

Supporting Information

A king and vassals' tale: molecular signatures of clonal integration in *Posidonia oceanica* under chronic light shortage

Miriam Ruocco^{1*}, Laura Entrambasaguas¹, Emanuela Dattolo¹, Alfonsina Milito¹, Lazaro Marín-Guirao^{1,2†}, Gabriele Procaccini^{1†}

Section 1

Transcriptome sequencing, de-novo assembly and functional annotation

The Illumina sequencing generated 498,017,114 single-end reads (average length = 75 bp). Raw reads were quality-trimmed to obtain a final amount of 421,487,471 HQ reads (84.63%) and provided as input for the *de-novo* assembly. The newly assembled transcriptome consisted of 281,925 transcripts (≥ 200 bp), with an overall size of 156.5 Mb, a mean length of 993 bp and N50 = 1,964 (Table S1). A total of 167,446 transcripts (59.39%) significantly matched to known proteins. The annotation process also retrieved information on GO terms (as Biological Processes - BPs, Molecular Functions - MFs and Cellular Components - CCs). Based on sequence homology, 140,569 sequences (49.86%) were successfully assigned to at least one GO term (Table S2); top 20 GO term distribution can be retrieved from Fig. S1. KEGG annotation was obtained for 8,398 transcripts (3%), whereas 46,495 transcripts (16.5%) retrieved a significant EC annotation (Table S2 and Fig. S2).

Section 2

Table S1. Summary statistics of the combined *P. oceanica* transcriptome. % GC = the proportion of guanidine and cytosine nucleotides among total nucleotides; N50 = the length of the longest contig such that all contigs of at least that length compose at least 50% of the bases of the assembly

| Basic statistics of the <i>P. oceanica</i> transcriptome | |
|---|-------------|
| Total number of transcripts | 281,925 |
| Number of Trinity “genes” | 157,553 |
| GC (%) | 41.07 |
| Mean (bp) | 993.44 |
| Minimum contig length (bp) | 204 |
| Maximum contig length (bp) | 17,138 |
| N50 value | 1,964 |
| Total assembled bases | 156,519,687 |

Table S2. Summary of annotation results for the combined *P. oceanica* transcriptome

| Functional annotation of the merged <i>P. oceanica</i> transcriptome | |
|---|---------------------|
| | Number of sequences |
| Blast hits | 26,877 (10%) |
| GO mapping | 31,061 (11%) |
| Blast2GO annotation | 109,508 (39%) |
| KEGG annotation | 8,398 (3%) |
| Enzyme code (EC) | 46,495 (16.5%) |

Figure S1. Top 20 GO term distribution as biological processes (BP), molecular functions (MF) and cellular components (CC) retrieved for the merged *P. oceanica* transcriptome

