							
Gene category	Variable	Factor	df	MS	F	P	SNK pairwise tests
Stress-related genes	*HSP90*	**T**	2	4.74	37.77	**0.00**	**NP ≠ C (P < 0.01)**
		Error	6	0.13			**P ≠ C (P < 0.01)**
							P = NP (P > 0.05)
	SHSP	**T**	2	10.23	6.98	**0.03**	**NP ≠ C (P < 0.05)**
		Error	6	1.47			**P ≠ C (P < 0.05)**
							P = NP (P > 0.05)
	AOX	**T**	2	14.32	12.89	**0.01**	**NP ≠ C (P < 0.01)**
		Error	6	1.11			**P ≠ C (P < 0.01)**
							P = NP (P > 0.05)
	MSD	T	2	2.15	4.83	0.06	
		Error	6	0.44			
	DDB	**T**	2	2.83	14.06	**0.01**	**NP ≠ C (P < 0.05)**
		Error	6	0.20			**P ≠ C (P < 0.01)**
							P = NP (P > 0.05)
Photosynthesis-related genes	*CAB-151*	T	2	1.49	3.68	0.09	
		Error	6	0.40			
	POR	T	2	0.89	1.44	0.31	
		Error	6	0.62			
	FD	**T**	2	3.47	5.80	**0.04**	NP = C (P > 0.05)
		Error	6	0.60			**P ≠ C (P < 0.05)**
							P = NP (P > 0.05)
	psbA	T	2	2.30	3.27	0.11	
		Error	6	0.70			
	PSBS	T	2	1.29	3.15	0.12	
		Error	6	0.41			
Epigenetics-related genes	*ATX2*	T	2	2.02	1.65	0.27	
		Error	6	1.22			
	ATRX7	**T**	2	2.23	6.46	**0.03**	**P ≠ C (P < 0.05)**
		Error	6	0.35			NP = C (P > 0.05)
							P = NP (P > 0.05)
	ASHL2	**T**	2	3.32	14.23	**0.01**	**NP ≠ C (P < 0.05)**
		Error	6	0.23			**P ≠ C (P < 0.01)**
							P = NP (P > 0.05)
	SETD3	**T**	2	3.16	6.69	**0.03**	**NP ≠ C (P < 0.05)**
		Error	6	0.47			**P ≠ C (P < 0.05)**
							P = NP (P > 0.05)
	DME	**T**	2	1.56	5.07	**0.05**	**P ≠ C (P < 0.05)**
		Error	6	0.31			P = NP (P > 0.05)

re-occurring heat stress event than non-primed seedlings and offered some insights into the molecular basis of thermo-priming in seagrass seedlings. During the triggering stimulus (i.e. the second exposure to high temperatures), these seedlings experienced lower thermal pigment degradation than non-primed seedlings, kept their carbon balance unaltered through a complete respiratory homeostasis and increased their growth rates leading to larger seedlings. In addition, the altered expression levels of epigenetic-related genes pointed to the potential involvement of chromatin remodeling processes as the basis of the acquired primed status revealing that early life stages of seagrasses may have the potential for long-term storage of stress responses.

In this study, the maximum quantum yield (Fv/Fm) of both P and NP experienced a significant reduction during the trigging stimulus confirming that 32 °C is a stressful temperature for *P. oceanica* seedlings, likely close to the lethal temperature limit of the species (Guerrero-Meseguer et al., 2020; Hernán et al., 2017; Olsen et al., 2012; Pereda-Briones et al., 2019). Interestingly, the effective photochemical capacity ($\Delta F/Fm'$) of thermo-primed seedlings (P) was higher than controls and NP seedlings indicating an improved capacity to move electrons along the photosynthetic electron transport chain (ETC). These seedlings, contrarily to NP, increased the expression level of Ferredoxin (FD) which is a key gene of the chloroplast electron transport chain encoding for the photosynthetic electron carrier, and thus, likely responsible of the observed photochemical enhancement. FD plays an important role in the final step of the linear electron flow, thanks to its ability to divert electrons to cyclic or alternative electron flow pathways, sustaining photosynthesis and minimizing damaging ROS production (Munekage et al., 2004); although it can alternatively increase ROS production by transferring electrons to oxygen through the Mehler reaction or the Water-Water cycle (Foyer and Noctor, 2000; Asada, 2006). In *P. oceanica* adult plants, thermal stress affects the regulation of ROS scavengers as a response to protect cells from the potential oxidative damage caused by heat-induced ROS production (Traboni et al., 2018; Tutar et al., 2017).

Here, despite the enhanced photochemistry, P seedlings showed lower photosynthetic capacity (O_2 production), although not significantly, suggesting the lack of acclimation in photosynthetic carbon fixation to imposed warming conditions. The inconsistency between the two photosynthetic parameters (i.e. photochemical efficiency and photosynthetic capacity) can be due to the potential activation/deactivation of alternative electron transport pathways (e.g., photorespiration, Water–Water cycle, cyclic electron transport; Niyogi, 2000) under stressful conditions, as already described in seagrasses under different abiotic stress conditions (e.g. Dattolo et al., 2017; Marín-Guirao et al., 2013; Silva et al., 2013). Alternatively, it might be related to changes in leaf absorbance, which can be promoted by changes in leaf pigment content and leaf morphology (Enríquez, 2005). During the exposure to anomalous high temperatures, thermo-primed seedlings experienced a lower generalized pigment degradation than non-primed seedlings, evidencing an improved tolerance to warming. We cannot determine whether this response is or not the result of de novo synthesis of thermally stable isoforms of proteins during the warming exposure (Somero, 1995). In fact, only primed seedlings activated genes (although not significantly) for the synthesis of photosynthetic pigments. Although the expression of pigment-related genes was not statistically significant, it could be reflecting a stronger activation during the early responses to warming. Significant changes in protein abundance through gene expression requires time, as the increase in protein abundance of the thermally stable isoform can take several days since the strong activation of the related gene (Degen et al., 2021).

