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*Supplement of*

# Response of key stress-related genes of the seagrass *Posidonia oceanica* in the vicinity of submarine volcanic vents

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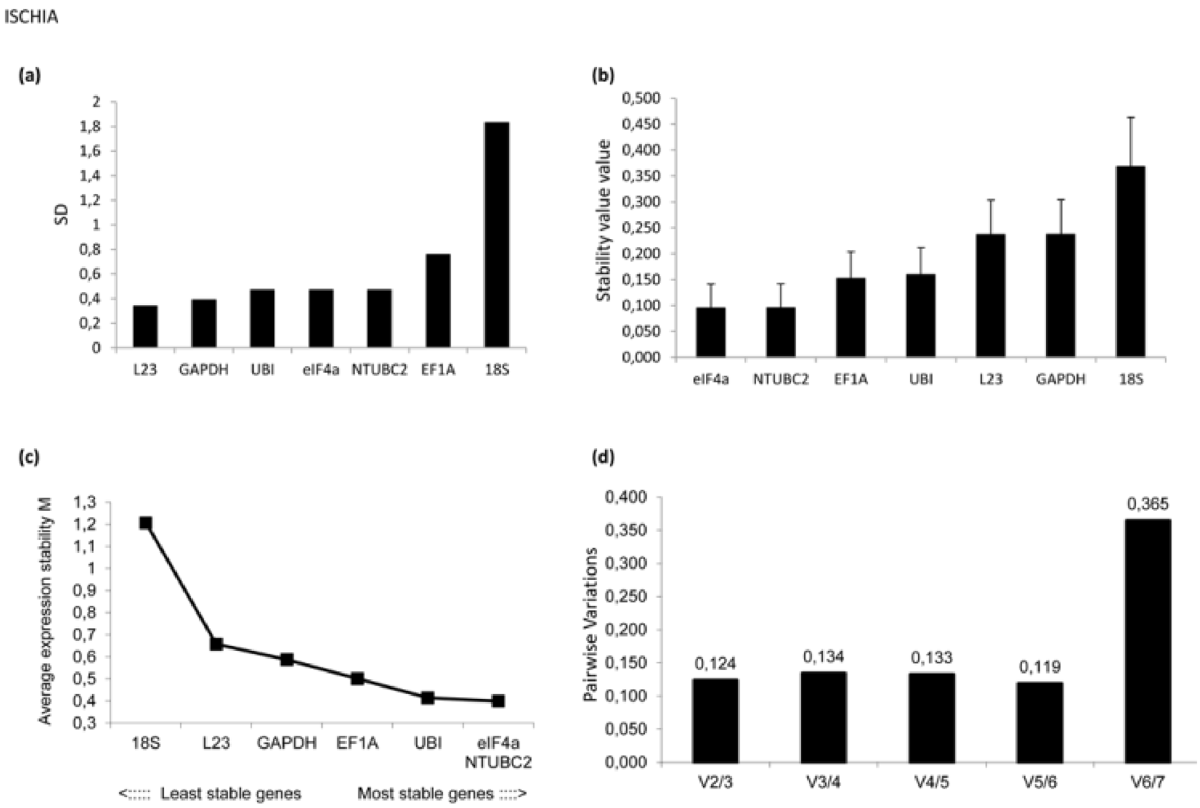

**Figure 1S.** Ranking of the best reference genes (RGs) in *P. oceanica* collected at Ischia Island. (a) Ranking of the best RGs based on standard deviation of the Ct values given by BestKeeper. (b) The NormFinder algorithm ranks the candidate RGs according to their expression stability. Lower stability values indicate more stable genes. (c) The stepwise exclusion of genes that are more variable among samples using the geNorm program. More stable genes with the lowest average expression stability value are indicated by the arrow (Vandesompele *et al.*, 2002). (d) Pairwise variation (V) to evaluate the effect of adding another RG to the best couple already analyzed using geNorm (e.g. adding a third gene V2/3, a fourth V3/4, etc.). The inclusion of additional RGs was not required below the cutoff value of 0.15.

