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Alex Santillán-Sarmiento[a†], Jessica Pazzaglia[a,b], Miriam Ruocco[a], Emanuela Dattolo[a], Luca Ambrosino[c], Gidon Winters[d,e], Lázaro Marin-Guirao[a,f]*, Gabriele Procaccini[a]*[†]

[a] Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, 80121, Naples, Italy

[b] Department of Life Sciences, University of Trieste, Trieste, Italy

[c] Research Infrastructure for Marine Biological Resources Department, Stazione Zoologica Anton Dohrn, 80121, Naples, Italy

[d] Dead Sea and Arava Science Center (DSASC), Masada National Park, Mount Masada, Israel 8698000, Israel.

[e] Eilat Campus, Ben-Gurion University of the Negev, Hatmarim Blv, Eilat, 8855630 Israel

[f] Seagrass Ecology Group, Oceanographic Center of Murcia, Spanish Institute of Oceanography (IEO-CSIC), Murcia, Spain

[†]Corresponding authors: alex.renato.santillan@gmail.com; gpro@szn.it

*These authors contributed equally to the paper

Abstract

The continuous worldwide seagrasses decline calls for immediate actions in order to preserve this precious marine ecosystem. The main stressors that have been linked with decline in seagrasses are 1) the increasing ocean temperature due to climate change and 2) the continuous inputs of nutrients (eutrophication) associated with coastal human activities. To avoid the loss of seagrass populations, an "early warning" system is needed. We used Weighed Gene Co-expression Network Analysis

(WGCNA), a systems biology approach, to identify potential candidate genes that can provide an early warning signal of stress in the Mediterranean iconic seagrass *Posidonia oceanica*, anticipating plant mortality. Plants were collected from both eutrophic (EU) and oligotrophic (OL) environments and were exposed to thermal and nutrient stress in a dedicated mesocosm. By correlating the whole-genome gene expression after 2-weeks exposure with the shoot survival percentage after 5-weeks exposure to stressors, we were able to identify several transcripts that indicated an early activation of several biological processes (BP) including: protein metabolic process, RNA metabolic process, organonitrogen compound biosynthetic process, catabolic process and response to stimulus, which were shared among OL and EU plants and among leaf and shoot apical meristem (SAM), in response to excessive heat and nutrients. Our results suggest a more dynamic and specific response of the SAM compared to the leaf, especially the SAM from plants coming from a more stressful environment appeared more dynamic than the SAM from a more pristine environment. A vast list of potential molecular markers is also provided that can be used as targets to assess field samples.

Keywords: Seagrasses, Posidonia oceanica, global warming, eutrophication, transcriptomics, gene co-expression network analysis, early warning indicators

1. Introduction

Seagrasses are marine angiosperms that support complex food webs along the coastline. Seagrasses meadows provide valuable ecosystem services and host a rich diversity of organisms (Costanza et al., 2014; Mtwana Nordlund et al., 2016). Due to their sessile nature, and the proximity of most meadows to intense and growing coastal development, seagrass meadows are particularly exposed to stress, and cannot easily "escape" from adverse conditions, thus facing a rapid decline worldwide (Waycott et al., 2009). During the last 100 years, nearly one third of the known seagrasses meadow's extension has been lost, and the global rate of seagrass loss has accelerated from ~ 1%

yr^{-1} before 1940 to 7% yr^{-1} since 1990 (Waycott et al., 2009). Current rates of seagrass decline (7% yr^{-1}) are higher than the global declines estimated for coral reef (4 – 9% yr^{-1}) and tropical forests (0.5% yr^{-1}; Duarte et al., 2008). Global and local anthropogenic factors are progressively threatening the survival of slow growing seagrasses in particular, among the others the Mediterranean iconic *Posidonia oceanica* (Jordà et al., 2012). Stressors can occur alone or in combination, and their effect can be magnified, as observed for sea warming and nutrient inputs from the fast urbanisation of coastal areas (Helber et al., 2021; Pazzaglia et al., 2020). Understanding the response of complex systems such as seagrass meadows to external perturbations is critically important in the current context of rapid global environmental change.

Ecosystems and the organisms inhabiting them have a range of tolerance or display different levels of phenotypic plasticity in response to external perturbations (Pazzaglia et al., 2021; Pigliucci, 1996; Weissmann and Shnerb, 2016). However, after passing certain thresholds or "tipping points", catastrophic regime shifts may take place, in which a relatively small change in environmental conditions triggers a sudden jump from a steady state to another accompanied by hysteresis and often being irreversible (Drake and Griffen, 2010; Scheffer et al., 2009; Weissmann and Shnerb, 2016). Those sudden changes may lead to the loss of entire populations and as a consequence the loss of resilience of the entire ecosystem (Bellwood et al., 2004; Drake and Griffen, 2010). Climate change may affect seagrasses directly through seawater temperature increase that can have detrimental effects on plant physiology, for instance by reducing the photosynthetic rate and growth (Lefcheck et al., 2017; Moore et al., 2014; Nejrup and Pedersen, 2008). However, seagrasses are also exposed to direct anthropogenic stressors such as eutrophication, that affects carbon balance, photosynthetic and growth rates (Mvungi and Pillay, 2019; Pazzaglia et al., 2020).

Current global seagrass monitoring programs, such as "SeagrassNet" (https://www.seagrassnet.org), or "Seagrass Watch" (McKenzie et al., 2021) follow changes in species composition, percentage of cover and biomass, parameters that are relatively slow to respond to stressors, thus are only able to

track changes in seagrass meadows that have already taken place. We still lack parameters that can provide an early warning signal, long before conditions become irreversible and possibly un-restorable (i.e., in proximity to, or even pass a tipping point (Macreadie et al., 2014; Weissmann and Shnerb, 2016). Therefore, we urgently need to develop new efficient tools for early detection of stress in seagrass, such as molecular markers identified with next-generation sequencing technologies (NGS; Macreadie et al., 2014). These stress markers must be applied to monitor the status of seagrasses and contribute to their conservation.

The use of NGS technologies (e.g., RNA-seq; Wang et al., 2009) has contributed to our understanding of the molecular basis of processes involved in stress response (e.g. thermal stress, Franssen et al., 2014; Marín-Guirao et al., 2017, 2019). Most of these studies analysed gene expression at the peak or at the end of stress exposures period (Franssen et al., 2011) possibly missing the early response to the stress. Thus, a systematic approach in finding markers that could be used as early warning molecular signals of stress is still missing. Several studies have demonstrated the efficiency of Weighted Gene Co-expression Network Analysis (WGCNA) (Zhang and Horvath, 2005) in finding new genes involved in human pathologies such as cancer and osteoarthritis (Chou et al., 2014; Emilsson et al., 2008; Mueller et al., 2017; Qiu et al., 2019). WGCNA is a systems biology approach that merges network theory with gene expression data analysis for describing patterns of correlation among genes in large-scale gene expression data sets such as whole transcriptome data (Fuller et al., 2011). Genes or transcripts with similar expression patterns (co-expressed) may participate in regulatory and signalling circuits (Eisen et al., 1998). Under this premise, networks of co-expressed genes are built to facilitate the understanding of such pathways and the identification of key genes involved in them (Fuller et al., 2011). Within gene networks, it is possible to detect modules (groups of tightly co-expressed genes) that can be subsequently related to specific phenotypic traits (e.g. growth rates or mortality). Additionally, central genes or "hub genes" can be found within modules, which are the most interconnected genes

in a specific module or genes that interact with many other genes in a specific module (Zhao et al., 2016). WGCNA has already been applied to identify hub genes involved in nitrogen use, mild drought, chilling and heat stress, in terrestrial plants (Cheng et al., 2020; Goel et al., 2018; Greenham et al., 2017; Mishra et al., 2021). For instance, a set of 36 genes involved in oxidative stress, photosynthesis and circadian rhythms were selected as early markers of drought stress by applying WGCNA to RNA-seq data in the crop species *Brassica rapa* (Greenham et al., 2017). This approach has also been employed in corals to find the basis of plasticity and the genes involved in bleaching (Kenkel and Matz, 2017; Rose et al., 2016). WGCNA has never been applied in seagrasses.

The Mediterranean endemic seagrass *Posidonia oceanica* forms dense meadows, with a rich associated plant and animal community, at depths from 1 to 45 m (Telesca et al., 2015). It is a key ecosystem engineer that provides essential ecosystem goods and services to the whole Mediterranean basin (Campagne et al., 2015). Also, it is considered an important contributor to blue carbon accumulation, with increasing relevance for climate change mitigation (Pergent-Martini et al., 2021). *P. oceanica* meadows are exposed to extreme environmental pressure, particularly in the western Mediterranean, where a significant regression has been observed (Boudouresque et al., 2009). Recent projections estimated a functional extinction of the species by year 2100 due to climate change (Chefaoui et al., 2018). The recolonization of lost areas can be extremely slow, since *P. oceanica* is a long-lived species with slow rhizome elongation (Boudouresque et al., 2009).

The objective of this study was to explore molecular indicators in *P. oceanica* that can provide early signals of stress before mortality occurs. Pazzaglia et al. (2022, 2020) recently demonstrated an organ-specific vulnerability to abiotic stressors and a decrease in long-term survival depending on plants' origin. Moreover, it was recently recognised that the shoot apical meristems of this species, rather than the leaves, might be the potential sentinel tissue suitable for use in early warning stress monitoring (Ruocco et al., 2021). Thus, we hypothesize that the transcriptomic profiles of plants

undergoing stress exposure contain anticipatory molecular signals of stress related to shoot survival. These signals may change in plants with different stress history and among different tissues, and can be exploited as potential early indicators of disruptive stress in seagrass species. We utilised for the first time WGCNA to analyse whole transcriptomic profiles obtained from leaves and shoot apical meristems (SAM) of *P. oceanica* plants, collected from both oligotrophic and eutrophic sites and exposed to stress conditions caused by high temperatures and excess nutrient levels. Samples (leaves and SAMs) were collected after two weeks of exposure to treatments and their transcriptomic response was correlated to the shoot survival percentage recorded at the end of the experiment (after five weeks of exposure) to explore and select anticipatory molecular signals of stress-induced mortality. Shoot survival was chosen as an integrative parameter of stress response leading to seagrass decline, since other parameters might indicate reversible responses to stress (Ceccherelli et al., 2018).

2. Materials and methods

2.1. Experimental design

For a complete description of sampling and experimental design see Pazzaglia et al., (2020). Briefly, meadows from two nearby locations were chosen for sampling according to their differences in anthropogenic pressure: Spiaggia del Poggio (Bacoli) in the Gulf of Pozzuoli (Italy, 40 47.9300 N; 14 05.1410 E), that was identified as more impacted by eutrophication (EU); and Castello Aragonese off the Island of Ischia (Italy, 4044.1140N; 1357.8660 E), that was identified as pristine and oligotrophic (OL; see Pazzaglia et al., 2020; Helber et al., 2021 for environmental information of sites). *P. oceanica* fragments (ramets) bearing ca. 20 vertical shoots were collected by SCUBA-diving during the spring of 2019. Collected ramets were transferred immediately to the mesocosms facility at Stazione Zoologica Anton Dohrn (SZN, Naples, Italy) and distributed into 12 glass aquaria (500 L) filled with natural filtered and UV-treated seawater (see Ruocco et al., 2019 for a complete description of the mesocosm facility). Four ramets (two for each meadow) were

allocated in each of the 12 tanks, attached to the bottom of a basket filled with coarse sediment. An orthogonal design was used to apply the four different treatments: control (C), which consisted of seawater with no added nutrient at 24 °C; increased temperature (T), which consisted of a gradual increase from 24 to 30 °C; nutrients excess (N), in which a nutrient solution prepared using Osmocote® Pro fertilizer pellets 6 months release (19% N – 3.9% P – 8.3% K, ICL Specialty Fertilizers) was added to tanks; and a treatment combining both conditions (NT). See Pazzaglia et al. (2020) for more details on the experiment. Stress exposure lasted five weeks, while detection of early transcriptomic response to stress was performed after two weeks. As explained below, the molecular response after two weeks of stress exposure was analysed with WGCNA and correlated to shoot survival after 5-week of stress exposure to identify potential anticipatory signals of mortality in *P. oceanica* (Figure 1).

