

**TRANSCRIPTOMICS OF POST-ANTHESIS COLOUR CHANGE IN *LOTUS***

by

Mannfred M.A. Boehm

B.Sc, Nova Scotia Agricultural College (Dalhousie University), 2014

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## Abstract

Post-anthesis colour change (PACC) is widely thought to be an adaptation to signal floral viability to pollinators, and may be a developmental pre-pattern for bird pollination in *Lotus*. *Lotus filicaulis* and *Lotus sessilifolius* are insect-pollinated herbaceous legumes with flowers that open yellow, shift to orange and finally, red. This thesis examines the ecological and molecular basis for floral colour change in these *Lotus* species.

*L. filicaulis* was cultivated in a glasshouse from which pollinating insects (bees) were excluded, and the rate of colour change was recorded in both unpollinated and manually pollinated flowers. Unpollinated flowers from both the yellow stage and red stage were sampled for sequencing. The transcriptomes of *L. filicaulis* and *L. sessilifolius* of both colour stages were analyzed for differentially expressed genes and enriched ontologies.

Pollination significantly accelerates PACC in *L. filicaulis*, consistent with the hypothesis that PACC increases pollination efficiency by directing pollinators to unpollinated flowers. RNA-seq results show the synchronized upregulation of the entire cyanidin biosynthesis pathway in the red stage of PACC of both *Lotus* species – possibly at the expense of adjacent pathways competing for the same substrate. This thesis is the foundation for understanding the molecular evolution of PACC in *Lotus* and will be useful in testing the hypothesis that PACC is a preadaptation to bird pollination in *Lotus*.

## Preface

This thesis is a draft manuscript that will be submitted for publication: Boehm, M.M.A., Ojeda D.I., and Cronk Q.C.B. 2016. Dissecting the ‘bacon-and-eggs’ phenotype: transcriptomics of floral colour change in *Lotus*. The idea came from discussions with Q.C.B Cronk and D.I. Ojeda. M.M.A. Boehm designed the analytical pipeline, performed all the analyses, conducted the hand pollination experiment and wrote the manuscript. D.I. Ojeda designed the transcriptomics experiment, collected plant material and processed tissue for RNA sequencing. Q.C.B. Cronk contributed to experimental and analytical design, contributed to the analysis of data, and provided feedback on this manuscript.

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## 1 Introduction

### 1.1 What is post anthesis colour change (PACC)?

In angiosperms, flowers are typically left on display until pollination or senescence (i.e. until the pollen becomes inviable or the stigma non-receptive), at which time the flower will generally wilt and cease reward production. Often the corolla abscises at this point, thus effectively excluding any further visits from pollinators (Faegri & Vanderpilj, 1979). However, instead of abscission, some species retain the corolla past the cessation of reward production and modify petal colour, a trait I herein refer to as post-anthesis colour change (PACC) (Mohan Ram & Mathur, 1984; Farzad et al., 2003). I use “post-anthesis colour change” instead of the broader “floral colour change” as many flowers change colour between pre-anthesis (bud) and anthesis (flower opening). This early maturation colour change is unrelated to PACC. Several species of *Lotus* L. (Fabaceae) exhibit yellow flowers at anthesis followed by a transition to red (Ojeda et al., 2013) thus these species have sometimes acquired the English vernacular name ‘bacon and eggs’ (Grigson, 1975) (Figure 1). A well-studied example is the North American lotoid legume *Acmispon glaber* (Vogel) Brouillet (*Lotus scoparius* (Nutt.) Ottley) (Jones & Cruzan, 1999).

### 1.2 Historical record of PACC

The widespread (Weiss, 1995) and striking phenomenon of PACC has long interested botanists. One of the first scientific observations of PACC dates to a letter written from Fritz Müller to Charles Darwin, discussing the pollination ecology of *Lantana camara* L. (Müller, 1877; see also Weiss, 1991; Jones & Cruzan 1999). Müller notes that *L. camara* flowers open as yellow and over a three-day period shift to orange and finally purple. Tellingly, he also writes that butterflies tend to visit yellow flowers most often, occasionally visiting the orange flowers, and never the purple flowers. More recently the phenomenon has been documented in a variety of studies and reviews (Mohan Ram & Mathur, 1984; Weiss, 1991; Ojeda et al., 2013; Brito et al. 2015).