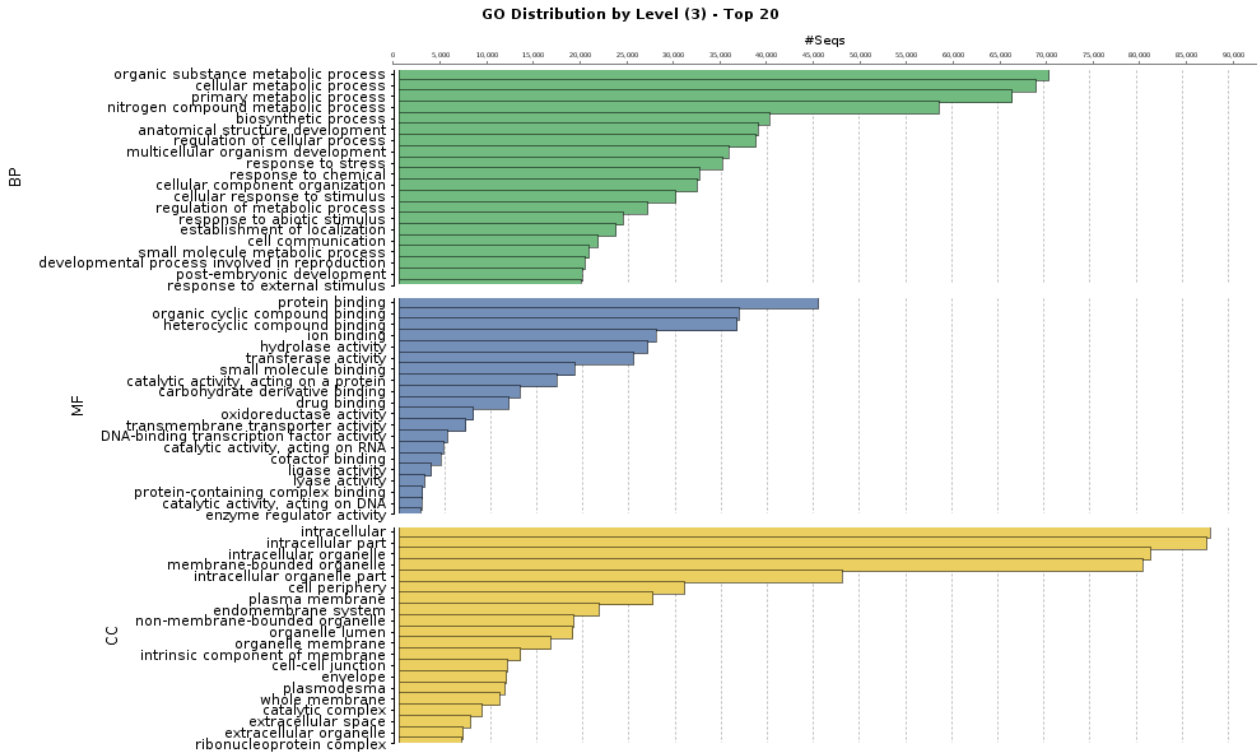


Figure S2. Enzyme Code (EC) distribution of the merged *P. oceanica* transcriptome

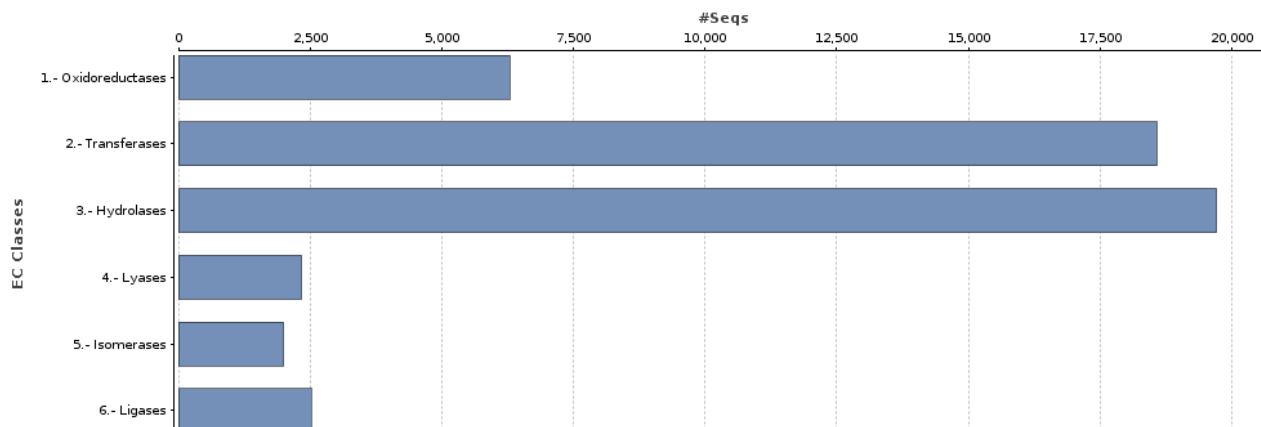


Figure S3. In (a) heatmap and hierarchical cluster analysis of gene-expression patterns across all analysed samples. Deeper colours indicate higher up (red) or down (blue) regulation. In (b) PCA based on expression values of the different biological replicates of leaves and SAMs under control and LL conditions. A_C = leaf of apical shoot, control; A_LL = leaf of apical shoot, low light; V_C = leaf of vertical shoot, control; V_LL = leaf of vertical shoot, low light; MA_C = SAM of apical shoot, control; MA_LL = SAM of apical shoot, low light; MV_C = SAM of vertical shoot, control; MV_LL = SAM of vertical shoot, low light