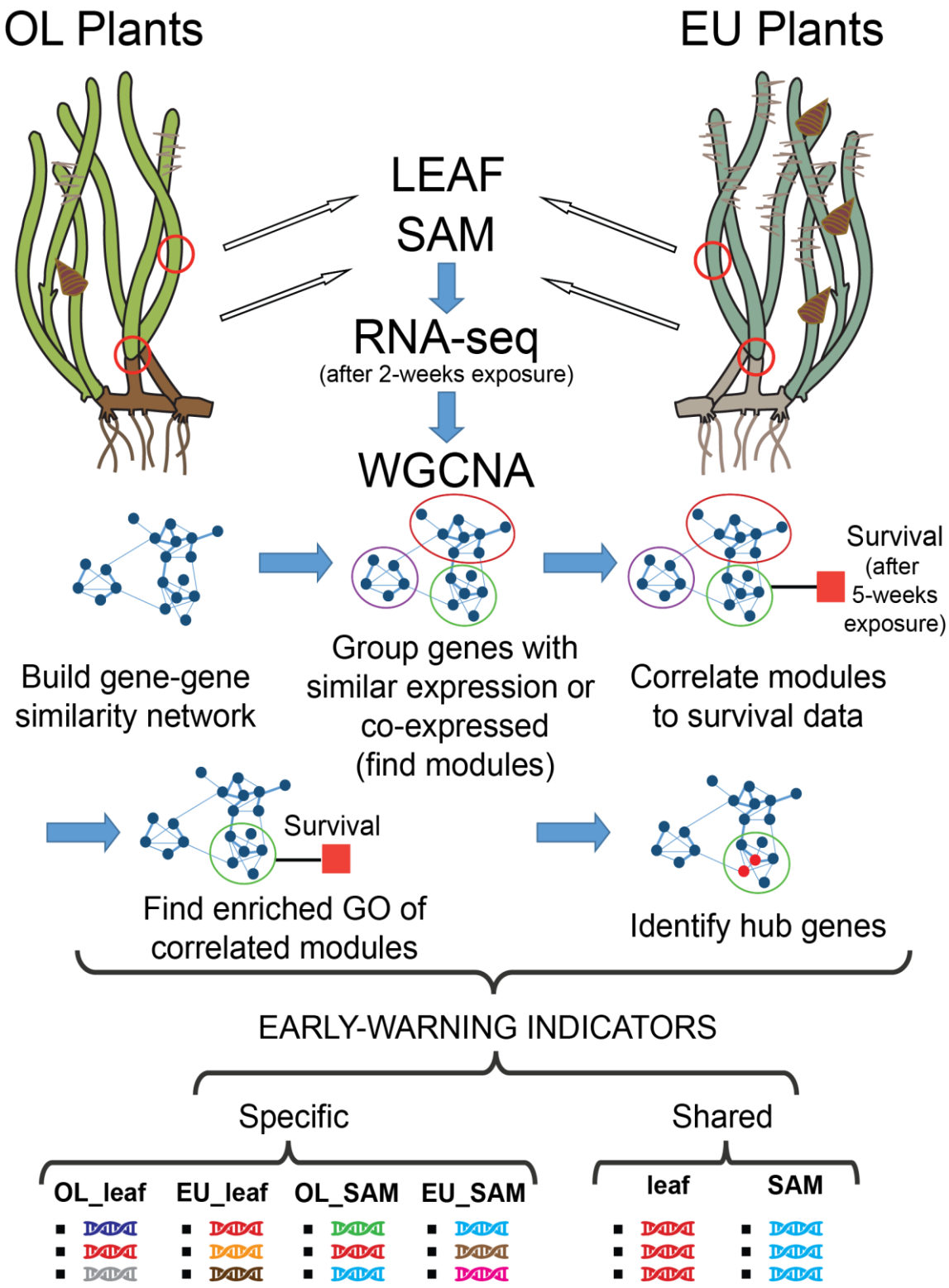


Figure 1. *Workflow of early warning indicators' selection method. After RNA extraction and sequencing from samples exposed for 2 weeks to stress. WGCNA was run using read counts to build 4 networks of gene co-expression. Then modules were identified within each network and correlated with shoot survival measured after 5 weeks of exposure to stress. Hub genes were identified within each module with a significant correlation with shoot survival.*

2.2. RNA extraction and sequencing

To analyse the early response to stress of the whole-genome expression pattern, fragments of 6 cm from the second ranked leaves (n = 3) and of 0.5 cm form the most apical portion of the rhizome tip (n = 3) containing the shoot apical meristem (SAM), were collected from orthotropic shoots after two weeks-exposure to treatments. Leaves were cleaned from epiphytes, immersed in RNA-later solution (Ambion, Life Technologies), held at 4 °C for one night and then stored at -20 °C. SAM material was cleaned from dead leaf tissue and sediment and then immediately frozen at -80 °C with liquid N. Total RNA was extracted from both plant tissues using the Aurum™ Total RNA Mini Kit (BIO-RAD) according to manufacturer's instructions. Concentration and purity of RNA were assess using a NanoDrop® (ND-1000) spectrophotometer (Thermo Fisher Scientific) and checked by 1.5% agarose gel electrophoresis. Only extracts with 260/280 nm and 260/230 nm absorbance ratios between 1.8 and 2.2 were selected for integrity analysis. Integrity of RNA extracts was assessed with an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc.). Samples with a RNA integrity number (RIN) $\geq$ 7 were used for library preparation. A total of 24 libraries were constructed for all treatments (C, T, N, NT; n = 3) for both sampling locations (OL, EU) per plant tissue (leaf, SAM) with the QuantSeq 3'mRNA-Seq Library Prep Kits (Lexogen, GmbH), according to manufacturer's specifications and using the Ion Chef™ system (ThermoFisher Scientific). Libraries were sequenced with an Ion GeneStudio S5 System (ThermoFisher Scientific).

2.3. Reads quality control and cleaning

The *P. oceanica* sequencing reads came from four different datasets divided into two different plant tissues: leaf and meristem, and two different collection sites: Ischia (OL) and Bacoli (EU). All datasets included 4 experimental conditions: Control, Temperature, Nutrient and Nutrient + Temperature (n = 3). The reads quality check was performed using FastQC (Andrews, 2010). Reads with a phred-score lower than 15 and a length lower than 50bp were trimmed using Trimmomatic (Bolger et al., 2014).

2.4. Mapping, counting and annotation

All the cleaned reads were mapped, independently, on the reference *P. oceanica* transcriptome (see (Ruocco et al., 2021) dataset using the Bowtie2 aligner with default settings (Langmead and Salzberg, 2012). Reads count calculation for each replicate was performed using the eXpress software (Roberts and Pachter, 2013). The entire reference transcriptome was aligned versus the SwissProt database using the BLASTx software (Camacho et al., 2009), with an e-value threshold of $1e^{-3}$.

2.5. Weighted gene co-expression network analysis

All four datasets were filtered to remove transcripts that did not reach a value of 10 counts in all the replicates in order to remove low abundant or non-varying genes that introduce noise into the analysis. Counts from filtered transcripts for all conditions in each dataset were used to generate four co-expression networks using the WGCNA package in R (Langfelder and Horvath, 2008). Independent unsigned networks were obtained from Ischia meristem (OL_SAM), Ischia leaves (OL_leaf), Bacoli meristem (EU_SAM) and Bacoli leaves (EU_leaf) samples. The adjacency matrix was constructed using a soft threshold power of 18 (See supplemental figure 1). Adjacency values were then transformed to topological overlap measure (TOM) using the *blockwiseModules* function (Zhang and Horvath, 2005) that constructs the gene networks and identifies modules. Modules within networks were identified using the dynamic tree cut algorithm (Langfelder et al., 2008) setting a minimum cluster size (*minModuleSize* parameter) of 30 and a merging threshold function (*mergeCutHeight* parameter) of 0.25.

2.6. Relating modules to shoot survival

To relate the plant traits after 5-week of stress exposure with the gene networks, the module eigengenes (ME, first principal component of each module) were correlated to the mortality data

(expressed as percentage of control shoot survival) reported in Pazzaglia et al., (2020). A *Student* asymptotic *p*-value for correlation was used by selecting the WGCNA *corPvalueStudent* function.

2.7. Functional analysis

To identify global patterns of responses between different datasets, a Gene Ontology (GO) enrichment analysis was performed with Ontologizer software (Bauer et al., 2008) in modules showing a significant correlation with shoot survival. The threshold used to identify significantly enriched functional terms was $P < 0.05$. The top 20 most represented GO terms in each module for the "Biological process" (BP) ontology were used for comparative analysis and visualised using the Treemap function of the Revigo online tool (Supek et al., 2011).

2.8. Hub transcripts selection

Central genes or "hubs" were identified within the modules that showed a significant correlation with shoot survival data. Highly connected genes were identified in two different ways: i) by selecting the transcripts from the top 20 most represented GO terms in each module; and ii) by using their module membership (MM) value, also known as eigengene-based connectivity k_{ME} (intramodular connectivity; Fuller et al., 2007), which represents the correlation between the expression level of a particular gene/transcript and the module eigengene (Horvath and Dong, 2008). Transcripts with the highest MM are highly connected within a particular module (Fuller et al., 2011). All the transcripts pooled from the modules were used to find shared transcripts between localities and between plant tissues and then to select the candidate marker genes.

3. Results

Gene co-expression

Each of the four datasets yielded a network with a different number of differentially expressed transcripts, being lower in OL than EU and ranging from 13,669 in OL_leaf to 16,705 in EU_SAM (Table 1). Accordingly, a different number of co-expression modules were identified within the four

datasets, being lower in OL for both plant tissues (leaf: 16 and SAM: 27) compared to EU (leaf: 24 and SAM: 46). Also, the amount of total co-expressed transcripts obtained from the analysis of leaves was lower compared to SAM for both sampled localities (Table 1).

Table 1. *Summary of the network transcripts data across the four datasets*

Network	N. of transcripts	N. of co-expression modules	Modules correlated to shoot survival
OL_Leaf	13669	16	1
OL_SAM	15025	27	2
EU_Leaf	14541	24	2
EU_SAM	16705	46	7

Module analysis

Several modules showed a significant correlation ($R > 0.6$; $P < 0.05$) with shoot survival and were selected for further analysis (Table 2). The module Turquoise was selected from the network built from OL_leaf, while in EU_leaf the modules selected for subsequent GO enrichment analysis and selection of hub genes were the Blue and Lightcyan. In the networks generated from SAM, the modules Lightgreen and Pink were selected in OL_SAM and modules Cyan, Darkmagenta, Lightyellow, Plum1, Purple, Red and Violet were selected in EU.

Table 2. *Modules selected according correlation with shoot survival for OL_leaf, EU_leaf, OL_SAM and EU_SAM networks. Correlation coefficient (R) and P-value are shown for each selected module.*

Network	Module	*R*	*p*
OL_LEAF	Turquoise	-0.62	0.03
EU_LEAF	Lightcyan	-0.71	0.01
	Blue	-0.68	0.02
OL_SAM	Lightgreen	0.87	0.0003
	Pink	0.66	0.02
EU_SAM	Darkmagenta	-0.8	0.008
	Violet	-0.7	0.01
	Red	-0.7	0.02
	Cyan	-0.7	0.01
	Lightyellow	-0.6	0.04

	Purple	-0.9	0.0002
	Plum1	0.6	0.04

GO enrichment

Many similarities were observed among datasets for the top 20 most represented enriched Biological Process (BP; Figure 2). In leaves, GO terms related to metabolic processes and response to stress were the most common (Figure 2a and b). Similarly, several metabolic and catabolic processes were found among the most common terms in OL_SAM and EU_SAM (Figure 2c and d). On the other hand, differences between leaves and meristems were more evident. In the OL plants, processes related to the metabolism of nitrogen compounds were more represented in SAM rather than in leaf (Figure 2a and c). Also, fewer processes associated with the response to stress or response to stimulus were found in OL_SAM than in OL_leaf. In contrast, in EU plants, processes associated with the response to stress were equally represented in SAM and in leaves (Figure 2b and d). Additionally, other processes, such as “cation transmembrane transport” (GO:0098655) and “signalling” (GO:0023052), were represented only in EU_SAM compared to the other datasets.