### **1.3 Evolutionary ecology of PACC**

As anticipated by Müller, PACC is likely an adaptation to redirect pollinators at close range while maintaining long-distance appeal of the plant floral display (Weiss, 1991; Jones & Cruzan, 1999), hence the retention of the corolla instead of wilting or abscission. Indeed, in the North American *Acmispon glaber* (formerly known as *Lotus scoparius*) PACC is accelerated by pollination and corresponds temporally with the termination of reward production (Jones & Cruzan 1999). By differentiating between viable and unviable flowers, it is thought that plants are able to signal to pollinators which flowers possess a food reward (Weiss, 1991). This is of potential benefit for both organisms as the pollinator forages with greater efficiency, while the plant may receive a higher rate of successful pollination events. Pollinators could, of course, be directed to young flowers by the wilting or abscission of old flowers, but it is hypothesized that PACC provides a selective advantage by aiding long-distance attractiveness of the overall floral display.

### **1.4 Insect vision and colours of PACC**

The flower colours involved in effective signalling are not random; PACC tends to be a shift from shorter reflective wavelengths (e.g. yellow), to longer ones (e.g. red). Like many floral traits, it is plausible that PACC has evolved through interaction with pollinators, and thus the characteristics of insect vision may be implicated in the colour palette of PACC. Generally, bees (Hymenoptera: Apidae) have three kinds of light receptors with peak sensitivities at 340nm (ultraviolet, Sreceptor), 430nm (blue, M-receptor), and 540nm (green, L-receptor) (Chittka, 1996). However, the physiology of hymenopteran light receptors (Chittka, 1996) and colour-reward experiments (Weiss, 1991; Chittka & Waser, 1997; MartínezHarms et al., 2010) suggest that bees are not incapable of detecting red light (620750nm), and electroretinogram recordings of *Bombus dahlbomii* (Apidae) have demonstrated that red light can induce an L-receptor response (Martínez-Harms et al., 2010). Indeed, in the wild *B. dahlbomii* is known to visit a variety of plant species with flowers that look red to humans, and they can be trained to visit artificial red targets *ex situ* (Martínez-Harms et al., 2010). Results of colourreward experiments suggest that bees are able to detect red flowers by achromatic contrast (Chittka & Waser, 1997; Martínez-Harms et al., 2010). That is, red flowers likely appear as a dark, hue-less patches

against a background of green plant foliage, providing sufficient contrast to locate such flowers (Martínez-Harms et al., 2010).

Given that a pollinator spends a finite amount of time at any one plant, PACC may be an adaptation to attract insect pollinators at long range by deceptively retaining flowers, regardless of viability, while honestly directing pollinators at short range to the flowers most likely to benefit from pollen transfer (discussed in Brito et al., 2015). In numerous plant taxa the visual physiology of pollinators has mediated the evolution of flower colour (Rausher, 2008); in the case of PACC, it is conceivable that natural selection would favour a post-anthesis colour that is relatively less attractive than at anthesis, but not so unattractive as to be indistinguishable from its surroundings.

## 1.5 Molecular basis of PACC

The timing of PACC is potentially tied to a host of environmental and biochemical triggers that influence pigment pathways in conjunction with the cessation of food rewards. In *Lantana camara*, *Gossypium hirsutum* L., and *Viola cornuta* L. PACC has been found to a result from changes in flavonoid biosynthetic processes, specifically those branches that produce anthocyanins and their methylated derivatives (Mohan Ram & Mathur, 1984; Farzad et al., 2003; Tan et al., 2013). The anthocyanin biosynthetic pathway (ABP) is a highly conserved and versatile pathway known to produce the orange, red and purple colouration in floral tissues - in addition to a range of other functions not related to pigmentation (Kong, 2003). The regulation of PACC at the genetic level remains poorly known, however, presuming the anthocyanin pathway is involved, it is possible that PACC is regulated by MYB transcription factors (e.g. PAP1 and PAP2, Feller et al. 2011) as well as WD40s and basic helix-loop-helix proteins (Ramsay & Glover, 2005). These three proteins are known to produce a MYB-bHLH-WD40 transcription complex capable of regulating a range of target genes in numerous plant species (Ramsay & Glover, 2005), including those involved in anthocyanin biosynthesis (Spelt et al., 2002). Additionally, many flavonoid biosynthetic pathway genes are represented as gene families with multiple duplicated copies (Ober, 2005) and whether all or some of these copies are involved in PACC remains to be determined.

## 1.6 The genus *Lotus*

*Lotus* is a genus with a diverse range of floral traits to attract pollinators (Ojeda et al., 2013), including varying sugar compositions, corolla shapes, petal micromorphologies, and petal pigmentations (Ojeda et al., 2012; Ojeda et al., 2013).

