Supporting Information

Franssen et al. 10.1073/pnas.1107680108

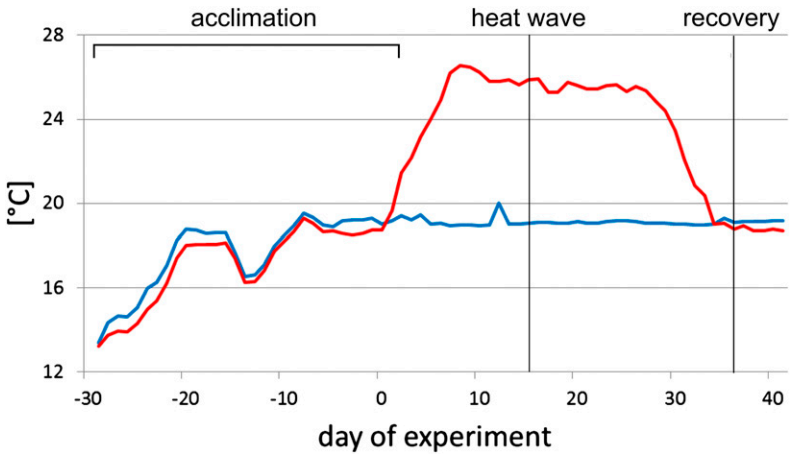

Fig. S1. Temperature profile of the heat-wave simulation. Blue indicates control, and red indicates temperature in heat-stress treatments. Time points for sampling of RNA during (day 16) and after the heat wave (day 36) are indicated by vertical lines.