The contrasting ability to regulate respiration under increased temperatures between thermo-primed and unprimed seedlings pointed to a lower heat-sensitivity in the former (Marín-Guirao et al. 2016, 2018). This evidence was also supported by the complete respiratory homeostasis achieved by these seedlings under warmed waters. Respiratory homeostasis is a functional trait associated with the tolerance to heat in seagrasses, as previously shown for *P. oceanica* and other seagrass species (e.g. Collier et al., 2011; Marín-Guirao et al., 2016, 2018). Through this metabolic acclimative response P seedlings were able to maintain the carbon balance unaltered (i.e. photosynthetic to respiratory ratio) under warming conditions, allowing the availability of fixed carbon for primary plant processes, such as growth and carbon storage. Carbon storage is a key process for the species survival since the ability of plants for overwintering tightly depends on the energy reserves stored during the summer growing season (Alcoverro et al., 2001). The evidence that P seedlings performed better during the triggering stimulus than NP seedlings was clearly reflected by their higher growth rates that ultimately led to larger seedlings. As demonstrated for terrestrial plants, one of the main advantages for inducing a priming status is the activation of thermo-tolerance mechanisms allowing the generation of more productive and larger individuals able to better cope with stressful conditions (*Triticum aestivum*, Wang et al., 2014). In contrast, non-primed seedlings experienced a dramatic carbon balance reduction driven mainly by an increased respiratory activity. However, they grew similarly to controls and attained also a similar size. This result suggests that seedling growth was sustained by the mobilization of carbohydrate reserves stored on seeds, which constitute a functional part of seedlings for several months, making young seedlings relatively independent from external conditions (Celdrán and Marín, 2013). Moreover, since the photosynthetic activity of seeds also contribute to seedlings growth and their metabolic (respiratory) activity varies with environmental changes (e.g. Temperature; Celdrán and Marín, 2011), it would be interesting to also study seed responses when exploring thermo-priming strategies in seagrass seedlings. An accelerated respiratory metabolism leads to excessive production of ROS causing progressive oxidative damage and ultimately cell death (Mittler et al., 2004). The strong activation in heated seedlings of the alternative oxidase (AOX) pathway of the mitochondrial ETC can be interpreted as a metabolic response for alleviating ROS production. AOX activity appears to increase under stressful conditions that cause oxidative stress, including heat stress (Del-Saz et al., 2018; Saha et al., 2016), and helps to dissipate excessive reducing equivalents and limit respiratory ROS production (Scafaro et al., 2021); although it can also reduce ATP production due to electrons flowing to AOX bypass the proton pumping complexes (Millar et al., 2011). The induction of AOX under heat stress supports their pivotal role in mediating seagrass stress acclimation as previously suggested in *P. oceanica* adult plants (Marín-Guirao et al., 2017; Ruocco et al., 2019; Tutar et al., 2017). Moreover, since the induction of AOX is dependent on ROS accumulation, the much higher AOX induction in non-primed with respect to thermo-primed seedlings, could be revealing that the former were suffering a greater heat-stress level and thus, greater heat-induced ROS production. Primed seedlings, indeed, activated a stronger antioxidant defense when subjected to increased temperatures and induced a stronger expression of small heat shock proteins (SHSP), doubling the expression level of nonprime seedlings. SHSPs, as ubiquitous molecular chaperones, are involved in the heat stress response of plants and provide an effective and low-cost thermo-protection, responsible for downstream plant thermo-tolerance (Sun et al., 2002; Wang et al., 2004). In *P. oceanica*, the SHSP seems to be the HSP with higher responsiveness under heat stress (Tutar et al., 2017; Traboni et al., 2018; Marín-Guirao et al., 2016). Additionally, the higher constitutive expression level of SHSP observed in shallow thermal-tolerant genotypes in comparison to deep-sensitive genotypes suggest its role in a pre-adaptive defense strategy of the species against heat stress (Marín-Guirao et al., 2017; Tutar et al., 2017).

While heat stress-inducible genes are essential to investigate the acquired priming status (He and Li, 2018; Lin et al., 2014), exploring epigenetic regulation under priming treatment is essential for the analysis of the subsequent storage of the information of priming cues which is known as stress-memory (Lämke and Bäurle, 2017; Liu et al., 2015). In this regard, methylation/demethylation of the histone H3 is known to be linked to gene regulation and memory of stress responses in primed plants (Ding et al., 2012), conferring to modifications of the