It is a promising genus for studying the evolution of pollination syndromes, and PACC is particularly interesting because it has been suggested that it is a preadaptation (developmental ‘pre-pattern’), for the evolution of bird pollination in *Lotus* section Rhyncholotus (Ojeda et al., 2013). *Lotus* includes the well-studied *L. corniculatus* L., which is of considerable economic importance as a forage crop (Duke, 1981) and exhibits PACC. However, *L. corniculatus* is a polyploid and therefore a complex organism for studying gene expression.

The anthocyanin pathway in *Lotus japonicus* (Regel) K. Larsen (*L. corniculatus* L. var. *japonicus* Regel) has been well studied, but it does not exhibit PACC. Chromatographic analysis of *L. japonicus* aerial tissue has revealed that cyanidin and peonidin make up the flavonoid profile of this species (Suzuki et al., 2008); both of these pigments are known to reflect red and purple light and it is possible these same branches of the anthocyanin pathway are involved in the *Lotus* spp. that exhibit PACC. For this reason I have chosen to leverage the genomic resources available for *L. japonicus* by studying PACC in the closely related *L. filicaulis* Durieu, a diploid perennial herbaceous legume (Ferreira & Pedrosa-Harand, 2014) notable for its post-anthesis transition from yellow to red flowers (Figure 1). For comparison I also investigate another species, *Lotus sessilifolius* DC, which has been the subject of previous studies (Ojeda et al., 2013) and may play a significant role in the evolution of pollination syndromes in *Lotus* (Ojeda et al. 2012). Additionally, an analysis of the pigment composition of *L. sessilifolius* floral tissue suggests that *FLAVONOL SYNTHASE* (FLS) may play an important role in PACC of this species (Ojeda et al. 2013).

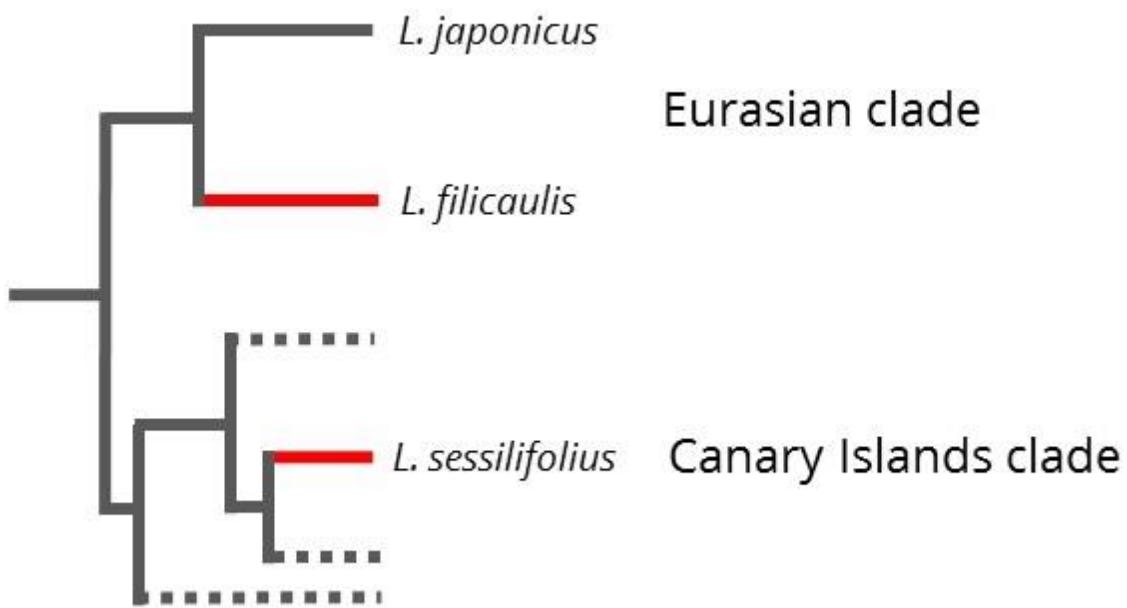
## 1.7 This Study

In this study I first investigate whether PACC is triggered by pollination in *Lotus filicaulis*. I then compare RNA expression at anthesis (yellow) and during PACC (red) in *Lotus filicaulis* and *Lotus sessilifolius* to describe the pigment pathways involved, and propose several candidate genes and pathways that I believe play a key role in post-anthesis colour change in these *Lotus*