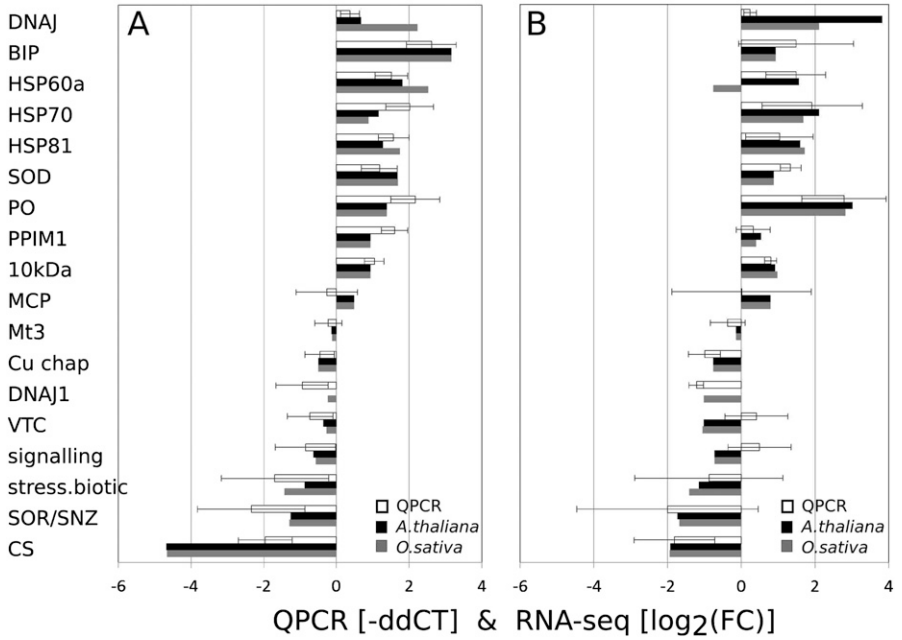

Fig. S2. Validation of the technical accuracy for determining changes in expression based on direct 454 cDNA sequencing with subsequent mapping of read counts. Expression changes in response to heat treatment were measured during the heat wave for the northern (*A*) and the southern (*B*) populations by quantitative real-time PCR (QPCR). Delta-delta cycle threshold ($\Delta\Delta C_T$) values are shown by white bars, and RNA-sequencing count data [$\log_2$ fold-change (FC)] are shown by black (reference proteome *Arabidopsis thaliana*) and gray (reference proteome *Oryza sativa*) bars. RNA-sequencing data for each library were obtained from pooled samples of six to eight genotypes; quantitative real-time PCR results are based on a subset of five biological replicates. Positive values indicate higher expression in response to heat treatment in comparison with the control treatment. Nine of the genes assessed using quantitative real-time PCR are taken from ref. 1; one was taken from ref. 2; and eight additional ones were developed for this study. Full gene names and *Arabidopsis* orthologue reference nos are as follows: 10kDa – *chloroplast chaperonin 10* (at5g20720), 70kDa – *binding protein 70kDa* (at3g25230), BIP – *luminal binding protein* (at5g28540), *copper_chaperone* (at3g56240, CS – *carotenoid synthesis gene* (at4g27030), dnaJ - *heat shock protein dnaJ* (at3g08910), dnaJ1 - *heat shock protein dnaJ1* (at5g16650), Hsp60a – *heat shock protein 60a* (at3g23990), Hsp70 – *heat shock protein 70* (at5g02500), Hsp81 –*heat shock protein 81* (at5g56030), MCP – *mitochondrial carrier protein* (at1g07030), M13 – *metallothionein 3 gene* (at3g15353), PO – *proline oxidase* (at3g30775), signalling – *GF14 signalling protein* (at1g35160), SOD – *sodium oxide dismutase* (at3g10920), SOR/SNZ – *SOR/SNZ family protein* (at5g01410), stress.biotic – *Stress and disease responsive protein* (at3g13650), VTC2 – *vitamine C defective gene 2* (at4g26850).

1. Bergmann N, et al. (2010) Population-specificity of heat stress gene induction in northern and southern eelgrass *Zostera marina* populations under simulated global warming. *Mol Ecol* 19:2870–2883.
2. Winters G, Nelle P, Fricke B, Rauch G, Reusch TBH (2011) The effects of a simulated heat wave on the photophysiology and gene expression of high and low-latitude populations of Zostera marina. *Mar Ecol Prog Ser* 435:83–95.

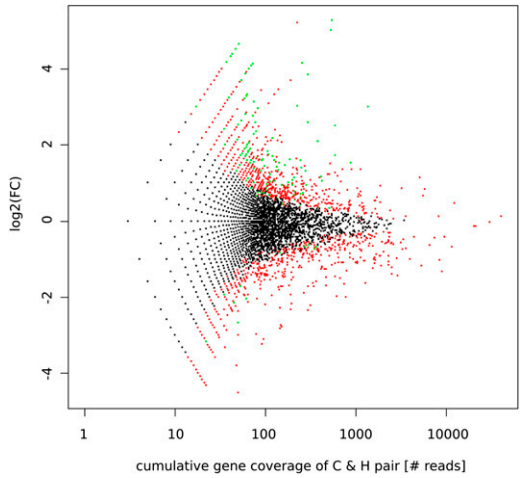

Fig. S3. Funnel plot of gene expression in *Zostera marina* as a function of absolute transcript abundance. Log_2 fold-changes (FC) in gene expression between treatment pairs (control and heat) against the cumulative transcript abundance of the respective library pair (log-scale). Black indicates nondifferentially expressed genes [bootstrap analysis; false discovery rate (FDR) $\alpha < 0.01$]. Red and green indicate genes that were significantly differentially expressed (bootstrap analysis; FDR < 0.01). Green indicates genes that were identified as indicator genes supporting the three distinct clusters in the principal component analysis (Fig. 1). Positive fold-changes indicate higher expression in the heat stress-derived cDNA library; negative values indicate higher expression in the control library.

