

DYNAMIC DNA METHYLATION IN *POSIDONIA OCEANICA* UNDER MULTIPLE STRESSES

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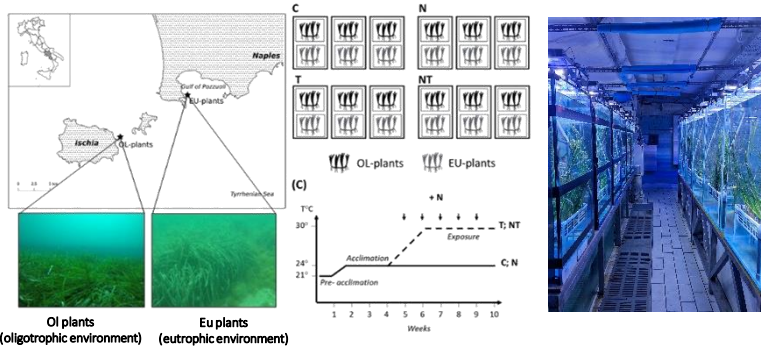
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INTRODUCTION

Posidonia oceanica is a seagrasses species particularly susceptible to environmental changes such as the intensification of warming and eutrophication events and the occurrence of rapid environmental changes are threatening natural populations. Here we explore the dynamics of 5mC-methylation patterns in *P. oceanica* collected from sites naturally experimentally exposed to multiple stresses. For this purpose, in a common garden experiment, we exposed *P. oceanica* plants to nutrient enrichment, temperature increase and their combination. Since in plants the modulation of epigenetic responses to environmental conditions represents a potential and robust mechanism to regulate gene expression network, we also aim to analyze expression profiles of epigenetic related genes to understand how DNA methylation patterns evolve under multiple stressors and their potential role in driving *P. oceanica* responses.

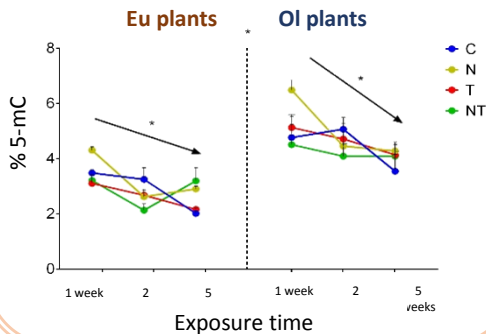
MATERIAL AND METHODS



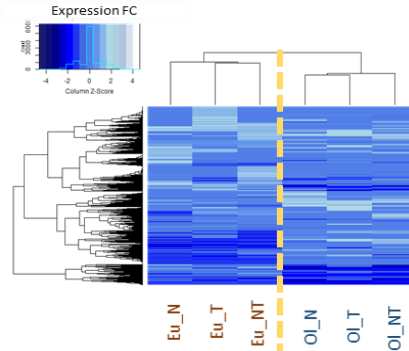
Plants from both locations (OL and EU plants) were placed in the same tank in triplicates per each experimental treatment/condition. The four treatments assayed were Control (C), Temperature (T), Nutrients (N) and Nutrients plus Temperature (NT). Genomic DNA was isolated from leaf tissue to analyze global DNA methylation colorimetrically at different time points during the exposure period. Gene expression profiles were analyzed by TAG sequencing technique which is an implementation of the LongSAGE protocol on the Illumina sequencing platform.

RESULTS

Global DNA-methylation assessment



Methylation-related genes profiles



The global % of methylated Cytosines (5-mC) significantly decreased during the exposure treatment. OL plants showed a higher % of 5-mC (average of 5%) in respect to Eu plants (3%). Gene expression patterns related to epigenetic modifications differed among plants with a consistent expression of genes related to histone modifications in respect to DNA modifications.

Chromatine modifications

CARM1
G117031
IDM1
LDL3
ATXR7
Kmt2b
SUVH2
ATXR3
JMJ15
SUVRS

DNA modifications

MET1B
eef1akmt1
DRD1
DDM1
NGAMT1

Eu plants

Chromatine modifications

G117031
JMJ16
IDM1
LDL3
SUVH2
JMJ15
SUVRS
B0103C08
ASHH2

DNA modifications

MET1B
eef1akmt1
DDM1
NGAMT1

OL plants

CONCLUSIONS

DNA methylation is a dynamic process that depends on surrounding environmental conditions. Results showed that the methylation levels decreased along the experiment with higher percentages measured at the beginning of the initial exposure to stress. Nutrients highly affected the DNA methylation level in both plant types suggesting that nutrients alone can be considered as the main stressor to trigger epigenetic responses. Local environmental conditions seemed to influence methylation levels and plants experiencing chronic exposure to eutrophication in the natural environment appeared less methylated than low nutrient plants. These findings were corroborated by gene expression profiles of methyl-related genes, that differed among plants and treatments. The higher presence of genes related to histone modifications could be at the basis of a transcriptional memory since chromatin activities allow plants to remember the exposure to pre-stress events improving their performances to subsequent stresses.