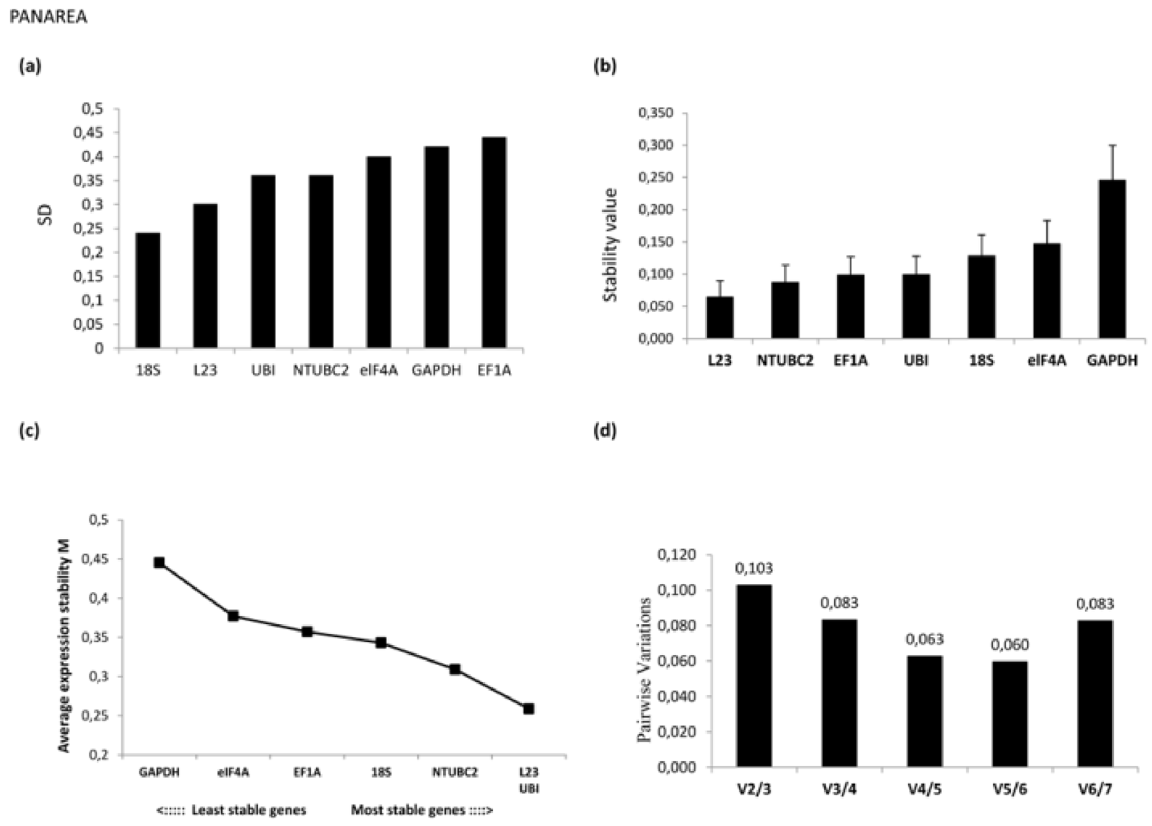

**Figure 2S.** Ranking of the best reference genes (RGs) in *P. oceanica* collected at Ischia Island. (a) Ranking of the best RGs based on standard deviation of the Ct values given by BestKeeper. (b) The NormFinder algorithm ranks the candidate RGs according to their expression stability. Lower stability values indicate more stable genes. (c) The stepwise exclusion of genes that are more variable among samples using the geNorm program. More stable genes with the lowest average expression stability value are indicated by the arrow (Vandesompele *et al.*, 2002). (d) Pairwise variation (V) to evaluate the effect of adding another RG to the best couple already analyzed using geNorm (e.g. adding a third gene V2/3, a fourth V3/4, etc.). The inclusion of additional RGs was not required below the cutoff value of 0.15.

**Table 1S.** List of genes of interest utilized for RT-qPCR experiments. For each gene, sequences of forward and reverse primers, amplicon length (L), efficiencies (E), correlation factor ($R^2$), accession numbers of Dr Zompo and GenBank databases are provided.