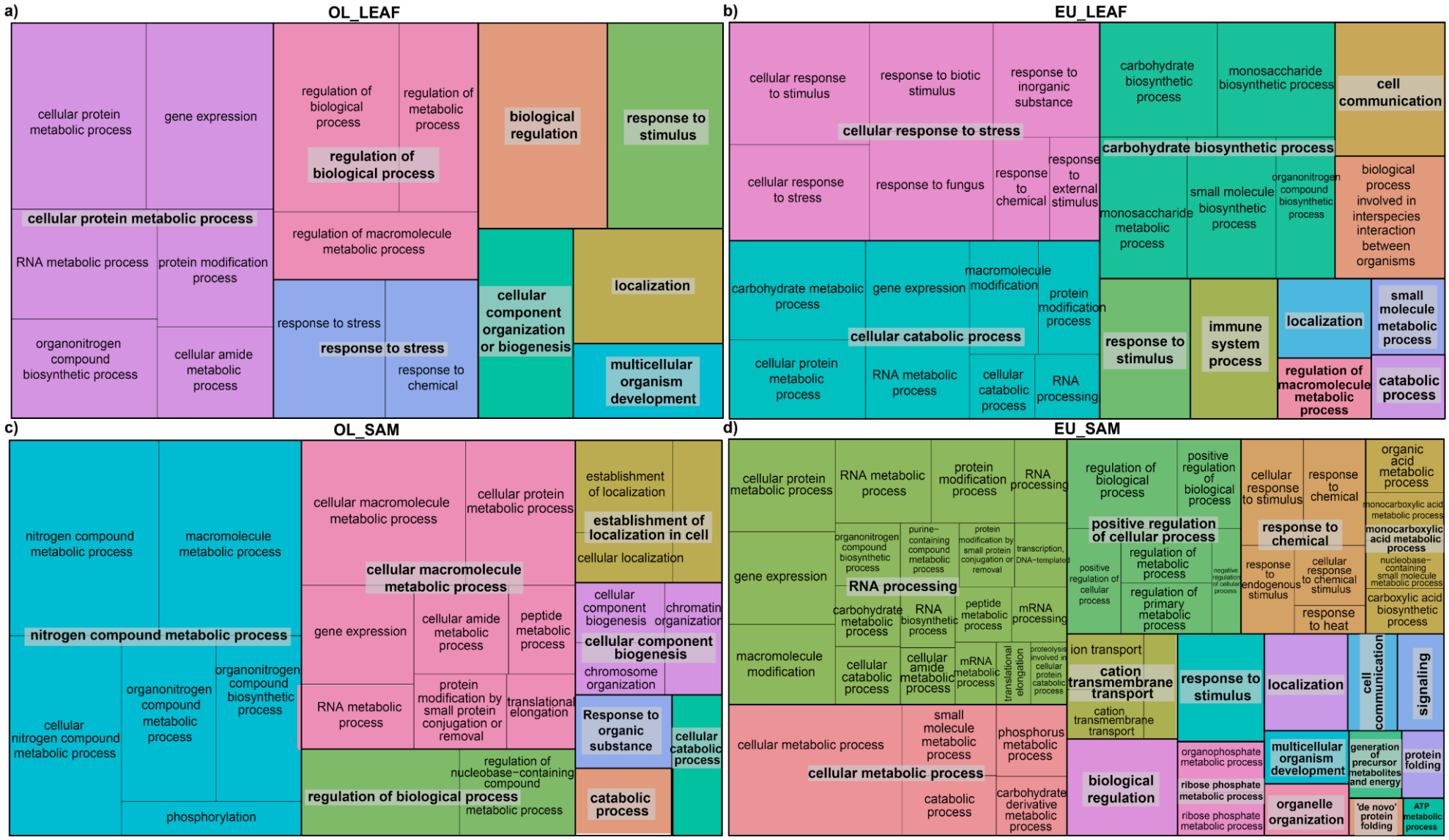


Figure 2*. Treemap visualization obtained with with the Revigo online tool of the most widespread GO enriched terms in the selected modules (based on samples exposed to 2 weeks of stress) that correlated with shoot survival (measured after 5 weeks of stress exposure) in a) OL_Leaf, b) EU_leaf, c) OL_SAM and d) EU_SAM.*

Several shared GO terms were found among the top 20 most represented BP ontologies in each of the modules selected from all datasets (Table 2). Five BPs were shared among all four datasets: "cellular protein metabolic process" (GO:0044267), "gene expression" (GO:0010467), "RNA metabolic process" (GO:0016070), "organonitrogen compound biosynthetic process" (GO:1901566) and "catabolic process" (GO:0009056). Shared BPs between OL_Leaf and EU_Leaf included "response to stimulus" (GO:0050896), "response to chemical" (GO:0042221), "regulation of macromolecule metabolic process" (GO:0060255), "regulation of primary metabolic process" (GO:0080090), while shared BPs between OL_SAM and EU_SAM included "cellular component biogenesis" (GO:0044085), "peptide metabolic process" (GO:0006518), "organic substance catabolic process" (GO:1901575), "amide biosynthetic process" (GO:0043604) among others. For a complete list of GO enriched terms for each module see supplemental File 1.

Table 3. *Top 20 most represented gene ontologies (Biological process: BP) for each module selected from all datasets. Shared BP terms within the same network are represented only once.*

GO_ID	**GO_description**	**Dataset**			
		OL_LEAF	EU_LEAF	OL_SAM	EU_SAM
GO:0044267	cellular protein metabolic process	√	√	√	√
GO:0010467	gene expression	√	√	√	√
GO:0016070	RNA metabolic process	√	√	√	√
GO:1901566	organonitrogen compound biosynthetic process	√	√	√	√
GO:0009056	catabolic process	√	√	√	√
GO:0050896	response to stimulus	√	√		√
GO:0060255	regulation of macromolecule metabolic process	√	√		√
GO:0036211	protein modification process	√	√		√
GO:0042221	response to chemical	√	√		√
GO:0080090	regulation of primary metabolic process	√	√		√
GO:0051179	Localization	√	√		√
GO:0043603	cellular amide metabolic process	√		√	√
GO:0050789	regulation of biological process	√		√	√
GO:0050794	regulation of cellular process	√		√	√
GO:0044248	cellular catabolic process		√	√	√
GO:2000112	regulation of cellular macromolecule biosynthetic process		√	√	√
GO:0044085	cellular component biogenesis			√	√
GO:0006518	peptide metabolic process			√	√
GO:1901575	organic substance catabolic process			√	√

GO:0043604	amide biosynthetic process			√	√
GO:0006414	translational elongation			√	√
GO:0070647	protein modification by small protein conjugation or removal			√	√
GO:0006412	Translation			√	√
GO:0043043	peptide biosynthetic process			√	√
GO:0065007	biological regulation	√			√
GO:0019222	regulation of metabolic process	√			√
GO:0031323	regulation of cellular metabolic process	√			√
GO:0051171	regulation of nitrogen compound metabolic process	√			√
GO:0007275	multicellular organism development	√			√
GO:0043412	macromolecule modification		√		√
GO:0044281	small molecule metabolic process		√		√
GO:0009889	regulation of biosynthetic process		√		√
GO:0031326	regulation of cellular biosynthetic process		√		√
GO:0006396	RNA processing		√		√
GO:0010556	regulation of macromolecule biosynthetic process		√		√
GO:0051716	cellular response to stimulus		√		√
GO:0007154	cell communication		√		√
GO:0044281	small molecule metabolic process		√		√
GO:0005975	carbohydrate metabolic process		√		√

Candidate genes selection

All the transcripts associated with the top 20 BPs from each selected module were pooled to find candidate markers according to their module membership (MM). A total of 3,673 transcripts were pooled in OL_leaf, compared to 262, 1,614 and 879 in OL_SAM, EU_leaf and EU_SAM, respectively (see supplemental File 2). The top 10 transcripts with the highest MM for each of the networks are reported in Table 4. In OL_Leaf, transcripts related to RNA processing (Swissprot accession: P29766 and Q943F3, respectively) were among the most central (i.e., hub transcripts with higher degree of connectivity) for the Turquoise module. Similarly, in OL_SAM the most central genes were related to RNA metabolism. In EU_leaf, hub transcripts related to “reactive oxygen metabolism” (P29448, Q9FE62, Q7XTY9), while in the meristem (EU_SAM) transcripts associated to the “synthesis of secondary metabolites” (Q9SD85), “defence response” (P93733, Q41350) and “response to stress” (Q93ZR6, P46423) were among the most central.

***Table 4**. Top ten hub transcripts per network: OL_leaf, EU_leaf, OL_SAM and EU_SAM. The transcript name, its associated swissprot protein ID and function, along with the module name and the value of module membership (MM), are shown.*

Transcript	Protein ID	Protein function	Module	MM
OL_LEAF				
pe_TR10932|c2_g1_i15	Q6ICX4	Polypyrimidine tract-binding protein homolog 3	Turquoise	0.974
pe_TR14440|c2_g1_i8	P29766	60S ribosomal protein L8	Turquoise	0.973
pe_TR37115|c1_g1_i9	P49689	40S ribosomal protein S30	Turquoise	0.970
pe_TR30735|c1_g1_i12	Q9SY02	Pentatricopeptide repeat-containing protein At4g02750	Turquoise	0.969
pe_TR16884|c1_g2_i2	P58684	Probable signal peptidase complex subunit 2 (EC 3.4.-.-)	Turquoise	0.967
pe_TR10288|c2_g6_i2	Q9D967	Magnesium-dependent phosphatase 1 (MDP-1) (EC 3.1.3.-)	Turquoise	0.964
pe_TR41959|c0_g2_i4	Q9XHS0	40S ribosomal protein S12	Turquoise	0.959
pe_TR24796|c0_g2_i1	P49027	Guanine nucleotide-binding protein subunit beta-like protein A	Turquoise	0.955
pe_TR45593|c0_g2_i6	Q0DJ99	Coatomer subunit delta-2 (Delta-coat protein 2)	Turquoise	0.953
pe_TR45392|c2_g1_i1	Q943F3	60S ribosomal protein L18a	Turquoise	0.953
OL_SAM				
pe_TR33281|c3_g1_i2	Q1MIC5	50S ribosomal protein L18	Pink	0.973
pe_TR30735|c1_g1_i1	Q9SY02	Pentatricopeptide repeat-containing protein At4g02750	Pink	0.970
pe2_TRINITY_DN55199_c0_g1_i1	O22922	U2 small nuclear ribonucleoprotein B" (U2 snRNP B")	Pink	0.968
pe_TR35140|c1_g3_i3	P93313	NADH-ubiquinone oxidoreductase chain 4 (EC 7.1.1.2)	Pink	0.965
se1_TR3178|c0_g1_i4	P27572	NADH-ubiquinone oxidoreductase chain 4 (EC 7.1.1.2)	Pink	0.963
pe_TR21334|c5_g3_i2	A6MMA7	ATP synthase subunit alpha, chloroplastic (EC 7.1.2.2)	Pink	0.960
pe_TR11939|c1_g2_i4	Q3ZCG8	ER membrane protein complex subunit 6	Pink	0.959
pe_TR6870|c0_g1_i6	P42322	Calcineurin subunit B (Calcineurin regulatory subunit)	Pink	0.950
pe_TR2303|c0_g1_i8	A0MDQ1	Ubiquitin-related modifier 1 homolog 1	LightGreen	0.945
pe_TR10138|c5_g2_i2	Q6ZJJ1	Probable L-ascorbate peroxidase 4, peroxisomal	Pink	0.942
EU_LEAF				
pe_TR44629|c5_g3_i8	P29448	Thioredoxin H1 (AtTrxh1)	Blue	0.962
pe_TR25097|c0_g1_i3	Q969N2	GPI transamidase component PIG-T (Phosphatidylinositol-glycan biosynthesis class T protein)	Blue	0.958
pe_TR21412|c3_g2_i3	Q8VC52	RNA-binding protein with multiple splicing 2	Blue	0.958
pe2_TRINITY_DN20851_c0_g1_i1	Q9ZRI7	Elongation factor 1-gamma 1 (EF-1-gamma 1)	Blue	0.949
pe_TR5428|c0_g1_i1	Q9FE62	Thioredoxin-like protein YLS8	Blue	0.947
pe_TR23348|c0_g6_i4	Q9M0V3	DNA damage-binding protein 1a	Blue	0.944
pe_TR38998|c0_g2_i4	Q9SRU2	Auxin transport protein BIG	Blue	0.943
pe_TR25369|c0_g1_i4	Q9LVI9	Dihydropyrimidine dehydrogenase (NADP(+)), chloroplastic	Blue	0.942
pe_TR32906|c3_g4_i3	Q7XTY9	Copper chaperone for superoxide dismutase, chloroplastic	Blue	0.941
pe_TR13479|c0_g1_i5	F4I096	Mediator of RNA polymerase II transcription subunit 13	Blue	0.941
EU_SAM				
pe_TR12992|c0_g1_i1	Q9SD85	Flavonoid 3'-monooxygenase (EC 1.14.14.82)	Purple	0.981
pe2_TRINITY_DN38505_c0_g1_i1	Q9FIJ4	DNA repair RAD52-like protein 2, chloroplastic	Purple	0.974
pe_TR43752|c5_g3_i9	P93733	Phospholipase D beta 1 (EC 3.1.4.4)	Purple	0.968

pe2_TRINITY_DN30268_c0_g2_i1	A2CIR7	BTB/POZ domain and ankyrin repeat-containing protein	Red	0.968
pe2_TRINITY_DN36199_c1_g1_i1	Q41350	Osmotin-like protein	Red	0.962
pe_TR39505|c4_g1_i1	Q9C9A6	U-box domain-containing protein 10 (EC 2.3.2.27)	Red	0.960
pe2_TRINITY_DN32264_c0_g1_i4	Q93ZR6	Wax ester synthase/diacylglycerol acyltransferase 1	Violet	0.958
pe_TR39962|c1_g2_i4	A2Z3C4	Probable 6-phosphogluconolactonase 4, chloroplastic	Purple	0.957
pe_TR39522|c0_g1_i3	O23627	Glycine--tRNA ligase, mitochondrial 1 (EC 6.1.1.14)	Red	0.957
pe_TR32334|c3_g1_i6	P46423	Glutathione S-transferase (EC 2.5.1.18)	Purple	0.957

In order to find shared transcripts between plant tissues from different sampling localities, transcript pools from OL and EU were compared. When comparing leaf networks, a total of 655 shared transcripts were found (Figure 3a). In contrast, only 11 shared transcripts resulted from the comparison between OL_SAM and EU_SAM (Figure 3b). The top 20 shared leaf transcripts according to the MM and the 11 transcripts shared in SAM from both localities are reported in Table 5.