chromatin structure a crucial role in driving the memorization of past stress events (Bäurle and Trindade, 2020). Here, we tested genes involved in chromatin modifications (ATX2, ATRX7, ASH2l, and SETD3) and a regulator of the active DNA methylation (DME) (Li et al., 2018; Pontvianne et al., 2010). In general, P seedlings showed slightly higher gene expression levels of all epigenetics-related genes in respect to NP seedlings. This is in contrast with what emerged from a recent study performed on *Posidonia australis* and *Zostera muelleri* adult plants (Nguyen et al., 2020), where methylation-related genes showed an opposite pattern and only ATX2 significantly differed among treatments in both species. It is worth underlining that in plants, trimethylation of lysine 4 of histone 3 (H3K4me3) is an important epigenetic mark that is associated to active chromatin states (Zhang et al., 2009) and transcriptional memory (Bhadouriya et al., 2021). In this study, the higher overexpression of genes related to H3K4 marks measured for P seedlings (ATRX7, ASH2l, and SETD3) gives first insights on: i) epigenetic changes induced by environmental stimuli in *P. oceanica* seedlings, ii) different epigenetic regulation under priming treatment of adult plants and seedlings, iii) the potential for juvenile stages of *P. oceanica* plants to memorize stress information through chromatin remodeling, overexpressing key genes in histone methylation and DNA demethylation. Regarding the first observation, in terrestrial plants dynamic epigenetic changes occur during embryo/seed development, germination, and early seedling development (Bouyer et al., 2017; Hannoufa et al., 2018; Kawakatsu et al., 2017). Thus, our results suggest that seedlings are more responsive to priming treatments in respect to adult plants. Moreover, since H3K4 marks tend to be accumulated after the exposure to heat stress in primed plants (*A. thaliana*, Lämke et al., 2016) and different regulation of key histone-modification related genes was already observed in *P. oceanica* during a heat-stress induced flowering event (Marín-Guirao et al., 2019), it appears that these genes are directly involved in driving epigenetic responses under stressful conditions, with the potential for storage of stress information.

In conclusion, this study revealed, for the first time in seagrass seedlings, that thermo-priming conferred higher tolerance to the occurrence of an extreme seawater-warming event. All the responses measured in our experiment at the physiological, metabolic and molecular levels, pointed to the acquisition of a priming status in *P. oceanica* seedlings by a previous exposure to increased temperatures. In fact, during the triggering stimulus, primed seedlings performed better than unprimed ones. They were able to enhance the photochemical efficiency, to attain respiratory homeostasis, to keep their carbon balance unaltered and to grow faster reaching larger sizes compared to non-primed seedlings. Since the induction of the thermo-priming status depends on the level of heat-stress experienced by plants during the priming stimulus, exploring the influence of different temperature levels and the duration of the exposure to these conditions could be a critical point and a further step for understanding the acquisition of a thermo-primed status in *P. oceanica* seedlings. Our findings about the acquisition of a thermo-priming status in *P. oceanica* seedlings were supported by the expression levels of key genes related to stress response, photosynthesis, and epigenetic modifications. The overexpression of key genes in histone modifications suggests that primed seedlings have the potential to store priming stress information for long-lasting memorization of the past stress event. Therefore, more studies are required to investigate specific stress-memory genes including epigenetic regulators to describe molecular mechanisms behind the acquisition of the priming status and to better describe the extent of the memorization of the past stress event. Since the priming approaches have been utilized in terrestrial systems to reinforce plants against different kinds of abiotic stresses (Kerchev et al., 2020), further studies are necessary to better explore tolerance-enhancing strategies in seagrasses through the induction of a priming stimulus under controlled conditions and the analysis of plants performance in natural environments. Indeed, seagrasses are exposed to multiple environmental changes in their natural environments along coastal areas where multiple anthropogenic pressures can co-occur (e.g. Pazzaglia et al., 2020). Thus, exploring metabolic and molecular mechanisms at the basis of the acquisition of the priming status are needed to optimize this approach, for improving conservation and restoration management of these highly valuable marine ecosystems.

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Jessica Pazzaglia: Conceptualization, Methodology, Formal analysis, Writing - Original draft
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Gabriele Procaccini: Conceptualization, Methodology, Writing - Review & editing, Supervision
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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.

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References

Abdusalam, A., Li, Q., 2018. Morphological plasticity and adaptation level of distylous Primula nivalis in a heterogeneous alpine environment. Plant Divers. 40, 284–291. https://doi.org/10.1016/j.pld.2018.11.003.

Alcoverro, T., Manzanera, M., Romero, J., 2001. Annual metabolic carbon balance of the seagrass Posidonia oceanica: the importance of carbohydrate reserves. Mar. Ecol. Prog. Ser. 211, 105–116. https://doi.org/10.3354/meps211105.

Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. In: Primer-E, L. (Ed.), PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods.

Arnaud-Haond, S., Duarte, C.M., Diaz-Almela, E., Marbà, N., Sintes, T., Serrão, E.A., 2012. Implications of extreme life span in clonal organisms: millenary clones in meadows of the threatened seagrass Posidonia oceanica. PLoS One 7. https://doi.org/10.1371/journal.pone.0030454.

Asada, K., 2006. Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiol. 141, 391–396. https://doi.org/10.1104/PP.106.082040.

Bäurle, I., Trindade, I., 2020. Chromatin regulation of somatic abiotic stress memory. J. Exp. Bot. https://doi.org/10.1093/jxb/eraa098.

Bhadouriya, S.L., Mehrotra, S., Basantani, M.K., Loake, G.J., Mehrotra, R., 2021. Role of chromatin architecture in plant stress responses: an update. Front. Plant Sci. https://doi.org/10.3389/fpls.2020.603380.

Balestri, E., Gobert, S., Lepoint, G., Lardicci, C., 2009. Seed nutrient content and nutritional status of *Posidonia oceanica* seedlings in the northwestern Mediterranean Sea. Mar. Ecol. Prog. Ser. 388, 99–109. https://doi.org/10.3354/meps08104.

Borg, M., Jacob, Y., Susaki, D., LeBlanc, C., Buendía, D., Axelsson, E., Kawashima, T., Voigt, P., Boavida, L., Becker, J., Higashiyama, T., Martienssen, R., Berger, F., 2020. Targeted reprogramming of H3K27me3 resets epigenetic memory in plant paternal chromatin. Nat. Cell Biol. 22, 621–629. https://doi.org/10.1038/s41556-020-0515-y.