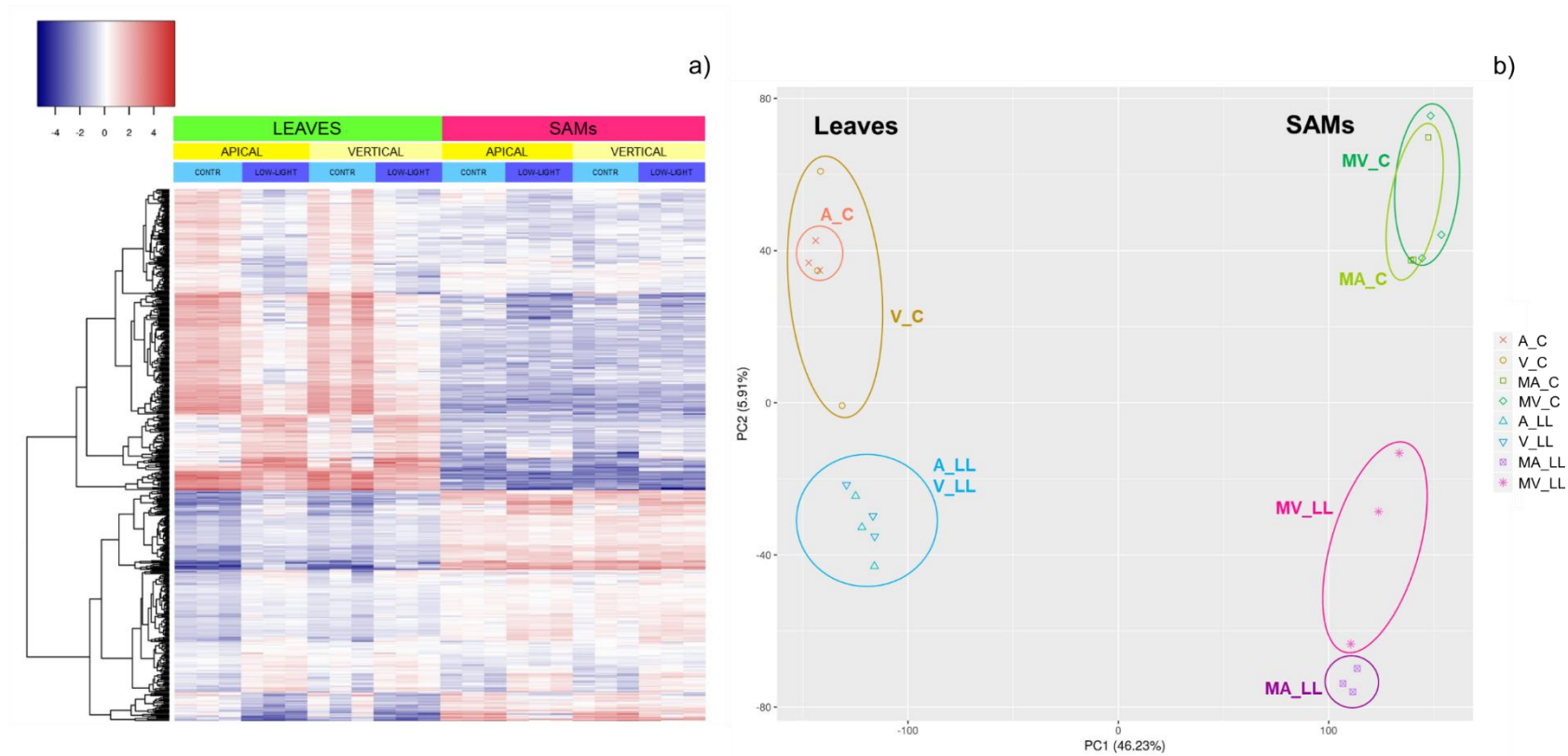


Table S4. Full list of enriched GO biological processes (BPs) in apical and vertical leaves under LL (FDR < 0.05). GO identifiers, BP names and FDR values are given. Shared enriched BPs between apical and vertical contrasts are in bold

