DIFFERENTIAL GENE EXPRESSION PROFILING UNDER DIFFERENT LIGHT CONDITIONS IN *POSIDONIA OCEANICA* (*L.*) DELILE BY SSH ANALYSIS

G. Procaccini [1]*, E. Dattolo [1], A. M. Innocenti [2], S. Mazzuca [2], A. Cavallini [3], L. Natali [3], J. Gu [4] and P. Bayer [4]
[1] Stazione Zoologica Anton Dohrn - gpro@szn.it
[2] Dipartimento di Ecologia, Università della Calabria, Arcavacata di Rende (CS), Italy
[3] Dipartimento di Biologia delle Piante Agrarie, Università di Pisa, Pisa, Italy
[4] Institute for Evolution and Biodiversity, University of Muenster, Huefferstrasse 1, D48149 Muenster, Germany

Abstract

A SSH (Suppression Subtractive Hybridization) cDNA library was constructed using samples collected from a continuous *Posidonia oceanica* meadow located in *Lacco Ameno* (Ischia, Gulf of Naples) at two different depths (-5 and -25 m). The aims are to identify differentially expressed genes associated with different light and temperature regimes and other potential stress-responsive gene networks important for adaptation. With ~1200 sequenced cDNA clones, 486 tentative unigenes were identified, of which only 28 are common to both shallow and deep libraries. Genes have been grouped in functional classes showing the highest differences between the two depths in primary metabolism, photosynthesis and stress defence genes.

Keywords: Genetics, Global Change, Phanerogams, Posidonia

Introduction

Seagrass meadows are among the most productive ecosystems [1] with *Posidonia oceanica* being the most important species in the Mediterranean coastline. This species forms extensive mono-specific meadows that are extremely sensitive to medium-high levels of disturbance [2] and are being threatened by fast environmental changes caused by global warming and increasing human activities. Collectively, the forces impose a strong selective pressure to the long living *P. oceanica* meadows, particularly those found at the extremes of the environmental and geographic limits of the species. Being sessile, seagrasses can not easily disperse from a given locality and must therefore adapt to selective regimes and acclimatize to variations in local environmental parameters. The goal of this study it to distinguish differentially expressed genes associated with different light and temperature regimes and to identify potential stress-responsive gene networks. In order to do that, we built a SSH cDNA library between two different depths (-5 and -25 m) in a continuous *P. oceanica* meadow located in *Lacco Ameno* (Ischia, Gulf of Naples). Existing data suggest that clones growing at different depths are genetically isolated and that plants growing above and below the summer thermocline experience strong differences in light and temperature exposure [3].

Material and Method

After the stabilization of the summer thermocline (about -15m depth), shoots were collected above and below this thermocline by SCUBA diving from a meadow located in Lacco Ameno, Ischia (Gulf of Naples) in July 2008. Plants found at -5m (shallow station) and -25m (deep station) depth were sampled. Total RNA were isolated from leaf tissue using a modified hexadecyltrimertihyl bromide (CTAB) method; poly(A+) mRNA was isolated from the total RNA using the Dynabeads mRNA Purification kit (DYNAL BIOTECH), and forward and reverse SSH libraries were built using the PCR-select cDNA subtraction kit (Clontech, Palo Alto, CA). A total of 1920 random clones (960 from the shallow and 960 from the deep library) were sequenced in double-strand (Richard Reinhardt, MPI Molecular Genetics, Berlin, Germany). EST raw sequences were quality-trimmed and assembled into tentative gene clusters (TC) using CAP3 [4]. Putative functions of the identified tentative unigenes (TUG), were assessed by performing homology searches against the following databases: Gene Ontology(GO), SWISSPROT, NR -NCBI (blastx-step, e-value 0.001) and DrZompo (blastn-step).

Results and Discussion

The final assembly of shallow and deep ESTs includes 486 TUGs with a successful mapping of 2279 ESTs. 364 ESTs could not be assigned to the TUGs library. The percentage of TUGs common to both libraries (shallow and deep, S and D) was only 5.86% (28/486); 205 TUGs were found in S while 309 TUGs were found in D. TUGs have been divided in seven functional categories, the percent abundance of which differed in the two libraries. Transcripts for Protein Turnover and Respiration are more abundant in S, suggesting a more rapid metabolism at higher irradiance, while transcripts for the other five categories are more abundant in D (Fig. 1).
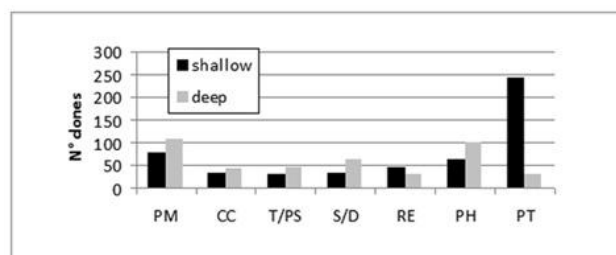

Fig. 1. Total number of ESTs assigned to functional categories. PM: Protein Metabolism; CC: Cell Component; T/PS : Transcription/Protein Synthesis; S/D : Stress/Defense; RE: Respiration; PH: Photosynthesis; PT: Protein Turnover

The presence of a higher number of transcripts assigned to Photosynthesis in the deep station is noteworthy, suggesting that a more complex and functional photosynthetic apparatus at lower irradiance is needed. Different stress responsive genes are present in the two libraries.

References

1 - Costanza R., d'Arge R., de Groot R., Farber S., Grasso M., 1997. The value of the world's ecosystem services and natural capital. *Nature*, 387: 253-260.
2 - Procaccini G., Buia MC., Gambi M.C.*, et al.*, 2003. Seagrass status and extent along the Mediterranean coasts of Italy, France and Spain. *In*: Green E.P., Short F.T. (eds.), World Atlas of Seagrasses, Univ. of California Press, Berkeley, pp 48-58.
3 - Migliaccio M., De Martino F., Silvestre F. and Procaccini G., 2005. Meadow-scale genetic structure in *Posidonia oceanica* L. (Delile). *Mar. Ecol. Prog. Series*, 304: 55-65.
4 - Huang X. and Madan A., 1999. CAP3: A DNA sequence assembly program. *Genome Res.*,9(9): 868-77.