Bouyer, D., Kramdi, A., Kassam, M., Heese, M., Schnittger, A., Roudier, F., Colot, V., 2017. DNA methylation dynamics during early plant life. Genome Biol. 18, 1–12. https://doi.org/10.1186/s13059-017-1313-0.

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.

Celdrán, D., Marín, A., 2013. Seed photosynthesis enhances Posidonia oceanica seedling growth. Ecosphere 4, 1–11. https://doi.org/10.1890/ES13-00104.1.

Celdrán, D., Marín, A., 2011. Photosynthetic activity of the non-dormant Posidonia oceanica seed. Mar. Biol. 1584 (158), 853–858. https://doi.org/10.1007/S00227-010-1612-4.

Champenois, W., Borges, A.V., 2019. Inter-annual variations over a decade of primary production of the seagrass Posidonia oceanica. Limnol. Oceanogr. 64, 32–45. https://doi.org/10.1002/lno.11017.
Chefaoui, R.M., Duarte, C.M., Serrão, E.A., 2018. Dramatic loss of seagrass habitat under projected climate change in the Mediterranean Sea. Glob. Chang. Biol. 24, 4919–4928. https://doi.org/10.1111/gcb.14401.
Chen, X., Hu, Y., Zhou, D.X., 2011. Epigenetic gene regulation by plant Jumonji group of histone demethylase. https://doi.org/10.1016/j.bbagrm.2011.03.004.
Chinnusamy, V., Zhu, J.K., 2009. Epigenetic regulation of stress responses in plants. Curr. Opin. Plant Biol. 12, 133–139. https://doi.org/10.1016/j.pbi.2008.12.006.
Collier, C.J., Waycott, M., 2014. Temperature extremes reduce seagrass growth and induce mortality. Mar. Pollut. Bull. 83, 483–490. https://doi.org/10.1016/j.marpolbul.2014.03.050.
Collier, C.J., Uthicke, S., Waycott, M., 2011. Thermal tolerance of two seagrass species at contrasting light levels: implications for future distribution in the Great Barrier Reef. Limnol. Oceanogr. https://doi.org/10.4319/lo.2011.56.6.2200.
Conrath, U., Beckers, G.J.M., Langenbach, C.J.G., Jaskiewicz, M.R., 2015. Priming for enhanced defense. Annu. Rev. Phytopathol. 53, 97–119. https://doi.org/10.1146/annurev-phyto-080614-120132.
Costanza, R., de Groot, R., Sutton, P., van der Ploeg, S., Anderson, S.J., Kubiszewski, I., Farber, S., Turner, R.K., 2014. Changes in the global value of ecosystem services. Glob. Environ. Chang. 26, 152–158. https://doi.org/10.1016/j.gloenvcha.2014.04.002.
D'Esposito, D., Orrù, L., Dattolo, E., Bernardo, L., Lamontanara, A., Orsini, L., Serra, I.A., Mazzuca, S., Procaccini, G., 2017. Corrigendum: transcriptome characterisation and simple sequence repeat marker discovery in the seagrass Posidonia oceanica. Sci. Data 4, 170025. https://doi.org/10.1038/sdata.2017.25.
Dattolo, E., Ruocco, M., Brunet, C., Lorenti, M., Lauritano, C., D'Esposito, D., de Luca, P., Sanges, R., Mazzuca, S., Procaccini, G., 2014. Response of the seagrass Posidonia oceanica to different light environments: insights from a combined molecular and photo-physiological study. Mar. Environ. Res. 101, 225–236. https://doi.org/10.1016/j.marenvres.2014.07.010.
Dattolo, E., Marín-Guirao, L., Ruiz, J.M., Procaccini, G., 2017. Long-term acclimation to reciprocal light conditions suggests depth-related selection in the marine foundation species Posidonia oceanica. Ecol. Evol. 7, 1148–1164.
Degen, G.E., Orr, D.J., Carmo-Silva, E., 2021. Heat-induced changes in the abundance of wheat rubisco activase isoforms. New Phytol. 229, 1298–1311. https://doi.org/10.1111/NPH.16937.
Del-Saz, N., Ribas-Carbo, M., McDonald, A., Lambers, H., Fernie, A., Florez-Sarasa, I., 2018. An in vivo perspective of the role(s) of the alternative oxidase pathway. Trends Plant Sci. 23, 206–219. https://doi.org/10.1016/J.TPLANTS.2017.11.006.
Ding, Y., Fromm, M., Avramova, Z., 2012. Multiple exposures to drought "train" transcriptional responses in arabidopsis. Nat. Commun. 3, 1–9. https://doi.org/10.1038/ncomms1732.
Doney, S.C., Ruckelshaus, M., Emmett Duffy, J., Barry, J.P., Chan, F., English, C.A., Galindo, H.M., Grebmeier, J.M., Hollowed, A.B., Knowlton, N., Polovina, J., Rabalais, N.N., Sydeman, W.J., Talley, L.D., 2012. Climate change impacts on marine ecosystems. Annu. Rev. Mar. Sci. 4, 11–37. https://doi.org/10.1146/annurev-marine-041911-111611.