| GO ID | GO – Biological process | FDR |
|-----------------|--|----------|
| <i>Apical</i> | | |
| 0080037 | negative regulation of cytokinin-activated signalling pathway | 3.31E-06 |
| 0009737 | response to abscisic acid | 5.52E-04 |
| 0046148 | pigment biosynthetic process | 1.13E-03 |
| 2000762 | regulation of phenylpropanoid metabolic process | 1.28E-03 |
| 0033386 | geranylgeranyl diphosphate biosynthetic process | 6.78E-03 |
| 0006857 | oligopeptide transport | 9.24E-03 |
| 0015698 | inorganic anion transport | 1.23E-02 |
| 0043693 | monoterpene biosynthetic process | 1.40E-02 |
| 0003006 | developmental process involved in reproduction | 2.22E-02 |
| 0033384 | geranyl diphosphate biosynthetic process | 4.20E-02 |
| 0009791 | post-embryonic development | 4.43E-02 |
| 0009269 | response to desiccation | 4.53E-02 |
| 0009812 | flavonoid metabolic process | 4.86E-02 |
| <i>Vertical</i> | | |
| 0006464 | cellular protein modification process | 2.58E-04 |
| 0006355 | regulation of transcription, DNA-templated | 7.44E-04 |
| 0006857 | oligopeptide transport | 9.75E-04 |
| 0009414 | response to water deprivation | 4.27E-03 |
| 0033554 | cellular response to stress | 6.28E-03 |
| 0080037 | negative regulation of cytokinin-activated signalling pathway | 9.12E-03 |
| 0048316 | seed development | 1.12E-02 |
| 0060560 | developmental growth involved in morphogenesis | 1.18E-02 |
| 0032101 | regulation of response to external stimulus | 1.29E-02 |
| 0016310 | Phosphorylation | 1.46E-02 |
| 0008202 | steroid metabolic process | 1.50E-02 |
| 0006970 | response to osmotic stress | 1.85E-02 |
| 0048588 | developmental cell growth | 1.89E-02 |
| 0090408 | phloem nitrate loading | 2.53E-02 |
| 0031347 | regulation of defense response | 2.67E-02 |
| 0019748 | secondary metabolic process | 2.67E-02 |
| 0009723 | response to ethylene | 2.74E-02 |
| 1901615 | organic hydroxy compound metabolic process | 2.81E-02 |
| 0043090 | amino acid import | 2.95E-02 |
| 0048437 | floral organ development | 3.07E-02 |
| 2000026 | regulation of multicellular organismal development | 3.18E-02 |
| 0035524 | proline transmembrane transport | 3.18E-02 |
| 0045862 | positive regulation of proteolysis | 3.55E-02 |
| 0051254 | positive regulation of RNA metabolic process | 3.61E-02 |
| 0065008 | regulation of biological quality | 3.64E-02 |
| 0015810 | aspartate transmembrane transport | 3.87E-02 |

| | | |
|---------|--|----------|
| 0010628 | positive regulation of gene expression | 3.87E-02 |
| 0006868 | glutamine transport | 4.11E-02 |
| 0048235 | pollen sperm cell differentiation | 4.11E-02 |
| 0015827 | tryptophan transport | 4.11E-02 |
| 0048545 | response to steroid hormone | 4.27E-02 |
| 0015825 | L-serine transport | 4.34E-02 |
| 0009556 | microsporogenesis | 4.50E-02 |
| 0044085 | cellular component biogenesis | 4.71E-02 |
| 0006955 | immune response | 4.89E-02 |
| 0006996 | organelle organization | 4.93E-02 |

Table S5. Reduced list of enriched GO-BPs (FDR < 0.01) in apical SAMs under LL. GO identifiers, BP names and FDR values are given. Shared