species. This is the first transcriptome-wide analysis of PACC to date. PACC appears to have evolved independently in *L. filicaulis* and *L. sessilifolius* (Ojeda et al. 2013; Figure 2) as they belong to different clades of *Lotus*. *L. sessilifolius* is in the Canary Island clade of *Lotus* and apparently a tetraploid (Ojeda and Santos-Guerra, 2011, Ojeda et al. 2013). However, *L. sessilifolius* (insect-pollinated) is the sister taxon to the bird pollinated ‘rhynchosilurus group’ in *Lotus* (Ojeda et al. 2013), and further studies on this species can provide additional information about the evolution of bird pollination in this group. I discuss the results in the context of observations regarding PACC in other plant species, the role of pollinators in driving the evolution of PACC, the role of gene duplication, and known molecular pathways of pigment biosynthesis.



**Figure 1** *Lotus filicaulis*, exhibiting post-anthesis colour change from yellow to red, a trait commonly referred to as the ‘bacon and eggs’ phenotype. Photo taken at the Horticulture Glasshouse, University of British Columbia.



**Figure 2** Simplified phylogeny of the *Lotus* spp. used in this study (modified from Ojeda *et al.* 2013, reproduced in Supplementary Figure S54). Red branches show clades where PACC has evolved, and dotted branches indicate the presence of adjacent clades.

## 2 Materials and Methods

### 2.1 Plant material and cultivation

The North African species, *Lotus filicaulis* Durieu (voucher: Ojeda 71, herb. UBC) was used for experiments on floral manipulation; *L. filicaulis* is sometimes regarded as synonymous with *L. tenuis* Willd. A second species, *Lotus sessilifolius* DC. (voucher: Ojeda 225, herb. UBC) from the Canary Islands, Spain, was added as a comparison for RNAseq experiments. Both species exhibit strong PACC with a yellow to red colour change. The plants were propagated from seeds in the horticulture glasshouse, University of British Columbia, Vancouver, Canada. All plants were grown in pots of 10-20 cm in diameter at 20-25 °C and were more than 6 weeks old when flowers were collected for analyses.

### 2.2 Hand pollination experiment

In order to standardize the stages of PACC, five genetic individuals of *L. filicaulis* were tagged for both control and treatment (hand pollination), monitored daily for colour change, and photographed to capture distinct colour stages. 3 flowers were measured per plant for both the control and treatment. Hand pollination was conducted one day after anthesis (flower opening) to ensure the keel petals had separated. Pollen was transferred between flowers using an implement of a Victorinox SwissToolTM (unpointed blade).

The rate of progression through these stages was modelled using the lme4 package (Bates et al. 2015) in R version 3.2.2 (R Development Core Team, 2008). Data were visualized using the ggplot2 package (Wickham, 2009) in R. The R scripts used in this analysis is available at [github.com/mannfred/Lotus](https://github.com/mannfred/Lotus).

A similar experiment was conducted on 21 individuals of *L. filicaulis*, but rather than tracking each stage of colour change, only the number of days for PACC to initiate was recorded. The results of this experiment can be found in Supplementary Table S42.

### 2.3 Transcriptome sequencing

The entire corollas (dorsal, lateral and ventral petals) of *L. filicaulis* and *L. sessilifolius* were separated from the rest of the flower at the same developmental stage (Stage 13 of anthesis, Ojeda et al., 2012), but with two different colour stages: 1) flowers at anthesis with a yellow color, and 2) flowers initiating flower color change to red. At least six unpollinated flowers of each type, yellow and red, were collected from one individual. Petals of each colour were removed from the flower and pooled to represent the phenotype across the entire individual. Flower petals were immediately placed on liquid nitrogen after collection and stored at 80°C until RNA extraction. RNA was extracted using the Pure LinkTM Plant RNA Reagent from Invitrogen following the manufacturer's protocol. RNA quantity and quality was determined using a QubitR 2.0 Invitrogen (Life Technologies) and with an Agilent 2100 Bioanalyzer, respectively. Samples with a RIN value of 7 or above were used for library construction. Paired end RNA libraries (Tables 2.1 and 2.2) were prepared for each individual sample with the Illumina kit according to the manufacturer's protocol. All samples were sequenced (100 base pair reads) in one lane of an Illumina Hi-Seq 2000 at the NextGen Sequencing Facility at the Biodiversity Research Centre, University of British Columbia (UBC).