Duarte, C.M., Agusti, S., Barbier, E., Britten, G.L., Castilla, J.C., Gattuso, J.-P., Fulweiler, R.W., Hughes, T.P., Knowlton, N., Lovelock, C.E., 2020. Rebuilding marine life. Nature 580, 39–51.
Duncan, E.J., Gluckman, P.D., Dearden, P.K., 2014. Epigenetics, plasticity, and evolution: how do we link epigenetic change to phenotype? J. Exp. Zool. Part B Mol. Dev. Evol. 322, 208–220. https://doi.org/10.1002/jez.b.22571.
Enríquez, S., 2005. Light absorption efficiency and the package effect in the leaves of the seagrass Thalassia testudinum. Mar. Ecol. Prog. Ser. 289, 141–150. https://doi.org/10.3354/MEPS289141.
Entrambasaguas, L., Ruocco, M., Verhoeven, K.J.F., Procaccini, G., Guirao, L.M., 2021. Gene body DNA methylation in seagrasses : inter- and intraspecific differences and interaction with transcriptome plasticity under heat stress. Sci. Rep. 1–15 https://doi.org/10.1038/s41598-021-93606-w.
Filbee-Dexter, K., Smajdor, A., 2019. Ethics of assisted evolution in marine conservation. Front. Mar. Sci. 6, 1–6. https://doi.org/10.3389/fmars.2019.00020.
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., Serrano, O., 2012. Seagrass ecosystems as a globally significant carbon stock. Nat. Geosci. 5, 505–509. https://doi.org/10.1038/ngeo1477.
Foyer, C.H., Noctor, G., 2000. Oxygen processing in photosynthesis: regulation and signalling. New Phytol. 146, 359–388. https://doi.org/10.1046/J.1469-8137.2000.00667.X.
Friedrich, T., Faivre, L., Bäurle, I., Schubert, D., 2019. Chromatin-based mechanisms of temperature memory in plants. Plant Cell Environ. https://doi.org/10.1111/pce.13373.
González-Grande, P., Suárez, N., Marín, O., 2020. Effect of salinity and seed salt priming on the physiology of adult plants of Solanum lycopersicum cv. 'Río Grande'. Rev. Bras. Bot. 43, 775–787. https://doi.org/10.1007/s40415-020-00636-1.
Guerrero-Meseguer, L., Marín, A., Sanz-Lázaro, C., 2020. Heat wave intensity can vary the cumulative effects of multiple environmental stressors on Posidonia oceanica seedlings: heat waves override other stressors. Mar. Environ. Res. 159 https://doi.org/10.1016/j.marenvres.2020.105001.
Guerrero-Meseguer, L., Marín, A., Sanz-Lázaro, C., 2017. Future heat waves due to climate change threaten the survival of P. oceanica seedlings. Environ. Pollut. 230, 40–45. https://doi.org/10.1016/j.envpol.2017.06.039.
Hannoufa, A., Canada, A.-F., Muleo, C.R., Xu, Y., Zhang, L., Wu, G., 2018. Epigenetic Regulation of Juvenile-to-Adult Transition in Plants. https://doi.org/10.3389/fpls.2018.01048.
He, Y., Li, Z., 2018. Epigenetic environmental memories in plants: establishment, maintenance, and reprogramming. Trends Genet. https://doi.org/10.1016/j.tig.2018.07.006.
Hepworth, J., Dean, C., 2015. Flowering locus C's lessons: conserved chromatin switches underpinning developmental timing and adaptation. Plant Physiol. 168, 1237–1245. https://doi.org/10.1104/pp.15.00496.
Hernán, G., Ortega, M.J., Gándara, A.M., Castejón, I., Terrados, J., Tomas, F., 2017. Future warmer seas: increased stress and susceptibility to grazing in seedlings of a marine habitat-forming species. Glob. Chang. Biol. 23, 4530–4543. https://doi.org/10.1111/gcb.13768.
Hilker, M., Schwachtje, J., Baier, M., Balazadeh, S., Bäurle, I., Geiselhardt, S., Hincha, D. K., Kunze, R., Mueller-Roeber, B., Rillig, M.C., Rolff, J., Romeis, T., Schmülling, T., Steppuhn, A., van Dongen, J., Whitcomb, S.J., Wurst, S., Zuther, E., Kopka, J., 2016. Priming and memory of stress responses in organisms lacking a nervous system. Biol. Rev. 91, 1118–1133. https://doi.org/10.1111/brv.12215.
Hobday, A.J., Alexander, L.V., Perkins, S.E., Smale, D.A., Straub, S.C., Oliver, E.C.J., Benthuysen, J.A., Burrows, M.T., Donat, M.G., Feng, M., Holbrook, N.J., Moore, P.J., Scannell, H.A., Sen Gupta, A., Wernberg, T., 2016. A hierarchical approach to defining marine heatwaves. Prog. Oceanogr. 141, 227–238. https://doi.org/10.1016/j.pocean.2015.12.014.
Hsu, P.D., Lander, E.S., Zhang, F., 2014. Development and applications of CRISPR-Cas9 for genome engineering. Cell. https://doi.org/10.1016/j.cell.2014.05.010.