BPs with vertical contrasts are in bold

| GO ID | GO – Biological process | FDR | GO ID | GO – Biological process | FDR |
|---------|---|----------|---------|--|----------|
| 0009637 | response to blue light | 1.08E-05 | 0009741 | response to brassinosteroid | 4.02E-03 |
| 0045893 | positive regulation of transcription, DNA-templated | 2.02E-05 | 0006098 | pentose-phosphate shunt | 4.10E-03 |
| 0009740 | gibberellic acid mediated signalling pathway | 2.66E-05 | 0009718 | anthocyanin-containing compound biosynthetic process | 4.42E-03 |
| 0009834 | plant-type secondary cell wall biogenesis | 6.41E-05 | 0030003 | cellular cation homeostasis | 4.43E-03 |
| 0010389 | regulation of G2/M transition of mitotic cell cycle | 9.28E-05 | 0043473 | pigmentation | 4.47E-03 |
| 1905393 | plant organ formation | 9.80E-05 | 0009788 | negative regulation of abscisic acid-activated signalling pathway | 5.07E-03 |
| 0009555 | pollen development | 1.17E-04 | 0010218 | response to far red light | 5.24E-03 |
| 0009958 | positive gravitropism | 1.28E-04 | 0048629 | trichome patterning | 5.61E-03 |
| 0052386 | cell wall thickening | 1.32E-04 | 0010315 | auxin efflux | 5.68E-03 |
| 0045892 | negative regulation of transcription, DNA-templated | 1.37E-04 | 0009269 | response to desiccation | 6.08E-03 |
| 0010417 | glucuronoxylan biosynthetic process | 1.99E-04 | 0009860 | pollen tube growth | 6.38E-03 |
| 0048826 | cotyledon morphogenesis | 2.86E-04 | 1901659 | glycosyl compound biosynthetic process | 7.09E-03 |
| 0007080 | mitotic metaphase plate congression | 2.95E-04 | 0055076 | transition metal ion homeostasis | 7.26E-03 |
| 0048354 | mucilage biosynthetic process involved in seed coat development | 5.00E-04 | 0009627 | systemic acquired resistance | 7.37E-03 |
| 0006306 | DNA methylation | 6.62E-04 | 0071668 | plant-type cell wall assembly | 7.43E-03 |
| 0000724 | double-strand break repair via homologous recombination | 6.64E-04 | 0000911 | cytokinesis by cell plate formation | 7.47E-03 |
| 0035556 | intracellular signal transduction | 7.72E-04 | 0009411 | response to UV | 7.48E-03 |
| 2000022 | regulation of jasmonic acid mediated signalling pathway | 1.20E-03 | 0006833 | water transport | 7.90E-03 |
| 0042547 | cell wall modification involved in multidimensional cell growth | 1.28E-03 | 0051555 | flavonol biosynthetic process | 8.14E-03 |
| 0015790 | UDP-xylose transmembrane transport | 1.80E-03 | 0048317 | seed morphogenesis | 8.80E-03 |

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|---------|---------------------------------|----------|---------|------------------------------|----------|
| 0009749 | response to glucose | 2.00E-03 | 0015698 | inorganic anion transport | 9.36E-03 |
| 0030243 | cellulose metabolic process | 2.25E-03 | 0009744 | response to sucrose | 9.61E-03 |
| 0010075 | regulation of meristem growth | 2.25E-03 | 0010089 | xylem development | 9.80E-03 |
| 0016114 | terpenoid biosynthetic process | 2.76E-03 | 0036258 | multivesicular body assembly | 9.80E-03 |
| 0090307 | mitotic spindle assembly | 2.84E-03 | | | |