### 2.4 Sequence analysis

Aligning RNA-seq data from *L. filicaulis* and *L. sessilifolius* has not yet been reported. Therefore, I compared two methods to discover differentially expressed genes relevant to PACC. In the first method, RNA-seq reads were aligned to the reference genome of a closely related model organism, *Lotus japonicus*. In the second method, RNA-seq reads were assembled into contigs (putative genes) and aligned to a reference transcriptome that was assembled *de novo* from the reads themselves. All scripts used in the analyses are available at [github.com/mannfred/Lotus](https://github.com/mannfred/Lotus).

### 2.5 TopHat Alignment

RNA-seq reads from *L. filicaulis* were analysed by TopHat. FASTQC Galaxy v0.63 (Andrews, 2010) was executed on a public Galaxy server (Goecks et al., 2010) to check the quality of reads and to identify sequencing artifacts. Sequencing reads were mapped to the *Lotus japonicus* genome release 2.5 (Sato et al., 2008) using TopHat2 Galaxy v0.9 (Kim et al., 2013). Sequence

Alignment Map (.sam) files were processed by HTSeq (Anders et al., 2015) to count the number of RNA-seq reads that mapped to each TopHat feature (gene), and differential expression (DE) was calculated using edgeR (Robinson et al., 2010). Raw counts per gene were used instead of FPKM transformed counts because edgeR incorporates its own dispersion-normalization procedure in its DE calculations (Robinson et al., 2010). EdgeR results were processed using a set of custom R scripts to filter significant results (Benjamini-Hochberg FDR <0.05 (Benjamini & Hochberg, 1995)) and differences in fold change.

DE genes were analyzed for ontology term enrichment (Yekutieli FDR<0.05 (Yekutieli & Benjamini, 1999)) using the Singular Enrichment Analysis tool with *Lotus japonicus* 2.5 genome reference in AgriGO (Du et al., 2010). Because the functional annotation of the *L. japonicus* genome is ongoing, genes associated with enriched GO categories were annotated for putative function by BLASTing the *Glycine max* (L.) Merr. CDS library (Williams 82 Assembly 2 version 1, Schmutz et al., 2010) sourced from Phytozome v10.3 (Goodstein et al., 2012).

## 2.6 STAR Alignment

RNA-seq reads from both *L. filicaulis* and *L. sessilifolius* were mapped to the *Lotus japonicus* genome Kazusa version 2.5 (Sato et al., 2008) using STAR v2.4 (Dobin et al., 2013). Successfully mapped transcripts were assembled and expression was estimated using RSEM v1.2.19 (Li & Dewey, 2011). Differential expression of the yellow and red flower transcriptomes was calculated using the same methods as above.

## 2.7 *De novo* Assembly by Trinity

Sequenced Illumina reads from *L. filicaulis* and *L. sessilifolius* were assembled *de novo* into contigs in Trinity v2.0.6 (Grabherr et al., 2011). Using assembled contigs as a reference transcriptome, contigs were measured for expression using Trinity's built-in RSEM tool (Li & Dewey, 2011) and DE was calculated as above. DE contigs were then identified by BLASTn (Altschul et al., 1997) as their top scoring *Lotus japonicus* homolog (expectation value of 1e-4) using the *L. japonicus* v2.5 CDS library sourced from Kazusa (Sato et al., 2008). Ontology term enrichment and annotation of associated genes was analysed as above.

## 2.8 Nucleotide Alignments

Trinity contigs BLASTing to *L. japonicus* genes of interest were investigated further by BLASTing the Legume IP database (Li et al., 2011) for *Glycine max* (v2.0) (Schmutz et al., 2010), *Medicago truncatula* Gaertn. (Mt4.0v1) (Young et al., 2011), and an outgroup *Arabidopsis thaliana* (L.) Heynh. (TAIR10) (Lamesch et al., 2011) to further identify homologous genes. Trinity contigs were trimmed before and after putative start and stop codons, respectively, which were identified by pairwise sequence alignment of their *L. japonicus* homolog using EMBOSS Needle (Rice et al., 2000). Coding sequences of putative homologs were compiled along with the relevant trimmed Trinity contig into a .fasta file and translated to amino acid sequences using TranslatorX (Abascal et al., 2010). Multiple sequence alignment was performed using the T-Coffee (Notredame et al., 2000) option in TranslatorX. The phylogenetic relationship of a given Trinity contig to its relatives was visualized using RAxML v8.2 (Stamatakis, 2014) in Mesquite (Maddison & Maddison, 2015).