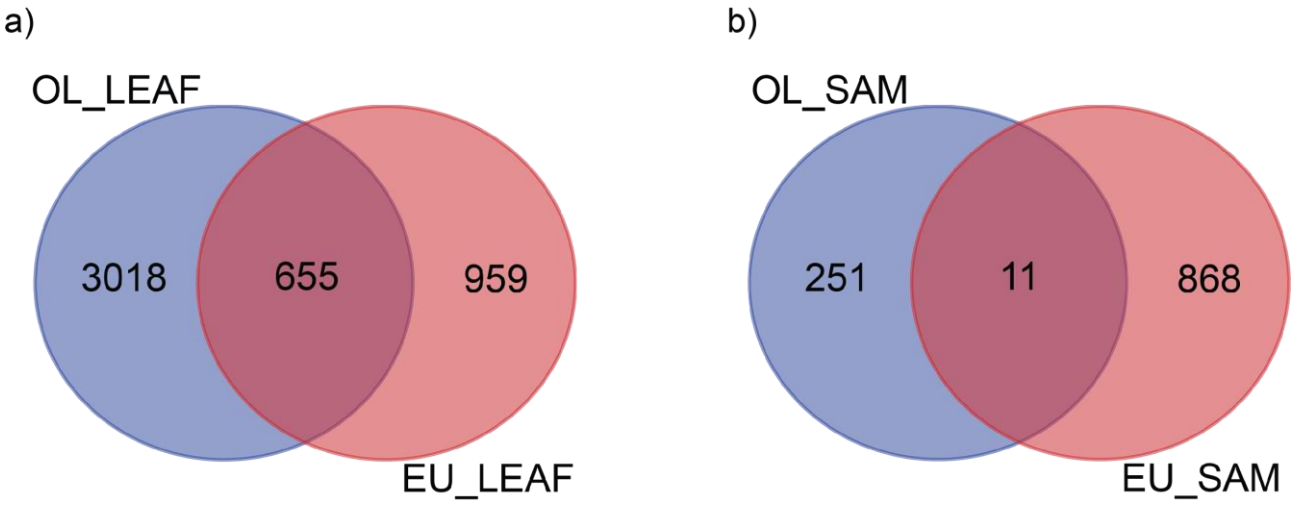


Figure 3*. Venn diagrams showing the number of unique and shared Hub transcripts in a) OL_leaf and EU_leaf; and b) OL_SAM and EU_SAM*

Table 5. *Hub transcripts shared between OL_leaf, EU_leaf (top) and OL_SAM and EU_SAM (bottom) with highest module membership (MM).*

Transcript	**Protein ID**	**Protein function**	**Module**	**MM**
LEAF				
pe_TR10288|c2_g6_i2	Q9D967	Magnesium-dependent phosphatase 1	Turquoise-OL_LEAF	0.964
pe_TR44629|c5_g3_i8	P29448	Thioredoxin H1 (AtTrxh1)	Blue-EU_LEAF	0.962
pe_TR21412|c3_g2_i3	Q8VC52	RNA-binding protein with multiple splicing 2	Blue-EU_LEAF	0.958
pe2_TRINITY_DN208	Q9ZRI7	Elongation factor 1-gamma 1	Blue-EU_LEAF	0.949

51_c0_g1_i1				
pe_TR5428|c0_g1_i1	Q9FE62	Thioredoxin-like protein YLS8	Blue-EU_LEAF	0.947
pe_TR34456|c2_g3_i1	Q9LJG8	Trihelix transcription factor ASIL2	Turquoise-OL_LEAF	0.945
pe_TR3549|c1_g1_i2	Q9LVP9	Vesicle transport v-SNARE 13	Turquoise-OL_LEAF	0.944
pe_TR32906|c3_g4_i3	Q7XTY9	Copper chaperone for superoxide dismutase, chloroplastic	Blue-EU_LEAF	0.941
pe_TR13479|c0_g1_i5	F4I096	Mediator of RNA polymerase II transcription subunit 13	Blue-EU_LEAF	0.941
pe_TR15144|c0_g1_i3	O75533	Splicing factor 3B subunit 1	Turquoise-OL_LEAF	0.940
pe_TR32719|c2_g1_i4	Q9SZL8	Protein FAR1-RELATED SEQUENCE 5	Blue-EU_LEAF	0.940
se1_TR12935|c0_g1_i6	Q941R4	GDP-mannose transporter GONST1	Blue-EU_LEAF	0.940
pe_TR42272|c5_g7_i2	O82782	1-(5-phosphoribosyl)-5-[(5-phosphoribosylamino)methylideneamino] imidazole-4-carboxamide isomerase, chloroplastic	Blue-EU_LEAF	0.939
pe2_TRINITY_DN35477_c1_g1_i2	Q6P4Y0	Threonylcarbamoyladenosine tRNA methylthiotransferase (EC 2.8.4.5)	Blue-EU_LEAF	0.938
pe_TR35097|c1_g2_i5	Q8S8F8	GLABRA2 expression modulator	Turquoise-OL_LEAF	0.936
pe_TR34459|c3_g9_i3	Q0JNR2	Cysteine proteinase inhibitor 12	Turquoise-OL_LEAF	0.933
se2_TRINITY_DN23173_c0_g2_i1	Q9FNR1	Glycine-rich RNA-binding protein 3, mitochondrial	Turquoise-OL_LEAF	0.932
pe_TR23092|c0_g1_i3	Q8LDP4	Peptidyl-prolyl cis-trans isomerase CYP19-4	Turquoise	0.928
pe_TR39052|c2_g1_i18	Q9ASP6	Heterogeneous nuclear ribonucleoprotein Q	Turquoise	0.927
pe_TR876|c0_g2_i8	Q9M2V1	Protein NCA1 (NO CATALASE ACTIVITY 1)	Turquoise	0.927
SAM				
pe_TR408|c0_g1_i5	O49697	NAC domain-containing protein 71	Violet-EU_SAM	0.932
se1_TR22293|c0_g4_i4	P42322	Calcineurin subunit B	Pink-OL_SAM	0.928
pe_TR2198|c1_g4_i8	P82163	30S ribosomal protein S13, chloroplastic	Pink-OL_SAM	0.926
pe_TR408|c0_g1_i6	O49697	NAC domain-containing protein 71	Violet-EU_SAM	0.920
pe_TR2198|c1_g4_i6	P82163	30S ribosomal protein S13, chloroplastic	Pink-OL_SAM	0.880
pe_TR39534|c2_g2_i2	O64530	Thiosulfate/3-mercaptopyruvate sulfurtransferase 1, mitochondrial	Pink-OL_SAM	0.812
pe_TR23056|c0_g2_i4	Q8VYB1	Mediator of RNA polymerase II transcription subunit 31	Pink-OL_SAM	0.797
pe_TR31925|c2_g2_i1	Q84WK5	Protein GID8 homolog	LightYellow-EU_SAM	0.787
pe_TR21175|c5_g1_i11	Q336M2	Cyclin-dependent kinase E-1	Cyan-EU_SAM	0.753
pe_TR15160|c1_g4_i2	Q9FNC9	Mitochondrial import receptor subunit TOM9-2	LightYellow-EU_SAM	0.694
pe_TR10527|c2_g1_i2	Q9M876	Tyrosine--tRNA ligase, chloroplastic/mitochondrial	Cyan-EU_SAM	0.654

As each network (OL_leaf, OL_SAM, EU_leaf and EU_SAM) was correlated with its corresponding shoot survival data for each group of plants, it was expected to find transcripts showing a different MM among the shared transcripts because they belonged to different modules in OL and EU networks (see supplemental file 2). In table 5, the value displayed corresponds to the highest MM for a particular module between the two networks (OL or EU), which means that the transcript was more central in the reported module within one of the two networks. Seven of the top

shared leaf transcripts shown in table 5, i.e. Q9D967 (Magnesium-dependent phosphatase 1), P29448 (Thioredoxin H1), Q8VC52 (RNA-binding protein with multiple splicing 2), Q9ZRI7 (Elongation factor 1-gamma 1), Q9FE62 (Thioredoxin-like protein YLS8), Q7XTY9 (Copper chaperone for superoxide dismutase, chloroplastic) and F4I096 (Mediator of RNA polymerase II transcription subunit 13) were also found in at least one of the networks reported in Table 4, meaning that even with a lower MM they were also present in the network of the other location (OL or EU). Transcripts that resulted unique in Table 4 have simply a higher MM compared to the ones reported in Table 5, but are not necessarily unique for the reported network. For the complete list of shared transcripts see supplemental file 2.

4. Discussion

In the present study, we have applied a system biology approach (WGCNA), based on the hypothesis that the transcriptome profiles of plants undergoing stress exposure could contain anticipatory molecular signals of stress related to shoot survival. These signals may change in plants with different stress history and can be exploited as potential early indicators of disruptive stress in the species. Our results confirm that the transcriptomic stress response of *P. oceanica* exhibits both common features (universality) and specific responses depending on plant tissue and plant stress history (Pazzaglia et al., 2022). Particularly, the response of the shoot apical meristem (SAM) appears to be more specific according to the collection site, and, therefore, the stress history of the plants. In contrast, leaf transcriptomes showed a greater convergence between locations in the response to stressors. Here, we discuss the resulting list of genes that show an anticipatory response to stress (mortality) and that could be potentially utilized as early warning indicators in seagrass management. Moreover, we discuss the implications of site-specific strategies to cope with stress, particularly heat and nutrient excess.

Global stress response in modules correlated with shoot survival

As observed in previous experiments (Helber et al., 2021; Pazzaglia et al., 2020), the physiological response to abiotic stress of plants from locations with different environmental conditions, is usually different. Therefore, in order to identify universal markers of stress, this study examined biological processes (BPs) that were shared between plants of different origin and under different abiotic stressors (excess nutrients and increased temperature) during their early stage of exposure to stress, when plant mortality was not yet visible. The "cellular metabolism" and "biosynthesis of nitrogenous compounds" GO terms were among the most widespread BPs shared in all datasets (leaf and SAM from both locations) (Table 3). These findings support the hypothesis of a continuous inorganic N assimilation by seagrasses in eutrophic waters, which causes a mobilization of carbon skeletons for the production of amino acids (Burkholder et al., 2007; Invers et al., 2004) which coincides with the decrease of non-structural carbohydrates observed by (Pazzaglia et al., 2020) after five-week exposure to N enrichment. In general, SAM response might be more dynamic than leaves, as by the larger amount of transcripts in the SAM networks and as previously observed in *P. oceanica* in response to light limitation (Ruocco et al., 2021). BPs involved in the response to external stimulus and chemical stimulus were shared in leaves and in EU_SAM, but not in OL_SAM. This supports previous observations (Pazzaglia et al., 2022), where the existence of regulatory defence machineries were already active in EU plants due to pre-exposure to higher nutrient inputs in the site of collection, which was also reflected in the physical weakness of rhizomes and shoots in contrast to OL plants (Helber et al., 2021; Pazzaglia et al., 2020), that resisted better the exposure to stress during the first two weeks of experiment.