IPCC, 2019. Technical summary. In: Pörtner, H.-O., Roberts, D.C., Masson-Delmotte, V., Zhai, P., Poloczanska, E., Mintenbeck, K., et al. (Eds.), IPCC Special Report on the Ocean and Cryosphere in a Changing Climate. IPCC, Geneva.
Jahnke, M., Olsen, J.L., Procaccini, G., 2015. A meta-analysis reveals a positive correlation between genetic diversity metrics and environmental status in the long-lived seagrass Posidonia oceanica. Mol. Ecol. 24, 2336–2348. https://doi.org/10.1111/mec.13174.
Jisha, K.C., Vijayakumari, K., Puthur, J.T., 2013. Seed priming for abiotic stress tolerance: an overview. Acta Physiol. Plant. 35, 1381–1396. https://doi.org/10.1007/s11738-012-1186-5.
Jueterbock, A., Boström, C., Coyer, J.A., Olsen, J.L., Kopp, M., Dhanasiri, A.K.S., Smolina, I., Arnaud-Haond, S., Van de Peer, Y., Hoarau, G., 2020. The seagrass methylome is associated with variation in photosynthetic performance among clonal shoots. Front. Plant Sci. 11, 1. https://doi.org/10.3389/fpls.2020.571646.
Kawakatsu, T., Nery, J.R., Castanon, R., Ecker, J.R., 2017. Dynamic DNA methylation reconfiguration during seed development and germination. Genome Biol. 18, 1–12. https://doi.org/10.1186/s13059-017-1251-x.
Kerchev, P., van der Meer, T., Sujeeth, N., Verlee, A., Stevens, C.V., Van Breusegem, F., Gechev, T., 2020. Molecular priming as an approach to induce tolerance against abiotic and oxidative stresses in crop plants. Biotechnol. Adv. 40, 107503 https://doi.org/10.1016/j.biotechadv.2019.107503.
Koressaar, T., Remm, M., 2007. Enhancements and modifications of primer design program Primer3. Bioinformatics 23, 1289–1291. https://doi.org/10.1093/bioinformatics/btm091.
Kotak, S., Larkindale, J., Lee, U., von Koskull-Döring, P., Vierling, E., Scharf, K.D., 2007. Complexity of the heat stress response in plants. Curr. Opin. Plant Biol. https://doi.org/10.1016/j.pbi.2007.04.011.
Kreps, J.A., Wu, Y., Chang, H.S., Zhu, T., Wang, X., Harper, J.F., 2002. Transcriptome changes for Arabidopsis in response to salt, osmotic, and cold stress. Plant Physiol. 130, 2129–2141. https://doi.org/10.1104/pp.008532.
Kumar, J., Rai, K.M., Pirseyedi, S., Elias, E.M., Xu, S., Dill-Macky, R., Kianian, S.F., 2020. Epigenetic regulation of gene expression improves fusarium head blight resistance in durum wheat. Sci. Rep. 10, 1–15. https://doi.org/10.1038/s41598-020-73521-2.
Lämke, J., Bäurle, I., 2017. Epigenetic and chromatin-based mechanisms in environmental stress adaptation and stress memory in plants. Genome Biol. https://doi.org/10.1186/s13059-017-1263-6.
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. https://doi.org/10.15252/embj.201592593.
Lauritano, C., Ruocco, M., Dattolo, E., Buia, M.C., Silva, J., Santos, R., Olivé, I., Costa, M. M., Procaccini, G., 2015. Response of key stress-related genes of the seagrass Posidonia oceanica in the vicinity of submarine volcanic vents. Biogeosciences 12, 4185–4195. https://doi.org/10.5194/bg-12-4185-2015.
Leuendorf, J.E., Frank, M., Schmülling, T., 2020. Acclimation, priming and memory in the response of Arabidopsis thaliana seedlings to cold stress. Sci. Rep. 10, 1–11. https://doi.org/10.1038/s41598-019-56797-x.
Li, Y., Kumar, S., Qian, W., 2018. Active DNA demethylation: mechanism and role in plant development. Plant Cell Rep. 37, 77–85. https://doi.org/10.1007/s00299-017-2215-z.
Lichtenthaler, H.K., Wellburn, A.R., 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochem. Soc. Trans. 11, 591–592. https://doi.org/10.1042/bst0110591.
Lin, M.Y., Chai, K.H., Ko, S.S., Kuang, L.Y., Lur, H.S., Charng, Y.Y., 2014. A positive feedback loop between HEAT SHOCK PROTEIN101 and HEAT STRESS-ASSOCIATED 32-KD PROTEIN modulates long-term acquired thermotolerance illustrating diverse heat stress responses in rice varieties. Plant Physiol. 164, 2045–2053. https://doi.org/10.1104/pp.113.229609.
Liu, J., Feng, L., Li, J., He, Z., 2015. Genetic and epigenetic control of plant heat responses. Front. Plant Sci. https://doi.org/10.3389/fpls.2015.00267.

