## S4 - Supporting information for differentially expressed transcripts

## 1 Comparing the expression of genes and transcripts

We checked the similarity of results when the quantification was done for the whole transcriptome, considering each individual isoform, or when we grouped expression levels at gene-level. The number of expressed genes and transcripts can be found on Table 1. In both cases, the number represents roughly 30% of the complete reference used.

|             | Number passing the expression filter | Percentage of total reference |
|-------------|--------------------------------------|-------------------------------|
| Genes       | 47676                                | 0.27                          |
| Transcripts | 133232                               | 0.30                          |

Table 1: Number of genes and transcripts kept after minimum expression filter for each of the quantification methods.

We also compared if the differentially expressed transcripts (DETs) corresponded to the differentially expressed genes (DEGs) when the analysis was performed grouping counts at the gene level. First, we used the contrast comparing biomass groups. The number of DEGs and DETs can be seen on Table 2. For 15,188 DEGs, at least one of its transcripts was differentially expressed (Figure 1).

|        | Genes | Transcripts |
|--------|-------|-------------|
| Down   | 10903 | 21996       |
| Not DE | 26602 | 90850       |
| Up     | 10171 | 20386       |

Table 2: Number of downregulated (Down), not differentially expressed (Not DE) and upregulated (Up) genes or transcripts .



Figure 1: Venn Diagram showing the overlap between the list of differentially expressed genes and differentially expressed transcripts.

We observed that the same six processes enriched among DEGs were also enriched in the DETs (Table 3). Analysis of DETs showed enrichment of terms related to transposition, defense response and enzymatic activities. Enriched terms related to photosynthesis (glyceraldehyde-3-phosphate dehydro-genase (NAD+) (phosphorylating) activity and geranylgeranyl-diphosphate geranylgeranyltransferase activity) that carrying out differential expression analysis of isoforms revealed more specific processes. In the case of glyceraldehyde-3-phosphate dehydrogenase (GADPH), we noted that only two non-DEGs presented DETs (Figure 4).

We focused the search of DEGs and DETs to a group of annotated genes with a common gene ontology term. We chose the *photosyntesis* biological process as an example. Only 18 DEGs had at least one isoform as differentially expressed, 12 genes found as differentially expressed did not have DETs and 47 genes had differentially expressed isoforms but were not differentially expressed when counts were grouped into the gene level (Figure 2). Five photosynthesis DEGs did not have at least one DET (Figure 3).

| Category   | Description  | Genes | DEGs | Transcripts DETs |      | Enriched | Enriched |
|------------|--|-------|------|------------------|------|----------|----------|
|            |  |       |      |                  |      | DEGs     | DETs     |
| GO:0002181 | cytoplasmic translation  | 81    | 58   | 129              | 59   | Yes      | Yes      |
| GO:0003964 | RNA-directed DNA poly-<br>merase activity  | 1212  | 592  | 1841             | 712  | Yes      | Yes      |
| GO:0004190 | aspartic-type endopepti-<br>dase activity  | 1045  | 500  | 1743             | 649  | Yes      | Yes      |
| GO:0004519 | endonuclease activity  | 945   | 457  | 1526             | 557  | Yes      | Yes      |
| GO:0004523 | RNA-DNA hybrid ribonu-<br>clease activity  | 653   | 326  | 878              | 357  | Yes      | Yes      |
| GO:0015074 | DNA integration  | 986   | 474  | 1562             | 592  | Yes      | Yes      |
| GO:0000943 | retrotransposon nucleocap-<br>sid  | 768   | 344  | 1217             | 423  | No       | Yes      |
| GO:0003676 | nucleic acid binding   | 1857  | 834  | 4312             | 1334 | No       | Yes      |
| GO:0003887 | DNA-directed DNA poly-<br>merase activity  | 384   | 179  | 626              | 233  | No       | Yes      |
| GO:0004310 | farnesyl-diphosphate farne-<br>syltransferase activity                           | 7     | 3    | 48               | 25   | No       | Yes      |
| GO:0004365 | glyceraldehyde-3-phosphate<br>dehydrogenase (NAD+)<br>(phosphorylating) activity | 16    | 11   | 55               | 30   | No       | Yes      |
| GO:0004514 | nicotinate-nucleotide<br>diphosphorylase (car-<br>boxylating) activity           | 4     | 1    | 13               | 10   | No       | Yes      |
| GO:0004540 | ribonuclease activity  | 227   | 101  | 311              | 120  | No       | Yes      |
| GO:0004674 | protein serine/threonine ki-<br>nase activity                                    | 1778  | 753  | 5026             | 1535 | No       | Yes      |
| GO:0004803 | transposase activity   | 108   | 50   | 244              | 110  | No       | Yes      |
| GO:0005524 | ATP binding  | 5497  | 2298 | 16431            | 4844 | No       | Yes      |
| GO:0006310 | DNA recombination  | 1090  | 504  | 1890             | 663  | No       | Yes      |
| GO:0006313 | transposition, DNA-<br>mediated  | 139   | 73   | 283              | 125  | No       | Yes      |
| GO:0006696 | ergosterol biosynthetic pro-<br>cess   | 19    | 12   | 67               | 36   | No       | Yes      |
| GO:0006952 | defense response   | 1840  | 765  | 4157             | 1352 | No       | Yes      |
| GO:0008171 | O-methyltransferase activ-<br>ity  | 57    | 29   | 164              | 77   | No       | Yes      |
| GO:0008270 | zinc ion binding   | 1686  | 699  | 4323             | 1308 | No       | Yes      |
| GO:0008615 | pyridoxine biosynthetic pro-<br>cess   | 7     | 4    | 19               | 14   | No       | Yes      |
| GO:0008825 | cyclopropane-fatty-acyl-<br>phospholipid synthase<br>activity                    | 2     | 1    | 10               | 9    | No       | Yes      |