Since WGCNA simultaneously analyses the response of transcripts not only to high nutrients treatment but also to the action of temperature and the combined stress, it is possible to attribute the presence of 'metabolic process', 'translation' and 'catabolic processes' found among the early response of both EU and OL plants, to heat stress, due to the acceleration of biochemical reactions triggered by increased temperature (Rasmusson et al., 2020). This could also contribute to

exacerbate the carbon imbalance of rhizomes and leaves after 5 weeks of exposure to stressors, particularly in EU plants, as previously highlighted by the comparison of physiological performance (Pazzaglia et al., 2020). The exposure to increased temperatures, indeed, induces the mobilisation and consumption of carbon reserves in *P. oceanica*, although the sensitivity of this response depends on the natural thermal environment in which the plants live (Marín-Guirao et al., 2018). OL plants appeared less susceptible to the experimental stress conditions, as no processes directly related to stress were observed among the most representative BPs in OL_SAM (Figure 2c). However, "response to heat" (GO:0009408) appeared among the most represented BPs only in EU_SAM (Figure 2d), showing that in general temperature stress affects more the SAM tissue of plants already stressed by eutrophic conditions (Pazzaglia et al., 2022). These complex interactions that occurred when both stressors were acting at the same time were reflected in the offset response of some physiological parameters reported in Pazzaglia et al. (2020).

Specific stress markers

Although similar biological processes were identified in all modules that correlated with shoot survival, the transcripts showing the highest MM differed among tissues and localities (Table 3). However, this did not necessarily mean that they were found exclusively in a particular dataset (see next section). Among the transcripts with highest MM in OL_Leaf, we found several genes involved in the processing of RNA (e.g. Pentatricopeptide repeat-containing protein), structural constituents of ribosomes (60S ribosomal protein L8, 40S ribosomal protein S30, 40S ribosomal protein S12 and 60S ribosomal protein L18a), splicing (Polypyrimidine tract-binding protein homolog 3) and protein modification and transport (Magnesium-dependent phosphatase 1, Signal peptidase complex subunit 2, Coatomer subunit delta-2). This seems to be related with the altered metabolism of OL plants and an increased organonitrogen compound biosynthesis rates due to temperature increase and nutrient excess. Additionally, some other specific markers were found in OL_Leaf (See additional file 2), such as a transcript annotated as Peroxiredoxin-2C (PRXIIC), a

thiol-specific peroxidase that plays a role in cell protection against oxidative stress by detoxifying peroxides (Cerveau et al., 2016). A transcript annotated for a "Flowering locus K homology domain" (FLK) was also found. This is a protein that operates in the autonomous flowering-promotive pathway in *Arabidopsis* by decreasing the transcript levels of the key flowering repressor "Flowering Locus C" (FLC, Ripoll et al., 2009), and a "FRIGIDA-like protein 3" (FRL3), which is involved in the upregulation of the expression of FLC (Jiang et al., 2009). Moreover, in addition to its role in flowering and development, FLK also contributes in regulating plants' defence against pathogens (Fabian et al., 2023). Flowering induction as early response to stress was already described in *P. oceanica* after experimental exposure to heat stress (Marín-Guirao et al., 2019; Ruiz et al., 2018) and is a common response observed in disturbed seagrass populations (Cabaço and Santos, 2012).

Constituents of the ribosome and splicing enzymes such as "50S ribosomal protein L18" (rplR) and "U2 small nuclear ribonucleoprotein B"" (U2B"), were also found among the top MM transcripts in OL_SAM. Transcripts involved in cellular respiration and primary metabolism such as "NADH-ubiquinone oxidoreductase chain 4" (ND4) and a "chloroplastic ATP synthase subunit alpha" (atpA) were also present. Increased respiration rate, and the consequent un-balance of P/R rate, in response to heat stress has already been described in *P. oceanica*, leading in the long term to a reduction in the carbohydrates content (Marín-Guirao et al., 2018, 2017, 2016). As a core subunit of the mitochondrial membrane respiratory chain NADH dehydrogenase (Complex I), ND4 is closely linked to cellular respiration and energy consumption which is also supported by atpA. Changes in the gene expression of atpA has been associated with mortality in a previous experiment in *P. oceanica* aimed to find early warning indicators of burial and nutrient stress (Ceccherelli et al., 2018). Also, the altered expression of these two genes has been related to the ability of *P. oceanica* to cope with increased temperatures (Marín-Guirao et al., 2017). Other transcripts involved in stress responses were found among the most central ones, e.g., a "BAG family molecular chaperone

regulator 4" (BAG4) and a "peroxisomal probable L-ascorbate peroxidase 4" (APX4). The former belongs to a family of proteins distinguished by a conserved BAG domain that directly interacts with Hsp70 and Hsc70 proteins, functioning as co-chaperones that participate in diverse cellular functions including stress responses, proliferation, migration, and cell death (Doukhanina et al., 2006; Irfan et al., 2021), the latter is a well-known antioxidant enzyme involved in the scavenging of reactive oxygen species (ROS). Annotated transcripts for protein modification included "ER membrane protein complex subunit 6" (EMC6) and various ubiquitination related proteins such as "ubiquitin-like-specific protease 2B" (ULP2B), which was shown to be unique for OL_SAM. The latter is a protease involved in Sumoylation (a post-transcriptional modification driven by small ubiquitin-like modifier: SUMO) and de-sumoylation which is involved in several responses to stress and nitrogen assimilation (Park et al., 2011).

In EU_leaf, the most central transcripts were related to the response to stress, growth and development. Among the others, we identified the "Thioredoxin-like protein YLS8" (Yellow Leaf Specific Gene 8), which has been previously suggested as a potential marker of senescence (Yoshida et al., 2001). The "chloroplastic copper chaperone for superoxide dismutase" (CCS) was also found. This gene has been already observed to respond to warming in *P. oceanica* as observed in a transcriptomic study and particularly in shallow-water plants (Marín-Guirao et al., 2017). It binds copper ions and delivers them specifically to Cu/Zn superoxide dismutases (SODs), the well-characterised scavengers of ROS, in order to activate them (Chu et al., 2005). Interestingly, a "Dihydropyrimidine dehydrogenase" (PYD1) was also found among the most central genes. This protein seems to be part of Amino-acid biosynthesis pathway (Cornelius et al., 2011). This may indicate that plants growing in the more eutrophic site have some mechanisms of nutrient metabolism already active, since this protein was also found in the meristematic tissue (not among the highest MM transcripts) but not in OL plants (see suppl. File 2). "The Auxin transport protein BIG" (BIG), required for auxin efflux and polar auxin transport, influences auxin-mediated

developmental responses (e.g. cell elongation, apical dominance, lateral root production, inflorescence architecture, general growth and development) and seems to be responsive to both high and low phosphate P conditions (Kanyuka et al., 2003; López-Bucio et al., 2005). Additionally, the important subunit 13 of the "mediator of RNA polymerase II transcription" (MED13), a coactivator involved in the regulated transcription of nearly all RNA polymerase II-dependent genes, was also central. This protein has been described as involved in several developmental processes such as embryo patterning, cotyledon organogenesis and flowering (Imura et al., 2012; Ito et al., 2011).

The meristem of plants growing under eutrophic conditions (EU_SAM) showed a more diverse response compared to other datasets as observed by the greater number of modules correlated with shoot survival (Table 1). Transcripts with the highest MM in the modules found for this dataset were mostly involved in senescence, defence and stress responses as it is the case of "phospholipase D beta 1" (PLDBETA1), "BTB/POZ domain and ankyrin repeat-containing protein NPR5" (NPR5) and "osmotin-like protein" (PR-5), all of them associated to the defence response to fungal pathogens (Anil Kumar et al., 2015; Yuan et al., 2007; Zhao et al., 2013). Another interesting transcript was found: a "wax ester synthase/diacylglycerol acyltransferase 1" (WSD1), which is involved in the cuticular wax biosynthesis (Li et al., 2008; Patwari et al., 2019) in turn associated to different responses to both defence to pathogens and to abiotic stress (Yeats and Rose, 2013). Additionally, a "glutathione S-transferase" (GST) was also found within the most central transcripts in the EU_SAM network. GST belongs to an ancient protein superfamily of detoxifying enzymes involved in diverse intracellular events such as primary and secondary metabolisms, stress metabolism, herbicide detoxification and plant protection against ozone damages, heavy metals, xenobiotics and microbial infections (Vaish et al., 2020). The higher incidence of transcripts related to pathogen defence and stress response among the most central for EU_SAM might indicate that they were experiencing a higher level of stress than OL plants even before collection, making them

prone to pathogen attack, which is in agreement with the results from the same experiment previously published (Pazzaglia et al., 2022, 2020), where EU plants showed a greater mortality at the end of the experiment (Pazzaglia et al., 2020). Also, transcripts such as "flavonoid 3'-monooxygenase" (CYP75B1) and "chalcone-flavanone isomerase 3" (CHI3), involved in the biosynthesis of flavonoids, were found. These transcripts are involved in a series of processes such as signalling pathways against pathogens and antioxidant metabolism (Brunetti et al., 2013). This is an interesting finding as the concentrations of flavonoids seems to be responsive to nutrient enrichment in *P. oceanica* (Cannac et al., 2006). All those transcripts support the hypothesis of a stronger and differential response of SAM (Ruocco et al., 2021), based on the environmental conditions at the collection site (Pazzaglia et al., 2022).

Shared stress markers

Although the networks obtained from SAM transcriptomes were larger in number of transcripts than the ones from leaves, the number of pooled transcripts in the modules correlated to shoot survival was lower in SAM (1,141) compared to leaf (5,287). Thus, we observe a greater number of shared "hub" transcripts between leaves than between SAMs from both locations. Among the shared transcripts for leaves, seven of those were also found among the most central for a specific network. For instance, the "thioredoxin-like protein YLS8", the "chloroplastic copper chaperone for superoxide dismutase" (CCS) and the "subunit 13 of the mediator of RNA polymerase II transcription", already discussed in the previous sections, are also shared in OL leaves and could be considered as early warning markers for leaf tissue (Figure 4). Other shared transcripts with high MM included a "GDP-mannose transporter" (GONST1) and a "heterogeneous nuclear ribonucleoprotein Q" (LIF2), both involved in defence from pathogens and immune responses (Le Roux et al., 2014; Yoo et al., 2020). Additionally, RNA processing transcripts such as the "glycine-rich RNA-binding protein 3" (RBG3) in the mitochondria and the "protein FAR1-related sequence 5" (FRS5) in the nucleus indicates the important role of transcriptional control in this tissue in

situations of stress (Kwak et al., 2005; Lin and Wang, 2004). Another important central transcript found to be shared in leaves is the "GLABRA2 expression modulator" (GEM), which is important in the control of "homeobox GLABRA2" (GL2), responsible for cell fate, via the maintenance of the repressor histone H3K9 methylation status (Caro et al., 2007). GEM has already been observed to be highly over expressed in *P. oceanica* after two weeks of exposure to heat (Marín-Guirao et al., 2019).