Liu, N., Fromm, M., Avramova, Z., 2014. H3K27me3 and H3K4me3 chromatin environment at super-induced dehydration stress memory genes of arabidopsis thaliana. Mol. Plant 7, 502–513. https://doi.org/10.1093/mp/ssu001.
McMahon, K., van Dijk, K.J., Ruiz-Montoya, L., Kendrick, G.A., Krauss, S.L., Waycott, M., Verduin, J., Lowe, R., Statton, J., Brown, E., Duarte, C., 2014. The movement ecology of seagrasses. Proc. R. Soc. B Biol. Sci. 281 https://doi.org/10.1098/rspb.2014.0878.
Marín-Guirao, L., Sandoval-Gil, J.M., Bernardeau-Esteller, J., Ruíz, J.M., Sánchez-Lizaso, J.L., 2013. Responses of the Mediterranean seagrass Posidonia oceanica to hypersaline stress duration and recovery. Mar. Environ. Res. 84, 60–75. https://doi.org/10.1016/j.marenvres.2012.12.001.
Marín-Guirao, L., Dattolo, E., Ruiz, J.M., Procaccini, G., 2015. In: Differential tolerance and resilience of Mediterranean seagrasses to short-term heat stress, p. 7287.
Marín-Guirao, L., Ruiz, J.M., Dattolo, E., Garcia-Munoz, R., Procaccini, G., 2016. Physiological and molecular evidence of differential short-Term heat tolerance in Mediterranean seagrasses. Sci. Rep. 6 https://doi.org/10.1038/srep28615.
Marín-Guirao, Lazaro, 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 https://doi.org/10.3389/fpls.2017.01142.
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. https://doi.org/10.1016/j.marpolbul.2018.07.050.
Marín-Guirao, L., Entrambasaguas, L., Ruiz, J.M., Procaccini, G., 2019. Heat-stress induced flowering can be a potential adaptive response to ocean warming for the iconic seagrass Posidonia oceanica. Mol. Ecol. 1–16 https://doi.org/10.1111/mec.15089.
Merilä, J., Hendry, A.P., 2014. Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. Evol. Appl. 7, 1–14. https://doi.org/10.1111/eva.12137.
Micheli, F., Halpern, B.S., Walbridge, S., Ciriaco, S., Ferretti, F., Fraschetti, S., Lewison, R., Nykjaer, L., Rosenberg, A.A., 2013. Cumulative human impacts on Mediterranean and black sea marine ecosystems : assessing current pressures and opportunities. PLoS One 8. https://doi.org/10.1371/journal.pone.0079889.
Millar, A., Whelan, J., KL, S., Day, D., 2011. Organization and regulation of mitochondrial respiration in plants. Annu. Rev. Plant Biol. 62, 79–104. https://doi.org/10.1146/ANNUREV-ARPLANT-042110-103857.
Mittler, R., Vanderauwera, S., Gollery, M., Van Breusegem, F., 2004. Reactive oxygen gene network of plants. Trends Plant Sci. https://doi.org/10.1016/j.tplants.2004.08.009.
Munekage, Y., Hashimoto, M., Miyake, C., Tomizawa, K.-I., Endo, T., Tasaka, M., Shikanai, T., 2004. Cyclic electron flow around photosystem I is essential for photosynthesis. Nature 4296991 (429), 579–582. https://doi.org/10.1038/nature02598.
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., Ralph, P.J., Marín-Guirao, L., Pernice, M., Procaccini, G., 2021. Seagrasses in an era of ocean warming: a review. Biol. Rev. 7 https://doi.org/10.1111/brv.12736.
Niyogi, K., 2000. Safety valves for photosynthesis. Curr. Opin. Plant Biol. 3, 455–460. https://doi.org/10.1016/S1369-5266(00)00113-8.
Oliver, E.C.J., Donat, M.G., Burrows, M.T., Moore, P.J., Smale, D.A., Alexander, L.V., Benthuysen, J.A., Feng, M., Sen Gupta, A., Hobday, A.J., Holbrook, N.J., Perkins-Kirkpatrick, S.E., Scannell, H.A., Straub, S.C., Wernberg, T., 2018. Longer and more frequent marine heatwaves over the past century. Nat. Commun. 9, 1324. https://doi.org/10.1038/s41467-018-03732-9.
Olsen, Y.S., Sánchez-Camacho, M., Marbà, N., Duarte, C.M., 2012. Mediterranean seagrass growth and demography responses to experimental warming. Estuar. Coasts 35, 1205–1213. https://doi.org/10.1007/s12237-012-9521-z.
Pastor, V., Luna, E., Mauch-Mani, B., Ton, J., Flors, V., 2013. Primed plants do not forget. Environ. Exp. Bot. 94, 46–56. https://doi.org/10.1016/j.envexpbot.2012.02.013.
Pazzaglia, J., Santillán-sarmiento, A., Helber, S.B., Ruocco, M., Terlizzi, A., Marín-guirao, L., Procaccini, G., 2020. Does warming likely enhance the effects of eutrophication in the seagrass *Posidonia oceanica*? Front. Mar. Sci. 7, 1–15. https://doi.org/10.3389/fmars.2020.564805.
Pazzaglia, J., Nguyen, H.M., Santillán-Sarmiento, A., Ruocco, M., Dattolo, E., Marín-Guirao, L., Procaccini, G., 2021a. The genetic component of seagrass restoration: what we know and the way forwards. Water 13, 829. https://doi.org/10.3390/w13060829.
Pazzaglia, J., Reusch, T.B.H., Terlizzi, A., Marín-Guirao, L., Procaccini, G., 2021b. Phenotypic plasticity under rapid global changes: the intrinsic force for future seagrasses survival. Evol. Appl. eva.13212 https://doi.org/10.1111/eva.13212.
Pereda-Briones, L., Terrados, J., Tomas, F., 2019. Negative effects of warming on seagrass seedlings are not exacerbated by invasive algae. Mar. Pollut. Bull. 141, 36–45. https://doi.org/10.1016/j.marpolbul.2019.01.049.
Pontvianne, F., Blevins, T., Pikaard, C.S., 2010. Arabidopsis histone lysine methyltransferases. Adv. Bot. Res. 1–22. https://doi.org/10.1016/s0065-2296(10)53001-5.
Procaccini, G., Ruocco, M., Marín-Guirao, L., Dattolo, E., Brunet, C., D'Esposito, D., Lauritano, C., Mazzuca, S., Costa, M.M., Barrote, I., Silva, J., Santos, R., Serra, I.A., Björk, M., Gullström, M., Rasmusson, L.M., Runcie, J.W., Buapet, P., Piro, A., Felisberto, P., Bernardo, L., Olivé, I., Beer, S., Gobert, S., 2017. Depth-specific fluctuations of gene expression and protein abundance modulate the photophysiology in the seagrass Posidonia oceanica. Sci. Rep. 7, 1–15. https://doi.org/10.1038/srep42890.