| Category   | Description  | Genes | DEGs | Transcripts DETs |     | Enriched<br>DEGs | Enriched<br>DETs |
|------------|--|-------|------|------------------|-----|------------------|------------------|
| GO:0009443 | pyridoxal 5'-phosphate sal-  | 4     | 2    | 20               | 14  | No               | Yes              |
| GO:0009870 | defense response signaling<br>pathway, resistance gene-<br>dependent | 225   | 94   | 452              | 161 | No               | Yes              |
| GO:0010942 | positive regulation of cell death                                    | 75    | 34   | 152              | 65  | No               | Yes              |
| GO:0016767 | geranylgeranyl-diphosphate<br>geranylgeranyltransferase<br>activity  | 6     | 3    | 37               | 22  | No               | Yes              |
| GO:0016866 | intramolecular transferase activity                                  | 33    | 18   | 88               | 44  | No               | Yes              |
| GO:0017148 | negative regulation of trans-<br>lation                              | 74    | 39   | 200              | 81  | No               | Yes              |
| GO:0019438 | aromatic compound biosyn-<br>thetic process                          | 28    | 14   | 73               | 36  | No               | Yes              |
| GO:0030598 | rRNA N-glycosylase activ-<br>ity                                     | 41    | 22   | 83               | 52  | No               | Yes              |
| GO:0032197 | transposition, RNA-<br>mediated                                      | 342   | 155  | 465              | 185 | No               | Yes              |
| GO:0032199 | reverse transcription in-<br>volved in RNA-mediated<br>transposition | 132   | 62   | 197              | 78  | No               | Yes              |
| GO:0032201 | telomere maintenance via<br>semi-conservative replica-<br>tion       | 3     | 2    | 7                | 7   | No               | Yes              |
| GO:0042301 | phosphate ion binding  | 10    | 8    | 24               | 17  | No               | Yes              |
| GO:0043531 | ADP binding  | 1199  | 517  | 2620             | 925 | No               | Yes              |
| GO:0043657 | host cell  | 9     | 6    | 18               | 14  | No               | Yes              |
| GO:0046718 | viral entry into host cell   | 29    | 17   | 42               | 26  | No               | Yes              |
| GO:0046905 | phytoene synthase activity   | 4     | 1    | 35               | 20  | No               | Yes              |
| GO:0046983 | protein dimerization activ-<br>ity                                   | 438   | 199  | 1245             | 444 | No               | Yes              |
| GO:0051286 | cell tip   | 10    | 9    | 12               | 10  | No               | Yes              |
| GO:0051996 | squalene synthase activity   | 7     | 3    | 48               | 25  | No               | Yes              |
| GO:0060548 | negative regulation of cell death                                    | 179   | 73   | 355              | 130 | No               | Yes              |
| GO:0070987 | error-free translession syn-<br>thesis                               | 2     | 2    | 7                | 7   | No               | Yes              |
| GO:0071768 | mycolic acid biosynthetic process                                    | 1     | 0    | 10               | 9   | No               | Yes              |
| GO:0075732 | viral penetration into host<br>nucleus                               | 19    | 12   | 23               | 17  | No               | Yes              |
| GO:0090305 | nucleic acid phosphodiester<br>bond hydrolysis                       | 136   | 64   | 214              | 83  | No               | Yes              |
| GO:0090729 | toxin activity   | 30    | 13   | 65               | 35  | No               | Yes              |

Table 3: Gene Ontology terms enriched among DEGs or DETs using the contrast between biomass groups. The number of genes, differentially expressed genes, transcripts and differentially expressed transcripts for each GO term are shown. The last two columns indicate if the term was enriched among DEGs and DETs, respectively.



Figure 2: Venn Diagram showing overlap between the DEGs and DETs of the photosynthesis biological process.



Figure 3: Genes associated to photosynthesis without differentially expressed transcripts. Expression, in counts per million, was measured for each biomass group. In the x - axis, transcripts passing the expression filter are shown beside the corresponding gene, indicated with the prefix **i**.



Figure 4: Expression differentially expressed transcripts corresponding to nondifferentially expressed glyceraldehyde-3-phosphate dehydrogenase (NAD+) (phosphorylating) activity related genes. The identificator of each gene provides the log of the counts per million of it. For the isoforms, the measure of expression is in counts per million shown as a proportion between the biomass groups, in different colors. The intensity of the color represents the logarithm of the counts per million (log CPM) of the transcript. Differentially expressed transcripts have their edges in black.