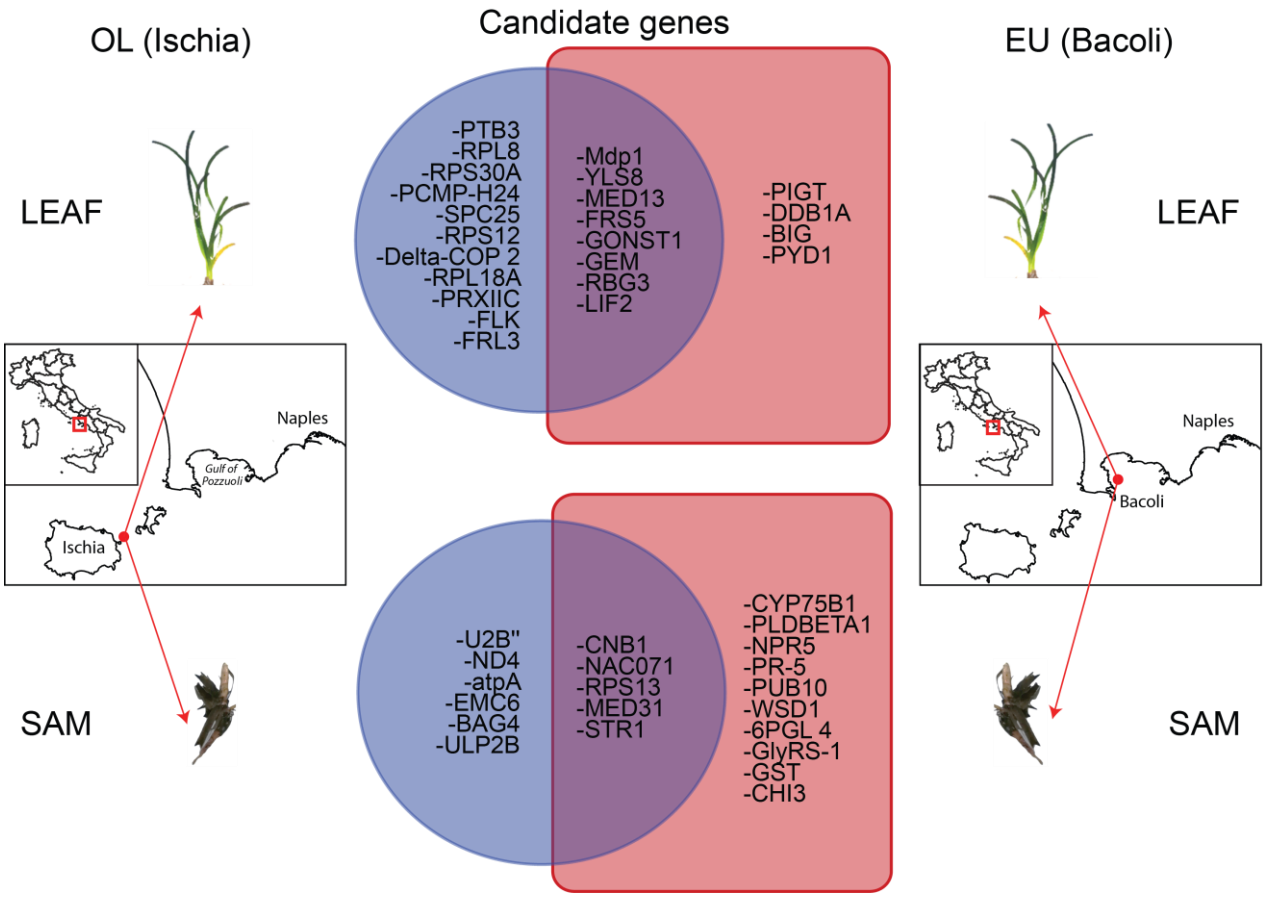


Figure 4*. Summary of potential early warning markers for heat and nutrient stress in P. oceanica. Shared and specific markers are show in the top Venn diagram for leaf tissue and in the bottom for SAMs. On the left side OL plants are reported and EU plants in the right side. See main text for acronyms.*

Contrary to leaves, only 11 transcripts were shared between SAM tissues from OL and EU. Two transcripts were annotated for a "NAC domain-containing protein 71" and two for a "chloroplastic 30S ribosomal protein S13", the former involved in the response to wounding and to auxin stimulus (Matsuoka et al., 2016; Pitaksaringkarn et al., 2014), the latter a constituent of the chloroplastic ribosome, responsible for the translation of the chloroplast genome-encoded proteins (Yamaguchi et

al., 2000). Also, the subunit 31 of the mediator of RNA polymerase II transcription (MED31) was observed in SAM, which implies the importance of the control of transcription as an important process in the early stages of stress responses. An important transcript involved in calcium signalling, the "calcineurin subunit B" (CNB1) was found to be central in both OL and EU apical meristems. This calcium-dependent, calmodulin-stimulated protein phosphatase has been previously observed to be regulated by stress signals such as drought, cold, and wounding (Jörg et al., 1999). Another interesting transcript found was the "thiosulfate/3-mercaptopyruvate sulfurtransferase 1" (STR1), which could contribute to oxidative stress resistance, mitochondrial respiratory function and the regulation of fatty acid metabolism (Pedre and Dick, 2021).

All these genes, with particular attention to the genes that have been already found to alter their expression levels (e.g., atpA, CCS, GEM) in response to stress such as high temperatures and light in *P. oceanica* (Ceccherelli et al., 2018; Marín-Guirao et al., 2019), will be targeted in further analysis as potential early warning indicators across environmental stress in *P. oceanica*.

Ecological significance

Evidence on the continuous decline of seagrasses points out the urgency for developing new efficient tools for monitoring and predicting stress signals in the marine environment. Seagrass restoration requires high number of resources and the success is not guaranteed (e.g., Pazzaglia et al., 2021; Boudouresque et al., 2021). Monitoring seagrass status, by means of indicators that are capable of detecting an early signal of stress, becomes of paramount importance particularly in slow growing and long living species such as *P. oceanica*. Traditional morphological and physiological methods cannot provide early enough signals of population decline (Macreadie et al., 2014; Ceccherelli et al., 2018). Thus, the application of WGCNA represents a first step towards developing high-throughput molecular monitoring tools. Since gene expression in response to stress can largely vary among organs and populations growing in different environments, only scanning a range of diverse populations and stress conditions would allow to find common early stress

indicators of population's decline. Our results provide a useful set of genes correlated to the survival of *P. oceanica*, which could contribute to detecting early population declines, identifying stress signals, and allowing the implementation of appropriate management strategies. Integrating similar analysis with seagrass monitoring programs can be a valuable resource for improving seagrass conservation efforts.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

Acknowledgment

This work was partially supported by the project SEA-Stress (Israeli-Italian Scientific and Technological Cooperation, MAECI, Italy), by the project Marine Hazard, PON03PE_00203_1, (Italian Ministry of Education, University and Research, MIUR). We thank the Molecular Biology and Sequencing and the Bioinforma Services of the Stazione Zoologica Anton Dohrn for transcriptome sequencing and bioinformatics analysis. We also thank the Secretariat of Higher Education, Science, Technology and Innovation (SENESCYT) for allowing AS taking part of this project.

References

Andrews, S., 2010. FastQC: a quality control tool for high throughput sequence data [WWW Document].

Anil Kumar, S., Hima Kumari, P., Shravan Kumar, G., Mohanalatha, C., Kavi Kishor, P.B., 2015. Osmotin: a plant sentinel and a possible agonist of mammalian adiponectin. Front. Plant Sci.

Bauer, S., Grossmann, S., Vingron, M., Robinson, P.N., 2008. Ontologizer 2.0—a multifunctional tool for GO term enrichment analysis and data exploration. Bioinformatics 24, 1650–1651. https://doi.org/10.1093/bioinformatics/btn250

Bellwood, D.R., Hughes, T.P., Folke, C., Nyström, M., 2004. Confronting the coral reef crisis. Nature 429, 827–833. https://doi.org/10.1038/nature02691

Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30, 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Boudouresque, C.F., Bernard, G., Pergent, G., Shili, A., Verlaque, M., 2009. Regression of Mediterranean seagrasses caused by natural processes and anthropogenic disturbances and stress: a critical review. Bot. Mar. 52, 395–418. https://doi.org/https://doi.org/10.1515/BOT.2009.057

Boudouresque, C.F., Blanfuné, A., Pergent, G., Thibaut, T., 2021. Restoration of seagrass meadows in the mediterranean sea: A critical review of effectiveness and ethical issues. Water (Switzerland). https://doi.org/10.3390/w13081034

Brunetti, C., Di Ferdinando, M., Fini, A., Pollastri, S., Tattini, M., 2013. Flavonoids as Antioxidants and Developmental Regulators: Relative Significance in Plants and Humans. Int. J. Mol. Sci. https://doi.org/10.3390/ijms14023540

Burkholder, J.M., Tomasko, D.A., Touchette, B.W., 2007. Seagrasses and eutrophication. J. Exp. Mar. Bio. Ecol. 350, 46–72. https://doi.org/https://doi.org/10.1016/j.jembe.2007.06.024

Cabaço, S., Santos, R., 2012. Seagrass reproductive effort as an ecological indicator of disturbance. Ecol. Indic. 23, 116–122. https://doi.org/https://doi.org/10.1016/j.ecolind.2012.03.022

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., Madden, T.L.,

2009. BLAST+: architecture and applications. BMC Bioinformatics 10, 421. https://doi.org/10.1186/1471-2105-10-421

Campagne, C.S., Salles, J.-M., Boissery, P., Deter, J., 2015. The seagrass *Posidonia oceanica*: Ecosystem services identification and economic evaluation of goods and benefits. Mar. Pollut. Bull. 97, 391–400. https://doi.org/https://doi.org/10.1016/j.marpolbul.2015.05.061

Cannac, M., Ferrat, L., Pergent-Martini, C., Pergent, G., Pasqualini, V., 2006. Effects of fish farming on flavonoids in *Posidonia oceanica*. Sci. Total Environ. 370, 91–98. https://doi.org/https://doi.org/10.1016/j.scitotenv.2006.07.016

Caro, E., Castellano, M.M., Gutierrez, C., 2007. A chromatin link that couples cell division to root epidermis patterning in *Arabidopsis*. Nature 447, 213–217. https://doi.org/10.1038/nature05763

Ceccherelli, G., Oliva, S., Pinna, S., Piazzi, L., Procaccini, G., Marin-Guirao, L., Dattolo, E., Gallia, R., La Manna, G., Gennaro, P., Costa, M.M., Barrote, I., Silva, J., Bulleri, F., 2018. Seagrass collapse due to synergistic stressors is not anticipated by phenological changes. Oecologia 186, 1137–1152. https://doi.org/10.1007/s00442-018-4075-9

Cerveau, D., Ouahrani, D., Marok, M.A., Blanchard, L., Rey, P., 2016. Physiological relevance of plant 2-Cys peroxiredoxin overoxidation level and oligomerization status. Plant. Cell Environ. 39, 103–119. https://doi.org/https://doi.org/10.1111/pce.12596

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/https://doi.org/10.1111/gcb.14401

Cheng, G., Zhang, L., Wang, H., Lu, J., Wei, H., Yu, S., 2020. Transcriptomic profiling of young cotyledons response to chilling stress in two contrasting cotton (*Gossypium hirsutum* l.) genotypes at the seedling stage. Int. J. Mol. Sci. 21, 1–26. https://doi.org/10.3390/ijms21145095

Chou, W.C., Cheng, A.L., Brotto, M., Chuang, C.Y., 2014. Visual gene-network analysis reveals the cancer gene co-expression in human endometrial cancer. BMC Genomics 15, 1–12. https://doi.org/10.1186/1471-2164-15-300

Chu, C.-C., Lee, W.-C., Guo, W.-Y., Pan, S.-M., Chen, L.-J., Li, H., Jinn, T.-L., 2005. A Copper Chaperone for Superoxide Dismutase That Confers Three Types of Copper/Zinc Superoxide Dismutase Activity in *Arabidopsis*. Plant Physiol. 139, 425–436. https://doi.org/10.1104/pp.105.065284

Cornelius, S., Witz, S., Rolletschek, H., Möhlmann, T., 2011. Pyrimidine degradation influences germination seedling growth and production of *Arabidopsis* seeds. J. Exp. Bot. 62, 5623–5632. https://doi.org/10.1093/jxb/err251

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/http://dx.doi.org/10.1016/j.gloenvcha.2014.04.002

Doukhanina, E. V, Chen, S., van der Zalm, E., Godzik, A., Reed, J., Dickman, M.B., 2006. Identification and Functional Characterization of the BAG Protein Family in *Arabidopsis thaliana*. J. Biol. Chem. 281, 18793–18801. https://doi.org/10.1074/jbc.M511794200

Drake, J.M., Griffen, B.D., 2010. Early warning signals of extinction in deteriorating environments. Nature 467, 456–459. https://doi.org/10.1038/nature09389

Duarte, C.M., Dennison, W.C., Orth, R.J.W., Carruthers, T.J.B., 2008. The Charisma of Coastal Ecosystems: Addressing the Imbalance. Estuaries and Coasts 31, 233–238. https://doi.org/10.1007/s12237-008-9038-7

Eisen, M.B., Spellman, P.T., Brown, P.O., Botstein, D., 1998. Cluster analysis and display of genome-wide expression patterns. Proc. Natl. Acad. Sci. 95, 14863–14868.