Table S6. Reduced list of enriched GO-BPs (FDR < 0.01) in vertical SAMs under LL. GO identifiers, BP names and FDR values are given. Shared BPs with apical contrasts are in bold

| GO ID | GO – Biological process | FDR | GO ID | GO – Biological process | FDR |
|---------|--|----------|---------|--|----------|
| 0045893 | positive regulation of transcription, DNA-templated | 3.16E-14 | 0034728 | nucleosome organization | 1.76E-03 |
| 0009658 | chloroplast organization | 2.38E-10 | 0010315 | auxin efflux | 1.80E-03 |
| 1900865 | chloroplast RNA modification | 1.00E-06 | 0035019 | somatic stem cell population maintenance | 1.80E-03 |
| 0043066 | negative regulation of apoptotic process | 1.23E-06 | 0006306 | DNA methylation | 2.01E-03 |
| 0031425 | chloroplast RNA processing | 1.30E-06 | 0044092 | negative regulation of molecular function | 2.38E-03 |
| 0009744 | response to sucrose | 2.16E-06 | 0010017 | red or far-red light signalling pathway | 2.60E-03 |
| 0000184 | nuclear-transcribed mRNA catabolic process, nonsense-mediated decay | 4.47E-06 | 0009942 | longitudinal axis specification | 2.67E-03 |
| 0010089 | xylem development | 5.50E-06 | 0010540 | basipetal auxin transport | 2.90E-03 |
| 0043488 | regulation of mRNA stability | 6.02E-06 | 0007281 | germ cell development | 2.96E-03 |
| 0010499 | proteasomal ubiquitin-independent protein catabolic process | 1.15E-05 | 0018279 | protein N-linked glycosylation via asparagine | 3.03E-03 |
| 0006413 | translational initiation | 1.58E-05 | 0007089 | traversing start control point of mitotic cell cycle | 3.25E-03 |
| 0019761 | glucosinolate biosynthetic process | 1.59E-05 | 0016554 | cytidine to uridine editing | 3.32E-03 |
| 2000031 | regulation of salicylic acid mediated signalling pathway | 1.92E-05 | 0009083 | branched-chain amino acid catabolic process | 3.37E-03 |
| 0009788 | negative regulation of abscisic acid-activated signalling pathway | 2.64E-05 | 0080001 | mucilage extrusion from seed coat | 3.59E-03 |
| 0043687 | post-translational protein modification | 2.65E-05 | 0009631 | cold acclimation | 3.83E-03 |
| 0050665 | hydrogen peroxide biosynthetic process | 6.21E-05 | 0000304 | response to singlet oxygen | 3.84E-03 |
| 0045492 | xylan biosynthetic process | 7.41E-05 | 0009958 | positive gravitropism | 4.04E-03 |
| 0006614 | SRP-dependent cotranslational protein targeting to membrane | 7.81E-05 | 0090307 | mitotic spindle assembly | 4.46E-03 |
| 0080156 | mitochondrial mRNA modification | 9.01E-05 | 0048767 | root hair elongation | 4.50E-03 |
| 0043617 | cellular response to sucrose starvation | 1.01E-04 | 0006749 | glutathione metabolic process | 4.57E-03 |
| 0006521 | regulation of cellular amino acid metabolic process | 1.02E-04 | 0061077 | chaperone-mediated protein folding | 4.60E-03 |