## 2.9 Gene family composition

Trinity (*de novo* assembly) sequences were BLASTed (1e-4) against the *L. japonicus* CDS library at LegumeIP to detect gene duplicates – specifically those not expressed at the time of sampling (this approach assumes no new gene duplications have occurred between *L. japonicus* and our two study species). BLAST results sharing the same annotation (i.e. gene name) as the top alignment were considered to be a part of the same gene family. Certain gene families (e.g. *DFR*, *CHS*) have been especially well-studied in *L. japonicus*, but CDS libraries are not necessarily updated in real time in accordance to ongoing *L. japonicus* research. In these cases, published literature on specific gene families took precedence over LegumeIP results.

**Table 1** Next-generation library construction for *L. filicaulis*.

| Library no. | Library ID<br>(Barcode) | Yellow / Red | Plant no. | Flower no.<br>(pooled) |
|-------------|-------------------------|--------------|-----------|------------------------|
| 1           | ATGAGC                  | Red          | 1         | $\geq 6$               |
| 2           | ACTGAT                  | Yellow       | 1         | $\geq 6$               |

**Table 2** Next-generation library construction for *L. sessilifolius*.

| Library no. | Library ID<br>(Barcode) | Yellow / Red | Plant no. | Flower no.<br>(pooled) |
|-------------|-------------------------|--------------|-----------|------------------------|
| 1           | CAAAAG                  | Red          | 1         | $\geq 6$               |
| 2           | ATTCCCT                 | Yellow       | 1         | $\geq 6$               |

### 3 Results

#### 3.1 Pollination accelerates colour change in *L. filicaulis*

Four distinct colour stages of PACC were observed; 1) Entirely yellow, 2) blush of orange, typically at the keel, 3) systemic orange colouration across the corolla, and 4) red, onset typically at the keel (Figure 2.1). The general linear model to test the effects of time (days) and treatment on colour was:

$$\text{COLOUR} = \text{CONSTANT} + \text{DAYS} + \text{TREATMENT} + \text{DAYS*TREATMENT}$$

Among plant standard deviation was effectively zero ( $s= 2.664 \text{ e-05}$ ) and was omitted as a random effect.

The number of days from anthesis was a significant predictor of colour ( $df= 1, 39; p= 1.69 \text{ e-09}$ ), as well as the effect of treatment through time ( $df= 1, 39; p= 5.06 \text{ e-04}$ ). No significant effect of treatment alone was detected ( $df= 1, 39; p= 0.851$ ).

The rate of progression through the four stages of PACC in the hand pollinated treatment is over twice that of the control (see Figure 2.1 caption).

#### 3.2 Illumina HiSeq library

A total of 12.5 million and 11.9 million .fastq reads were sequenced from the yellow-stage and red-stage RNA-seq libraries of *L. filicaulis*, respectively. GC content was 46% for the forward yellow-stage and red-stage reads and 45% for the reverse reads.

Similarly, 6.7 million and 12.0 million .fastq reads were sequenced from the yellow-stage and red-stage RNA-seq libraries of *L. sessilifolius*, respectively. GC content was 45% for the forward yellow-stage and red-stage reads and 44% for the reverse reads.

#### 3.3 *De novo* assembly of non-model organism RNA reads outperforms alignment of reads to a reference genome of a closely related species

Two methods were used to discover differential expression in PACC, 1) direct mapping of reads to the *L. japonicus* genome using two different splice junction mappers, and 2) *de novo* assembly

of reads to contigs followed by BLASTing these contigs to a *L. japonicus* CDS library. The evaluation of the two approaches is summarised in Tables 3.1 and 3.2. Direct alignment by STAR and TopHat achieved low mapping rates due to unsuccessful heterologous mapping from our study species to *L. japonicus*. However, the *de novo* assembly protocol achieved a high rate of gene identification, and produced independent contigs that could be verified for identity and biological relevance by amino acid alignment and phylogenetic methods. Trinity contigs of interest were found to align with homologous genes of related legumes, and their known phylogenetic relationship was successfully predicted by these alignments (Supplementary Figures S1-S53). A comparison of the most differentially expressed contigs of both stages and of both *Lotus* species can be found in Supplementary Tables S38-S41.