Emilsson, V., Thorleifsson, G., Zhang, B., Leonardson, A.S., Zink, F., Zhu, J., Carlson, S., Helgason, A., Walters, G.B., Gunnarsdottir, S., Mouy, M., Steinthorsdottir, V., Eiriksdottir, G.H., Bjornsdottir, G., Reynisdottir, I., Gudbjartsson, D., Helgadottir, A., Jonasdottir, Aslaug, Jonasdottir, Adalbjorg, Styrkarsdottir, U., Gretarsdottir, S., Magnusson, K.P., Stefansson, H., Fossdal, R., Kristjansson, K., Gislason, H.G., Stefansson, T., Leifsson, B.G., Thorsteinsdottir, U., Lamb, J.R., Gulcher, J.R., Reitman, M.L., Kong, A., Schadt, E.E., Stefansson, K., 2008. Genetics of gene expression and its effect on disease. Nature 452, 423–428. https://doi.org/10.1038/nature06758

Fabian, M., Gao, M., Zhang, X.-N., Shi, J., Vrydagh, L., Kim, S.-H., Patel, P., Hu, A.R., Lu, H., 2023. The Flowering Time Regulator FLK Controls Pathogen Defense in *Arabidopsis thaliana*. Plant Physiol kiad021. https://doi.org/10.1093/plphys/kiad021

Franssen, S.U., Gu, J., Bergmann, N., Winters, G., Klostermeier, U.C., Rosenstiel, P., Bornberg-Bauer, E., Reusch, T.B., 2011. Transcriptomic resilience to global warming in the seagrass *Zostera marina*, a marine foundation species. Proc. Natl. Acad. Sci. 108, 19276–19281. https://doi.org/10.1073/pnas.1107680108

Franssen, S.U., Gu, J., Winters, G., Huylmans, A.-K., Wienpahl, I., Sparwel, M., Coyer, J.A., Olsen, J.L., Reusch, T.B.H., Bornberg-Bauer, E., 2014. Genome-wide transcriptomic responses of the seagrasses *Zostera marina* and *Nanozostera noltii* under a simulated heatwave confirm functional types. Mar. Genomics 15, 65–73. https://doi.org/https://doi.org/10.1016/j.margen.2014.03.004

Fuller, T., Langfelder, P., Presson, A., Horvath, S., 2011. Review of Weighted Gene Coexpression Network Analysis, in: Lu, H.H.-S., Schölkopf, B., Zhao, H. (Eds.), Handbook of Statistical Bioinformatics. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 369–388. https://doi.org/10.1007/978-3-642-16345-6_18

Fuller, T.F., Ghazalpour, A., Aten, J.E., Drake, T.A., Lusis, A.J., Horvath, S., 2007. Weighted gene coexpression network analysis strategies applied to mouse weight. Mamm. Genome 18, 463–472. https://doi.org/10.1007/s00335-007-9043-3

Goel, P., Sharma, N.K., Bhuria, M., Sharma, V., Chauhan, R., Pathania, S., Swarnkar, M.K., Chawla, V., Acharya, V., Shankar, R., Singh, A.K., 2018. Transcriptome and Co-Expression Network Analyses Identify Key Genes Regulating Nitrogen Use Efficiency in *Brassica juncea* L. Sci. Rep. 8, 1–18. https://doi.org/10.1038/s41598-018-25826-6

Greenham, K., Guadagno, C.R., Gehan, M.A., Mockler, T.C., Weinig, C., Ewers, B.E., McClung, C.R., 2017. Temporal network analysis identifies early physiological and transcriptomic indicators of mild drought in brassica rapa. Elife 6, 1–26. https://doi.org/10.7554/eLife.29655

Helber, S.B., Procaccini, G., Belshe, E.F., Santillan-Sarmiento, A., Cardini, U., Bröhl, S., Schmid, M., Reuter, H., Teichberg, M., 2021. Unusually Warm Summer Temperatures Exacerbate Population and Plant Level Response of *Posidonia oceanica* to Anthropogenic Nutrient Stress . Front. Plant Sci.

Horvath, S., Dong, J., 2008. Geometric Interpretation of Gene Coexpression Network Analysis. PLOS Comput. Biol. 4, e1000117.

Imura, Y., Kobayashi, Y., Yamamoto, S., Furutani, M., Tasaka, M., Abe, M., Araki, T., 2012. CRYPTIC PRECOCIOUS/MED12 is a Novel Flowering Regulator with Multiple Target Steps in Arabidopsis. Plant Cell Physiol. 53, 287–303. https://doi.org/10.1093/pcp/pcs002

Invers, O., Kraemer, G.P., Pérez, M., Romero, J., 2004. Effects of nitrogen addition on nitrogen metabolism and carbon reserves in the temperate seagrass *Posidonia oceanica*. J. Exp. Mar. Bio. Ecol. 303, 97–114. https://doi.org/https://doi.org/10.1016/j.jembe.2003.11.005

Irfan, M., Kumar, P., Ahmad, I., Datta, A., 2021. Unraveling the role of tomato Bcl-2-associated athanogene (BAG) proteins during abiotic stress response and fruit ripening. Sci. Rep. 11, 21734. https://doi.org/10.1038/s41598-021-01185-7

Ito, J., Sono, T., Tasaka, M., Furutani, M., 2011. MACCHI-BOU 2 is Required for Early Embryo Patterning and Cotyledon Organogenesis in *Arabidopsis*. Plant Cell Physiol. 52, 539–552. https://doi.org/10.1093/pcp/pcr013

Jiang, D., Gu, X., He, Y., 2009. Establishment of the Winter-Annual Growth Habit via FRIGIDA-Mediated Histone Methylation at FLOWERING LOCUS C in *Arabidopsis*. Plant Cell 21, 1733–1746. https://doi.org/10.1105/tpc.109.067967

Jordà, G., Marbà, N., Duarte, C.M., 2012. Mediterranean seagrass vulnerable to regional climate warming. Nat. Clim. Chang. 2, 821–824. https://doi.org/10.1038/nclimate1533

Jörg, K., Qiang, X., Klaus, H., Wilhelm, G., Sheng, L., 1999. Genes for calcineurin B-like proteins in Arabidopsis are differentially regulated by stress signals. Proc. Natl. Acad. Sci. 96, 4718–4723. https://doi.org/10.1073/pnas.96.8.4718

Kanyuka, K., Praekelt, U., Franklin, K.A., Billingham, O.E., Hooley, R., Whitelam, G.C., Halliday, K.J., 2003. Mutations in the huge *Arabidopsis* gene BIG affect a range of hormone and light responses. Plant J. 35, 57–70. https://doi.org/https://doi.org/10.1046/j.1365-313X.2003.01779.x

Kenkel, C.D., Matz, M. V., 2017. Gene expression plasticity as a mechanism of coral adaptation to a variable environment. Nat. Ecol. Evol. 1, 1–7. https://doi.org/10.1038/s41559-016-0014

Kwak, K.J., Kim, Y.O., Kang, H., 2005. Characterization of transgenic *Arabidopsis* plants overexpressing GR-RBP4 under high salinity, dehydration, or cold stress. J. Exp. Bot. 56, 3007–3016. https://doi.org/10.1093/jxb/eri298

Langfelder, P., Horvath, S., 2008. WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics 9, 559. https://doi.org/10.1186/1471-2105-9-559

Langfelder, P., Zhang, B., Horvath, S., 2008. Defining clusters from a hierarchical cluster tree: The Dynamic Tree Cut package for R. Bioinformatics 24, 719–720. https://doi.org/10.1093/bioinformatics/btm563

Langmead, B., Salzberg, S.L., 2012. Fast gapped-read alignment with Bowtie 2. Nat. Methods 9, 357–359. https://doi.org/10.1038/nmeth.1923

Le Roux, C., Del Prete, S., Boutet-Mercey, S., Perreau, F., Balagué, C., Roby, D., Fagard, M., Gaudin, V., 2014. The hnRNP-Q Protein LIF2 Participates in the Plant Immune Response. PLoS One 9, e99343.

Lefcheck, J.S., Wilcox, D.J., Murphy, R.R., Marion, S.R., Orth, R.J., 2017. Multiple stressors threaten the imperiled coastal foundation species eelgrass (*Zostera marina*) in Chesapeake Bay, USA. Glob. Chang. Biol. 23, 3474–3483. https://doi.org/https://doi.org/10.1111/gcb.13623

Les, D.H., Cleland, M.A., Waycott, M., 1997. Phylogenetic Studies in Alismatidae, II: Evolution of Marine Angiosperms (Seagrasses) and Hydrophily. Syst. Bot. 22, 443–463. https://doi.org/10.2307/2419820

Li, F., Wu, X., Lam, P., Bird, D., Zheng, H., Samuels, L., Jetter, R., Kunst, L., 2008. Identification of the Wax Ester Synthase/Acyl-Coenzyme A:Diacylglycerol Acyltransferase WSD1 Required for Stem Wax Ester Biosynthesis in Arabidopsis. Plant Physiol. 148, 97–107. https://doi.org/10.1104/pp.108.123471

Lin, R., Wang, H., 2004. Arabidopsis FHY3/FAR1 Gene Family and Distinct Roles of Its Members in Light Control of Arabidopsis Development. Plant Physiol. 136, 4010–4022. https://doi.org/10.1104/pp.104.052191

López-Bucio, J., Hernández-Abreu, E., Sánchez-Calderón, L., Pérez-Torres, A., Rampey, R.A., Bartel, B., Herrera-Estrella, L., 2005. An Auxin Transport Independent Pathway Is Involved in Phosphate Stress-Induced Root Architectural Alterations in *Arabidopsis*. Identification of BIG as a Mediator of Auxin in Pericycle Cell Activation. Plant Physiol. 137, 681–691. https://doi.org/10.1104/pp.104.049577

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. Indic. 38, 279–281. https://doi.org/https://doi.org/10.1016/j.ecolind.2013.11.017

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/https://doi.org/10.1016/j.marpolbul.2018.07.050

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. https://doi.org/10.3389/fpls.2017.01142

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. 28, 2486–2501. https://doi.org/10.1111/mec.15089

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, 28615. https://doi.org/10.1038/srep28615

Matsuoka, K., Sugawara, E., Aoki, R., Takuma, K., Terao-Morita, M., Satoh, S., Asahina, M., 2016. Differential Cellular Control by Cotyledon-Derived Phytohormones Involved in Graft Reunion of *Arabidopsis* Hypocotyls. Plant Cell Physiol. 57, 2620–2631. https://doi.org/10.1093/pcp/pcw177

McKenzie, L.J., Collier, C.., Langlois, L.A., Yoshida, R.L., Uusitalo, J., Waycott, M., 2021. Marine Monitoring Program: Annual Report for Inshore Seagrass Monitoring 2018–19. Townsville.

Mishra, D.C., Arora, D., Kumar, R.R., Goswami, S., Varshney, S., Budhlakoti, N., Kumar, S., Chaturvedi, K.K., Sharma, A., Chinnusamy, V., Rai, A., 2021. Weighted gene co-expression analysis for identification of key genes regulating heat stress in wheat. Cereal Res. Commun. 49, 73–81. https://doi.org/10.1007/s42976-020-00072-7

Moore, K.A., Shields, E.C., Parrish, D.B., 2014. Impacts of Varying Estuarine Temperature and Light Conditions on *Zostera marina* (Eelgrass) and its Interactions With *Ruppia maritima* (Widgeongrass). Estuaries and Coasts 37, 20–30. https://doi.org/10.1007/s12237-013-9667-3

Mtwana Nordlund, L., Koch, E.W., Barbier, E.B., Creed, J.C., 2016. Seagrass Ecosystem Services and Their Variability across Genera and Geographical Regions. PLoS One 11, e0163091.

Mueller, A.J., Canty-Laird, E.G., Clegg, P.D., Tew, S.R., 2017. Cross-species gene modules emerge from a systems biology approach to osteoarthritis. npj Syst. Biol. Appl. 3, 1–14. https://doi.org/10.1038/s41540-017-0014-3

Mvungi, E.F., Pillay, D., 2019. Eutrophication overrides warming as a stressor for a temperate African seagrass (*Zostera capensis*). PLoS One 14, e0215129.

Nejrup, L.B., Pedersen, M.F., 2008. Effects of salinity and water temperature on the ecological performance of *Zostera marina*. Aquat. Bot. 88, 239–246. https://doi.org/https://doi.org/10.1016/j.aquabot.2007.10.006

Park, H.J., Kim, W.-Y., Park, H.C., Lee, S.Y., Bohnert, H.J., Yun, D.-J., 2011. SUMO and SUMOylation in plants. Mol. Cells 32, 305. https://doi.org/10.1007/s10059-011-0122-7

Patwari, P., Salewski, V., Gutbrod, K., Kreszies, T., Dresen-Scholz, B., Peisker, H., Steiner, U., Meyer, A.J., Schreiber, L., Dörmann, P., 2019. Surface wax esters contribute to drought tolerance in *Arabidopsis*. Plant J. 98, 727–744. https://doi.org/https://doi.org/10.1111/tpj.14269

Pazzaglia, J., Nguyen, H.M., Santillán-Sarmiento, A., Ruocco, M., Dattolo, E., Marín-Guirao, L., Procaccini, G., 2021. Review the genetic component of seagrass restoration: What we know and the way forwards. Water (Switzerland) 13. https://doi.org/10.3390/w13060829

Pazzaglia, J., Reusch, T.B.H., Terlizzi, A., Marín-Guirao, L., Procaccini, G., 2021. Phenotypic plasticity under rapid global changes: The intrinsic force for future seagrasses survival. Evol. Appl. 14, 1181–1201. https://doi.org/https://doi.org/10.1111/eva.13212

Pazzaglia, J., Santillán-Sarmiento, A., Helber, S.B., Ruocco, M., Terlizzi, A., Marín-Guirao, L., Procaccini, G., 2020. Does Warming Enhance the Effects of Eutrophication in the Seagrass *Posidonia oceanica*?. Front. Mar. Sci.

