This supplement accompanies the article

Genotype-specific responses to light stress in eelgrass *Zostera marina*, a marine foundation plant

Tiina Salo*, Thorsten B.H. Reusch, Christoffer Boström

*Corresponding author: tsalo@abo.fi

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Supplementary material

Selection criteria for target genes and primers

The new target genes were identified using the search function in the interactive seagrass sequence database Dr. Zompo (http://drzompo.uni-muenster.de, accessed during 14-24.1.2013). Primers were designed using Perlprimer (v1.1.21) and the selection criteria for primers were: primer length 20-24 bases, primer T_M 58-61, amplicon size 100-200 bases, CG content 40-60% and the primers were required to have a 3' GC clamp. The blastn- function at Dr Zompo -site was used to assure that the primers produced significant alignments only with the target sequences and possible homologues were tested using BioEdit Sequence Alignment Editor (v 7.1.9). RNA was isolated with InviTrap Spin Plant RNA Mini Kit (Stratec Molecular GmbH, Berlin, Germany), following standard protocol. The RNA concentration of each sample was quantified using a Nano-Drop spectrophotometer and ca. 200 ng RNA was used in reverse transcription. Reverse transcription was conducted using QuantiTect Reverse Transcription Kit (Qiagen) according to the standard protocol except that the total reaction volume of genomic DNA elimination reaction components was increased to 17.5 µl where of 3.5 µl was sampled after the incubation to be used as non-reverse-transcription control (NRTC).

The functioning and efficiency of primers were tested with quantitative real-time PCR (StepOne Plus, Applied Biosystems) using three dilution series (six threefold dilutions starting from 1:10) for the new primers and two dilution series (dilutions: 1:30 and 1:90) for the previously developed and used primers, of pooled cDNA from an extra genotype. The reactions (20 µl) included 10 µl SYBR green Master Mix, 0.8 µl of 100 µM of both forward and reverse primers, 4.4 µl H_2O and 4 µl diluted cDNA. Thermocycling started with 10 min at 95°C, followed by 40 cycles of 3 s at 95°C and 30 s at 60°C. The melt curve of the products was initiated by 15 s at 95°C, 1 min at 60°C +1°C and finished by 15 s at 95°C.

Target amplification and PCR-conditions

Prior to the analyses, specific target amplification of the samples was conducted using 3.8 µl specific target amplification pre-mix (containing 2X TaqMan PreAmp Master Mix, specific target amplification primer mix and H_2O in ratio of 10:2:3) and 1.3 µl cDNA sample in a 96 plate. The specific target amplification primer mix was prepared by mixing primers for all target genes with DNA suspension buffer so that the final concentration was 0.2 µM. In addition to the 80 samples, 2 dilution series (twofold dilutions with 5 concentrations starting from 1:2), 3 NRT, and 3 H_2O controls were prepared on the same plate. Four technical replicates were analyzed for each sample. The PCR protocol included a hot start with 10 min 95°C followed by 14 cycles of 15 s at 95°C and 4 min at 60°C. The PCR products were diluted 1:10. The real-time PCR analyses were conducted according to the Real-time PCR reference (Fluidigm) with a thermal mix (2400 s at 70°C and 30 s at 60°C), a hot start (60 s at 95°C), 30 x PCR cycle (5 s at 96°C, 20 s at 5°C), followed by melting (3 s at 60°C +1°C until 95°C was reached).