Radonić, A., Thulke, S., Mackay, I.M., Landt, O., Siegert, W., Nitsche, A., 2004. Guideline to reference gene selection for quantitative real-time PCR. Biochem. Biophys. Res. Commun. 313, 856–862. https://doi.org/10.1016/j.bbrc.2003.11.177.
Rakshit, A., Singh, H.B., 2018. Advances in seed priming. Advances in Seed Priming. https://doi.org/10.1007/978-981-13-0032-5.
Reyes, J.C., Hennig, L., Gruissem, W., 2002. Chromatin-Remodeling and Memory Factors. Plant Physiol, New regulators of plant development. https://doi.org/10.1104/pp.006791.
Ruiz, J.M., Marín-Guirao, L., García-Muñoz, R., Ramos-Segura, A., Bernardeau-Esteller, J., Pérez, M., Sanmartí, N., Ontoria, Y., Romero, J., Arthur, R., Alcoverro, T., Procaccini, G., 2018. Experimental evidence of warming-induced flowering in the Mediterranean seagrass Posidonia oceanica. Mar. Pollut. Bull. 134, 49–54. https://doi.org/10.1016/j.marpolbul.2017.10.037.
Ruocco, M., Marín-Guirao, L., Ravaglioli, C., Bulleri, F., Procaccini, G., 2018. Molecular level responses to chronic versus pulse nutrient loading in the seagrass Posidonia oceanica undergoing herbivore pressure. Oecologia 188, 23–39. https://doi.org/10.1007/s00442-018-4172-9.
Ruocco, M., De Luca, P., Marín-Guirao, L., Procaccini, G., 2019. Differential leaf age-dependent thermal plasticity in the keystone seagrass Posidonia oceanica. Front. Plant Sci. 10, 1556. https://doi.org/10.3389/fpls.2019.01556.
Saha, B., Borovskii, G., Panda, S.K., 2016. Alternative oxidase and plant stress tolerance. Plant Signal. Behav. 11 https://doi.org/10.1080/15592324.2016.1256530.
Salo, T., Pedersen, M.F., 2014. Synergistic effects of altered salinity and temperature on estuarine eelgrass (Zostera marina) seedlings and clonal shoots. J. Exp. Mar. Biol. Ecol. 457, 143–150. https://doi.org/10.1016/j.jembe.2014.04.008.
Serra, I.A., Lauritano, C., Dattolo, E., Puoti, A., Nicastro, S., Innocenti, A.M., Procaccini, G., 2012. Reference genes assessment for the seagrass Posidonia oceanica in different salinity, pH and light conditions. Mar. Biol. 159, 1269–1282. https://doi.org/10.1007/s00227-012-1907-8.
Scafaro, A.P., Fan, Y., Posch, B.C., Garcia, A., Coast, O., Atkin, O.K., 2021. Responses of leaf respiration to heatwaves. Plant Cell Environ. https://doi.org/10.1111/PCE.14018.
Silva, J., Barrote, I., Costa, M.M., Albano, S., Santos, R., 2013. Physiological responses of Zostera marina and Cymodocea nodosa to light-limitation stress. PLoS One 8, e81058. https://doi.org/10.1371/journal.pone.0081058.
Somero, G., 1995. Proteins and temperature. Annu. Rev. Physiol. 57, 43–68. https://doi.org/10.1146/ANNUREV.PH.57.030195.000355.
Sun, W., Van Montagu, M., Verbruggen, N., 2002. Small heat shock proteins and stress tolerance in plants. https://doi.org/10.1016/S0167-4781(02)00417-7.
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.
Tutar, O., Marín-Guirao, L., Ruiz, J.M., Procaccini, G., 2017. Antioxidant response to heat stress in seagrasses. A gene expression study. Mar. Environ. Res. https://doi.org/10.1016/j.marenvres.2017.10.011.
Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B.C., Remm, M., Rozen, S. G., 2012. Primer3-new capabilities and interfaces. Nucleic Acids Res. 40, e115. https://doi.org/10.1093/nar/gks596.
Walter, J., Jentsch, A., Beierkuhnlein, C., Kreyling, J., 2013. Ecological stress memory and cross stress tolerance in plants in the face of climate extremes. Environ. Exp. Bot. 94, 3–8. https://doi.org/10.1016/j.envexpbot.2012.02.009.
Wang, W., Vinocur, B., Shoseyov, O., Altman, A., 2004. Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. Trends Plant Sci. 9, 244–252. https://doi.org/10.1016/J.TPLANTS.2004.03.006.
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 inwheat seedlings. Plant Physiol. Biochem. 74, 185–192. https://doi.org/10.1016/j.plaphy.2013.11.014.
Waycott, M., Duarte, C.M., Carruthers, T.J.B., Orth, R.J., Dennison, W.C., Olyarnik, S., Calladine, A., Fourqurean, J.W., Heck, K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Short, F.T., Williams, S.L., 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. Proc. Natl. Acad. Sci. 106, 12377 LP–12381. https://doi.org/10.1073/pnas.0905620106.
Xie, F., Xiao, P., Chen, D., Xu, L., Zhang, B., 2012. miRDeepFinder: a miRNA analysis tool for deep sequencing of plant small RNAs. Plant Mol. Biol. 80, 75–84. https://doi.org/10.1007/s11103-012-9885-2.
Xu, S., Zhou, Y., Wang, P., Wang, F., Zhang, X., Gu, R., 2016. Salinity and temperature significantly influence seed germination, seedling establishment, and seedling growth of eelgrass Zostera marina L. PeerJ 2016. https://doi.org/10.7717/peerj.2697.
Zhang, X., Bernatavichute, Y.V., Cokus, S., Pellegrini, M., Jacobsen, S.E., 2009. Genome-wide analysis of mono-, di- and trimethylation of histone H3 lysine 4 in Arabidopsis thaliana. Genome Biol. 10, 1–14. https://doi.org/10.1186/gb-2009-10-6-r62.
Zieman, J.C., 1974. Methods for the study of the growth and production of turtle grass, Thalassia testudinum konig. Aquaculture 139–143.