Pazzaglia, J., Santillán-Sarmiento, A., Ruocco, M., Dattolo, E., Ambrosino, L., Marín-Guirao, L., Procaccini, G., 2022. Local environment modulates whole-transcriptome expression in the seagrass *Posidonia oceanica* under warming and nutrients excess. Environ. Pollut. 303, 119077. https://doi.org/https://doi.org/10.1016/j.envpol.2022.119077

Pedre, B., Dick, T.P., 2021. 3-Mercaptopyruvate sulfurtransferase: an enzyme at the crossroads of sulfane sulfur trafficking. Biol. Chem. 402, 223–237. https://doi.org/doi:10.1515/hsz-2020-0249

Pergent-Martini, C., Pergent, G., Monnier, B., Boudouresque, C.-F., Mori, C., Valette-Sansevin, A., 2021. Contribution of *Posidonia oceanica* meadows in the context of climate change mitigation in the Mediterranean Sea. Mar. Environ. Res. 165, 105236. https://doi.org/https://doi.org/10.1016/j.marenvres.2020.105236

Pigliucci, M., 1996. How organisms respond to environmental changes: from phenotypes to molecules (and vice versa). Trends Ecol. Evol. 11, 168–173.

Pitaksaringkarn, W., Matsuoka, K., Asahina, M., Miura, K., Sage-Ono, K., Ono, M., Yokoyama, R., Nishitani, K., Ishii, T., Iwai, H., Satoh, S., 2014. XTH20 and XTH19 regulated by ANAC071 under auxin flow are involved in cell proliferation in incised *Arabidopsis* inflorescence stems. Plant J. 80, 604–614. https://doi.org/https://doi.org/10.1111/tpj.12654

Qiu, J., Du, Z., Wang, Y., Zhou, Y., Zhang, Y., Xie, Y., Lv, Q., Fan, H., 2019. Weighted gene co-

expression network analysis reveals modules and hub genes associated with the development of breast cancer. Med. (United States) 98, 1–10. https://doi.org/10.1097/MD.0000000000014345

Rasmusson, L.M., Buapet, P., George, R., Gullström, M., Gunnarsson, P.C.B., Björk, M., 2020. Effects of temperature and hypoxia on respiration, photorespiration, and photosynthesis of seagrass leaves from contrasting temperature regimes. ICES J. Mar. Sci. 77, 2056–2065. https://doi.org/10.1093/icesjms/fsaa093

Ripoll, J.J., Rodríguez-Cazorla, E., González-Reig, S., Andújar, A., Alonso-Cantabrana, H., Perez-Amador, M.A., Carbonell, J., Martínez-Laborda, A., Vera, A., 2009. Antagonistic interactions between *Arabidopsis* K-homology domain genes uncover PEPPER as a positive regulator of the central floral repressor FLOWERING LOCUS C. Dev. Biol. 333, 251–262. https://doi.org/https://doi.org/10.1016/j.ydbio.2009.06.035

Roberts, A., Pachter, L., 2013. Streaming fragment assignment for real-time analysis of sequencing experiments. Nat. Methods 10, 71–73. https://doi.org/10.1038/nmeth.2251

Rose, N.H., Seneca, F.O., Palumbi, S.R., 2016. Gene networks in the wild: Identifying transcriptional modules that mediate coral resistance to experimental heat stress. Genome Biol. Evol. 8, 243–252. https://doi.org/10.1093/gbe/evv258

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/https://doi.org/10.1016/j.marpolbul.2017.10.037

Ruocco, M., Entrambasaguas, L., Dattolo, E., Milito, A., Marín-Guirao, L., Procaccini, G., 2021. 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., 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

Scheffer, M., Bascompte, J., Brock, W.A., Brovkin, V., Carpenter, S.R., Dakos, V., Held, H., Van Nes, E.H., Rietkerk, M., Sugihara, G., 2009. Early-warning signals for critical transitions. Nature 461, 53–59. https://doi.org/10.1038/nature08227

Supek, F., Bošnjak, M., Škunca, N., Šmuc, T., 2011. REVIGO Summarizes and Visualizes Long Lists of Gene Ontology Terms. PLoS One 6, e21800.

Telesca, L., Belluscio, A., Criscoli, A., Ardizzone, G., Apostolaki, E.T., Fraschetti, S., Gristina, M., Knittweis, L., Martin, C.S., Pergent, G., Alagna, A., Badalamenti, F., Garofalo, G., Gerakaris, V., Louise Pace, M., Pergent-Martini, C., Salomidi, M., 2015. Seagrass meadows (*Posidonia oceanica*) distribution and trajectories of change. Sci. Rep. 5, 12505. https://doi.org/10.1038/srep12505

Vaish, S., Gupta, D., Mehrotra, R., Mehrotra, S., Basantani, M.K., 2020. Glutathione S-transferase: a versatile protein family. 3 Biotech 10, 321. https://doi.org/10.1007/s13205-020-02312-3

Wang, Z., Gerstein, M., Snyder, M., 2009. RNA-Seq: a revolutionary tool for transcriptomics. Nat. Rev. Genet. 10, 57–63. https://doi.org/10.1038/nrg2484

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

Weissmann, H., Shnerb, N.M., 2016. Predicting catastrophic shifts. J. Theor. Biol. 397, 128–134. https://doi.org/10.1016/j.jtbi.2016.02.033

Yamaguchi, K., von Knoblauch, K., Subramanian, A.R., 2000. The Plastid Ribosomal Proteins: IDENTIFICATION OF ALL THE PROTEINS IN THE 30 S SUBUNIT OF AN ORGANELLE RIBOSOME (CHLOROPLAST) *. J. Biol. Chem. 275, 28455–28465. https://doi.org/10.1074/jbc.M004350200

Yeats, T.H., Rose, J.K.C., 2013. The Formation and Function of Plant Cuticles. Plant Physiol. 163, 5–20. https://doi.org/10.1104/pp.113.222737

Yoo, H., Greene, G.H., Yuan, M., Xu, G., Burton, D., Liu, L., Marqués, J., Dong, X., 2020. Translational Regulation of Metabolic Dynamics during Effector-Triggered Immunity. Mol. Plant 13, 88–98. https://doi.org/10.1016/j.molp.2019.09.009

Yoshida, S., Ito, M., Nishida, I., Watanabe, A., 2001. Isolation and RNA Gel Blot Analysis of Genes that Could Serve as Potential Molecular Markers for Leaf Senescence in *Arabidopsis thaliana*. Plant Cell Physiol. 42, 170–178. https://doi.org/10.1093/pcp/pce021

Yuan, Y., Zhong, S., Li, Qun, Zhu, Z., Lou, Y., Wang, L., Wang, J., Wang, M., Li, Qiaoli, Yang, D., He, Z., 2007. Functional analysis of rice NPR1-like genes reveals that OsNPR1/NH1 is the rice orthologue conferring disease resistance with enhanced herbivore susceptibility†. Plant Biotechnol. J. 5, 313–324. https://doi.org/https://doi.org/10.1111/j.1467-7652.2007.00243.x

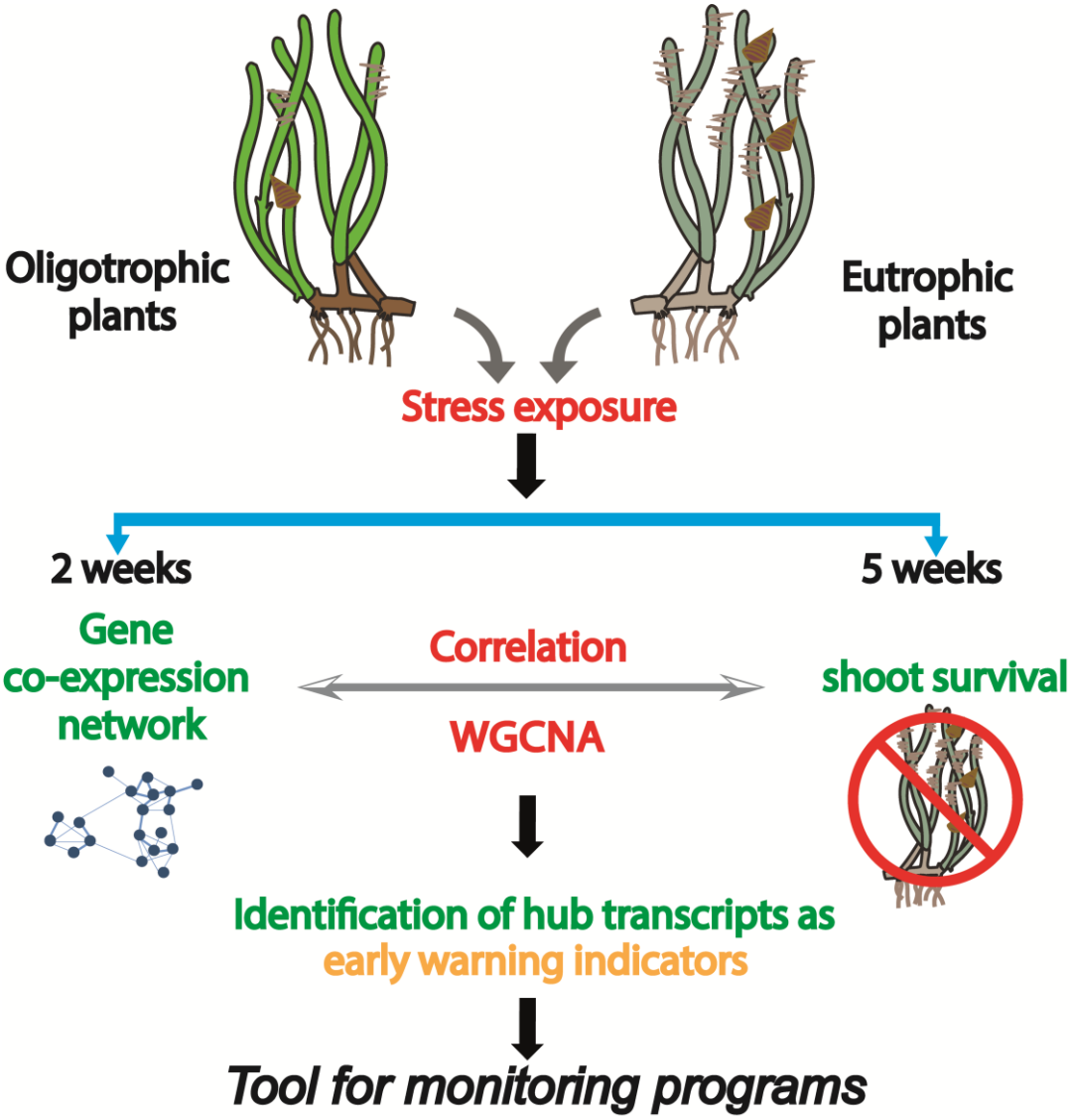
Zhang, B., Horvath, S., 2005. A General Framework for Weighted Gene Co- Expression Network Analysis. Stat. Appl. Genet. Mol. Biol. 4, Article17.

Zhao, J., Devaiah, S.P., Wang, C., Li, M., Welti, R., Wang, X., 2013. *Arabidopsis* phospholipase Dβ1 modulates defense responses to bacterial and fungal pathogens. New Phytol. 199, 228–240. https://doi.org/https://doi.org/10.1111/nph.12256

Zhao, X., Yu, H., Kong, L., Li, Q., 2016. Gene Co-Expression Network Analysis Reveals the Correlation Patterns Among Genes in Euryhaline Adaptation of *Crassostrea gigas*. Mar. Biotechnol. 18, 535–544. https://doi.org/10.1007/s10126-016-9715-7

Author Contributions Statement

Alex Santillán-Sarmiento: Methodology, Investigation, Writing - Original Draft.
Jessica Pazzaglia: Methodology, Investigation, Writing - Review & Editing
Miriam Ruocco: Methodology, Writing - Review & Editing.
Emanuela Dattolo: Methodology, Writing - Review & Editing.
Luca Ambrosino: Methodology, Writing -Review & Editing.
Gidon Winters: Conceptualization, Funding Acquisition, Writing - Review & Editing
Lázaro Marin-Guirao: Conceptualization, Supervision, Writing - Review & Editing
Gabriele Procaccini: Conceptualization, Supervision, Writing - Review & Editing, Funding acquisition, Project administration



Graphical abstract

Highlights

- Transcriptomic response comprises stress signals that could anticipate mortality
- Transcriptomic stress response exhibits both general and specific responses
- Meristematic tissue shows dynamic stress response depending on plant origin
- Found hub transcripts can be tested as potential marker of stress using RT-qPCR