### **3.4** The cyanidin pathway is upregulated in the red stage of PACC in *L. filicaulis* and *L. sessilifolius*

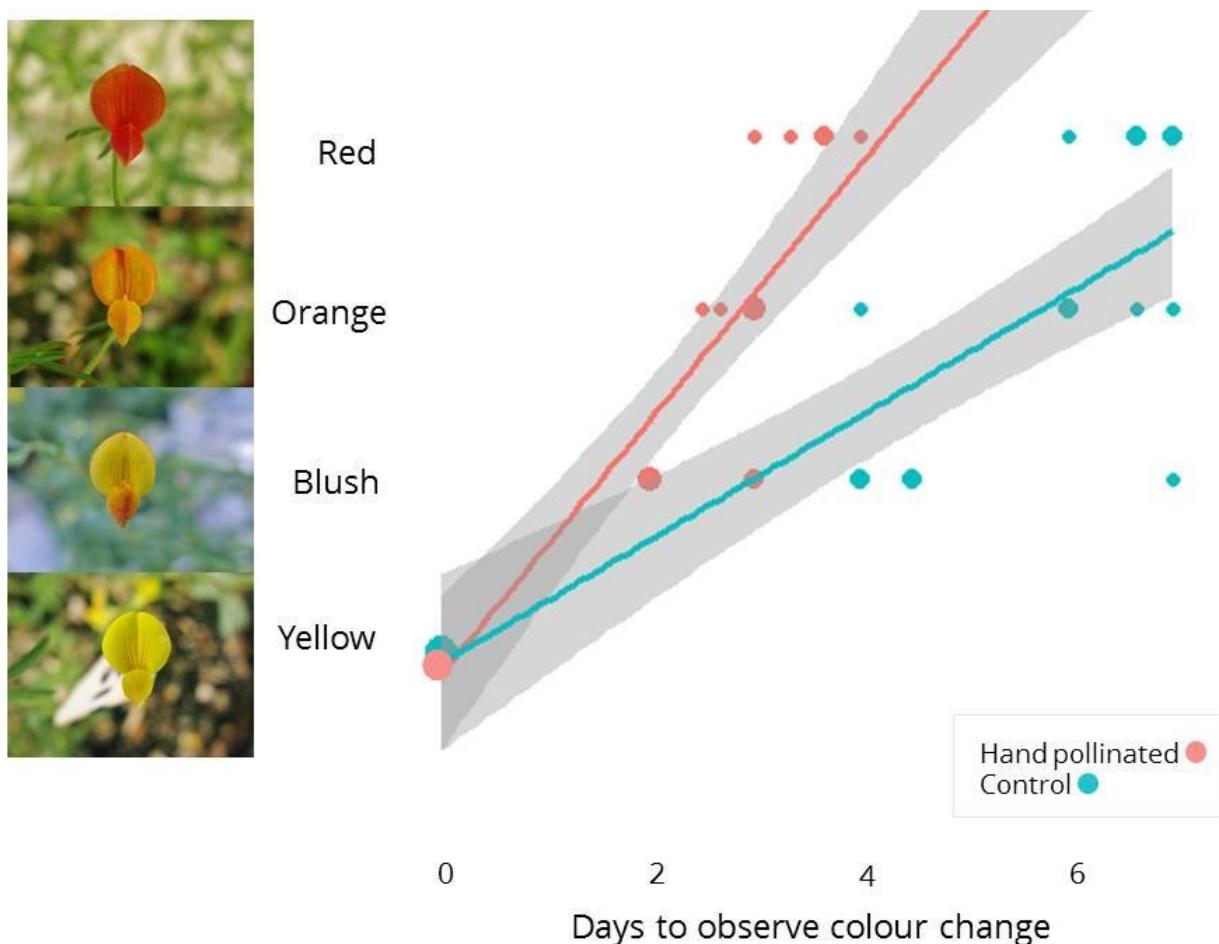
Regardless of the bioinformatics protocol, upregulation of numerous genes in the anthocyanin biosynthetic pathway (ABP) was detected in the red stage of PACC in both *Lotus* species examined (Tables 3.3 and 3.4, Figures 3.2 and 3.3). Except for anthocyanin synthase and flavonoid 3'-hydroxylase (both apparently single copy), gene duplicates or multiple gene families were identified in the *Lotus japonicus* genome for each of the genes of the ABP in both species (Figures 3.2 and 3.3). As a comparison the same analysis was carried out for the lignin biosynthetic pathway (LBP), which is not expected to be associated with PACC. There is no consistent upregulation of LBP genes during PACC. Generally, LBP genes are either not expressed, downregulated during PACC, or not differentially expressed (Figures 3.2 and 3.3).