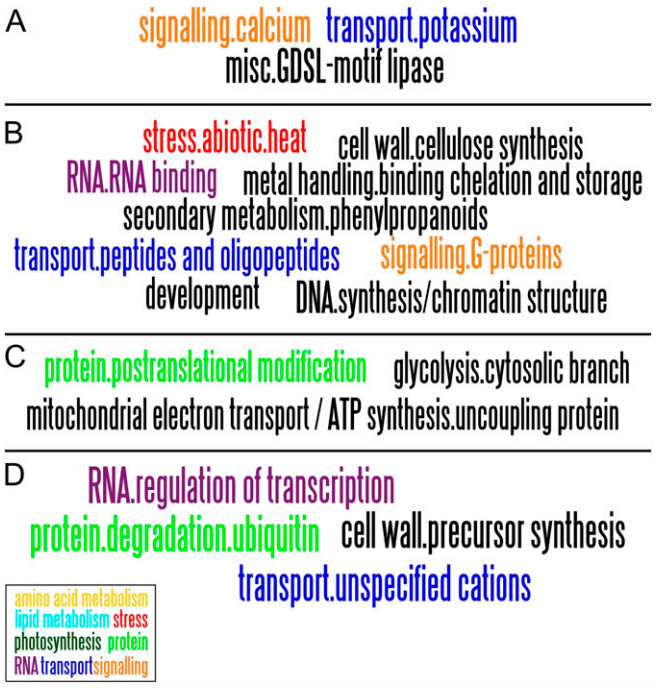

Fig. S4. Functional annotation of genes showing up- or down-regulation in a specific group of libraries, compared with all other groups (compare with Fig. 2). (*A*) Up-regulated genes in group 1, control expression (50.0% of genes not annotated). (*B*) Down-regulated genes in group 1, control expression (18.2% of genes not annotated). (*C*) Down-regulated genes in group 2, during heat stress (40.0% of genes not annotated). (*D*) Down-regulated genes in group 3, divergent early recovery (20.0% of genes not annotated). Gene sets were annotated with MapMan categories, and annotation is presented via term clouds, in which the annotation frequency is proportional to the word size. Gene categories are color coded: green, protein; purple, RNA; orange, signaling; red, stress; blue, transport; black, remaining categories.

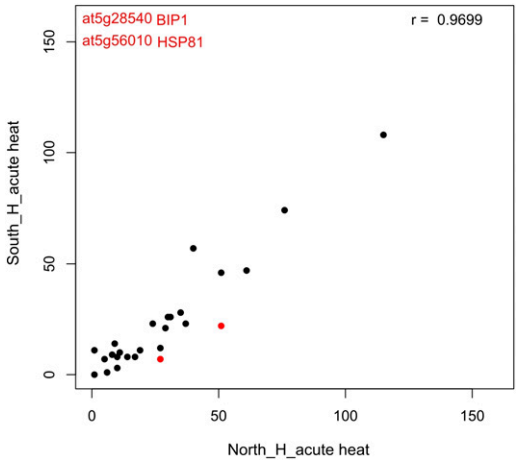

Fig. S5. Gene expression of 27 heat-shock and chaperone genes. The scatter plot compares the absolute number of mapped reads of the southern population against the northern population, corrected for small differences in the absolute size in read counts per library (Table S2). Two genes differentially expressed at an FDR of $\alpha < 0.05$ are displayed in red. Correlation coefficient between both populations, $r = 0.927$ ($P < 0.0001$).

Table S1. Overview of assembly and annotation success of sequence reads in *Zostera marina* cDNA libraries

Table S1

Table S2. Library sizes and sequence read quality parameters for all eight treatments

Table S2

Table S3. Overview of 1,872 tentatively differentially expressed (TDE) genes of eelgrass *Zostera marina* that showed a significant response to heat stress in at least one pairwise comparison, along with their respective annotations using the *Arabidopsis thaliana* proteome

Table S3

The absolute read counts, normalized for library size and rounded to next integer, for each of the eight experimental conditions are given. Differential expression was assessed using bootstrapping, applying a false discovery rate of $\alpha = 0.01$. Note that the table contains 1,890 gene annotations because in some tentative genes one gene identifier has two complementary MapMan (1) annotations and therefore occurs twice in the table.

1. Usadel B, et al. (2005) Extension of the visualization tool MapMan to allow statistical analysis of arrays, display of corresponding genes, and comparison with known responses. *Plant Physiol* 138:1195–1204.

Table S4. Overview of 234 indicator genes in *Zostera marina*, supporting the groupings in Figs. 1 and 2, and their putative function and *Arabidopsis thaliana*-based annotation

Table S4

The identification of these genes followed an indicator value analysis, implemented in the R package, procedure "indicspecies" (version 1.5.1). Only genes with correlations $r > 0.9$ were considered. Note: The table contains 236 gene annotations, because it is possible that one gene identifier has two complementary MapMan (1) annotations.

1. Usadel B, et al. (2005) Extension of the visualization tool MapMan to allow statistical analysis of arrays, display of corresponding genes, and comparison with known responses. *Plant Physiol* 138:1195–1204.