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|---------|---|----------|---------|--|----------|
| 0006415 | translational termination | 1.22E-04 | 0010114 | response to red light | 4.62E-03 |
| 0009860 | pollen tube growth | 1.24E-04 | 0007080 | mitotic metaphase plate congression | 4.83E-03 |
| 0010192 | mucilage biosynthetic process | 1.29E-04 | 0010889 | regulation of sequestering of triglyceride | 5.00E-03 |
| 0016259 | selenocysteine metabolic process | 1.87E-04 | 0048653 | anther development | 5.19E-03 |
| 2000022 | regulation of jasmonic acid mediated signalling pathway | 1.90E-04 | 0031048 | chromatin silencing by small RNA | 5.29E-03 |
| 0000090 | mitotic anaphase | 2.08E-04 | 0070370 | cellular heat acclimation | 5.41E-03 |
| 0009750 | response to fructose | 2.99E-04 | 0010264 | myo-inositol hexakisphosphate biosynthetic process | 5.41E-03 |
| 0006595 | polyamine metabolic process | 3.13E-04 | 0006650 | glycerophospholipid metabolic process | 5.57E-03 |
| 0030244 | cellulose biosynthetic process | 3.62E-04 | 0010413 | glucuronoxylan metabolic process | 6.22E-03 |
| 0010218 | response to far red light | 3.94E-04 | 0007265 | Ras protein signal transduction | 6.30E-03 |
| 0016926 | protein desumoylation | 4.22E-04 | 0010252 | auxin homeostasis | 6.32E-03 |
| 0000186 | activation of MAPKK activity | 4.40E-04 | 0008284 | positive regulation of cell proliferation | 6.46E-03 |
| 0010305 | leaf vascular tissue pattern formation | 4.85E-04 | 0010082 | regulation of root meristem growth | 6.69E-03 |
| 0006627 | protein processing involved in protein targeting to mitochondrion | 5.59E-04 | 0051775 | response to redox state | 6.98E-03 |
| 0009834 | plant-type secondary cell wall biogenesis | 5.70E-04 | 1903086 | negative regulation of sinapate ester biosynthetic process | 6.98E-03 |
| 0010182 | sugar mediated signalling pathway | 5.95E-04 | 0007155 | cell adhesion | 7.36E-03 |
| 0006006 | glucose metabolic process | 6.26E-04 | 0006997 | nucleus organization | 7.49E-03 |
| 0009825 | multidimensional cell growth | 6.33E-04 | 0048834 | specification of petal number | 7.56E-03 |
| 0051098 | regulation of binding | 7.14E-04 | 0048574 | long-day photoperiodism, flowering | 7.96E-03 |
| 0002223 | stimulatory C-type lectin receptor signalling pathway | 7.55E-04 | 0019395 | fatty acid oxidation | 8.10E-03 |
| 0006874 | cellular calcium ion homeostasis | 7.87E-04 | 0009251 | glucan catabolic process | 8.19E-03 |
| 0033209 | tumor necrosis factor-mediated signalling pathway | 8.20E-04 | 0006835 | dicarboxylic acid transport | 8.28E-03 |
| 0048359 | mucilage metabolic process involved in seed coat development | 8.60E-04 | 0031648 | protein destabilization | 8.32E-03 |
| 0010150 | leaf senescence | 8.65E-04 | 0098662 | inorganic cation transmembrane transport | 8.44E-03 |
| 0048010 | vascular endothelial growth factor receptor signalling pathway | 1.01E-03 | 0007143 | female meiotic nuclear division | 8.49E-03 |
| 0006977 | DNA damage response, signal transduction by p53 class mediator resulting in cell cycle arrest | 1.01E-03 | 0080086 | stamen filament development | 8.54E-03 |

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|---------|---|----------|---------|--|----------|
| 0043928 | exonucleolytic nuclear-transcribed mRNA catabolic process involved in deadenylation-dependent decay | 1.02E-03 | 0009765 | photosynthesis, light harvesting | 8.56E-03 |
| 0000084 | mitotic S phase | 1.03E-03 | 0006897 | endocytosis | 9.36E-03 |
| 0009827 | plant-type cell wall modification | 1.03E-03 | 0031124 | mRNA 3'-end processing | 9.45E-03 |
| 0000122 | negative regulation of transcription by RNA polymerase II | 1.23E-03 | 0043489 | RNA stabilization | 9.55E-03 |
| 0009640 | photomorphogenesis | 1.26E-03 | 0033108 | mitochondrial respiratory chain complex assembly | 9.57E-03 |
| 0052544 | defense response by callose deposition in cell wall | 1.28E-03 | 0008334 | histone mRNA metabolic process | 9.68E-03 |
| 0006470 | protein dephosphorylation | 1.45E-03 | 0006260 | DNA replication | 9.87E-03 |
| 0030422 | production of siRNA involved in RNA interference | 1.52E-03 | 0007076 | mitotic chromosome condensation | 1.00E-02 |
| 0009740 | gibberellic acid mediated signalling pathway | 1.55E-03 | 0033962 | cytoplasmic mRNA processing body assembly | 1.00E-02 |
| 0009785 | blue light signalling pathway | 1.68E-03 | | | |
| 0006636 | unsaturated fatty acid biosynthetic process | 1.72E-03 | | | |