| **Gene** | **Oligo F** | **Oligo R** | **L** | **E** | **$R^2$** | **Dr Zompo** | **GenBank** |
|---|---|---|---|---|---|---|---|
| HSP90 | CTCCATCTTGCTTCCCTCAG | TCAGTTTGGAGGAACCGAAC | 146 | 2 | 0.9948 | Pooc_contig353 | GO349004.1 |
| DNAJ | CACCTCCTGGAGCATCATCT | CGAAGGGCAAGCTCTACATC | 243 | 2 | 0.996 | Pooc_PC037B09 | GO348410.1 |
| HSP83 | CGTGTGATCCAAAGCCTTCT | ACGATCCGGATGAACAACTC | 248 | 1.9 | 0.999 | | |
| OzSP | AGATGCATCAGCCCATTTTC | GGAGGTACCACACCAACGAC | 175 | 1.96 | 0.997 | | |
| DEHSP | ATGAGAGTGGAGCTGCGTTT | AGCGACCCTTGCAGAACTTA | 176 | 1.98 | 0.9973 | | |
| LBP | ACCAGAGCTCGGTTTGAAGA | ATTCTGGTGCTTCCACCAAC | 137 | 1.95 | 0.9957 | | |
| SHSP | ACCGGAGGATGTGAAGATTG | AGCTTGCTGGACAAGGTGAT | 125 | 1.99 | 0.9832 | | |
| HSFA5 | GCTCCAACAACTCCAGCTTC | CCCCTTCACAAACTCGTCAT | 161 | 1.94 | 0.999 | | |
| ABC | GCTGCTGGTTCTTCCTTGAA | AGACAATGCTGCCTCCTGTT | 214 | 2 | 0.994 | Pooc_PC025C02 | GO346874.1 |
| CYP450 | CAAAACTTGGCCTGTTGGTT | GCAGAAACCACACCCTTCAT | 162 | 2 | 0.966 | Pooc_PC004H08 | GO346478.1 |
| ALDH | TGATTCTGCATGCACCAGTT | ATGGGAACGAGCTACCATTG | 219 | 2 | 0.99 | Pooc_PC042G01 | GO346135.1 |
| GST | ACCTTCGCTAGCTTCCCTTC | AGATGGCAACAATGGGGATA | 157 | 2 | 0.9963 | Pooc_PC030G07 | GO347013.1 |
| GPX | TTGGAAAGGGGAAGTTGTTG | CGGGTCAAACCCTGAGATAA | 232 | 2 | 0.9922 | Pooc_PC006G02 | GO346743.1 |
| GSH-S | TAGGTTTGGCCAATTCTTGC | AAGGGGTGGTTCTTCCAGAT | 213 | 2 | 0.992 | | |
| GR | AGTCCACACCAAATGGAAGC | AAGGGGAGGGAAGGGTTATT | 247 | 2 | 0.9952 | | |
| CAT | CATCACATGCTGGGTTTCAC | ACCGATCCTGGACATCTGAC | 178 | 2 | 0.9936 | Pooc_B_c426 | |
| SODCP | CAATGGCTGCATATCGACTG | TGCCGGACTTTATCTTCTGG | 157 | 2 | 0.9953 | Pooc_PC034C12 | GO347680.1 |
| CSD1 | GCTCCTGAGGATGAGATTCG | AGGCCAATAACACCACAAGC | 236 | 1.96 | 0.9988 | | |
| FSD | TCATGACTTCTTTTGGGAATCA | CCCAACCAGATCCAAACAGT | 153 | 2 | 0.9838 | | |
| MSD | GGCGGAGGTCATATAAACCA | ATAAGCAAGCCACACCCATC | 192 | 1.86 | 0.9923 | | |
| AR | CTGCTGATTGGAGCTTAGCC | AGGGGATTTTCCTTGAAAGTG | 102 | 1.93 | 0.9968 | | |
| APX | TCAGCTTGCTGGAGTTGTTG | CCCATGCGGTAAAAGATGTC | 156 | 1.95 | 0.9997 | | |
| sAPX | GCATGATGCTGGAACGTATG | AATTTTGGGACCTCCAGCTT | 228 | 2 | 0.9958 | | |
| Prx Q | AGGTGTCCAAGGGAGATGTG | TTGTGCGAAGCAGAATCATC | 229 | 2 | 0.995 | Pooc_Contig278 | GO347736.1 |
| GLP | GAAGTCGGGTTCATCACCAC | CTGCAGCTGACTGTTGAAGG | 153 | 2 | 0.996 | Pooc_B_c323 | |
| DSP5 | TCTCAGGTCCGGCACTAATC | GAAAGGCTTGCTCGTATTGC | 224 | 2 | 0.9992 | | |
| LPX | TGTAGCCACCGAAGGGATAC | GGTTGGTGGGATGAGGTAAA | 172 | 2 | 0.998 | Pooc_B_c10 | |
| FtsH2 | GCTCGAAGATACTCCGCAAC | GTCGGAGTGGTTCAGTTGGT | 210 | 2 | 0.9973 | | |
| HMA | GTGATGAGAACCCCAATGCT | CGACACCAGAGCTCAAACAA | 153 | 1.93 | 0.9993 | | |
| NRAMP1 | TACTTCCTATGGGCGAGTGC | TTGCCTATGGAATCTGAACCTT | 181 | 1.97 | 0.9916 | | |
| HMATPase | CACACCGAGCACATTGTACC | TGCTGCAGATGTAGGAATGG | 181 | 1.98 | 0.9983 | | |
| HMATPase5 | ATCGGACTCTCCTCGGTTTT | TGGCAGTGTTATGCTTCGTC | 222 | 2 | 0.96 | | JF811734.1 |
| MT3 | CATGTCGACCTGTGACAACTG | TATTAATGGCCACAGGTGCAG | 207 | 2 | 0.9954 | | |
| Fe-SP | CCATCTGATGAGGTAGTTTCAGG | CGCCAAGTCAACTTTGATAGC | 188 | 1.9 | 0.9968 | | |
| MTP | CTCGTTTCCTGAGGTTCTGC | TTTGCTGCTGTCATGGCTAC | 183 | 1.93 | 0.9989 | | |