### **3.5** The carotenoid pathway is upregulated in the yellow stage of PACC in *L. filicaulis* and *L. sessilifolius*

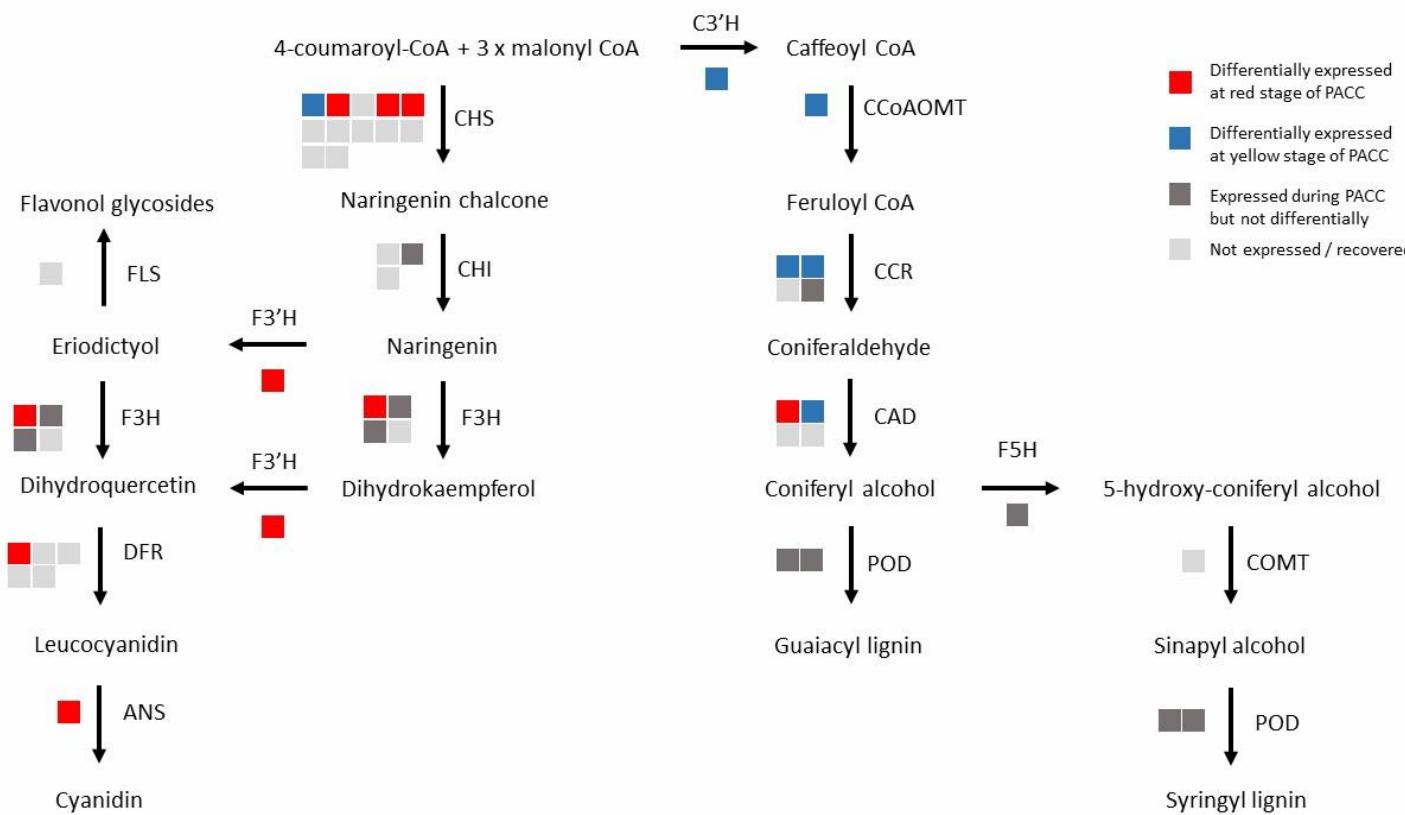
Genes annotated to the carotenoid biosynthetic process (GO:0016117) were significantly upregulated at the yellow stage (i.e. downregulated at the red stage of PACC) in both *Lotus* species (Supplementary Tables S35-S36). The core carotenoid pathway is represented, including the two major branches producing zeaxanthin and lutein (Figures 3.4 and 3.5).

### 3.6 Differences between *L. filicaulis* and *L. sessilifolius*

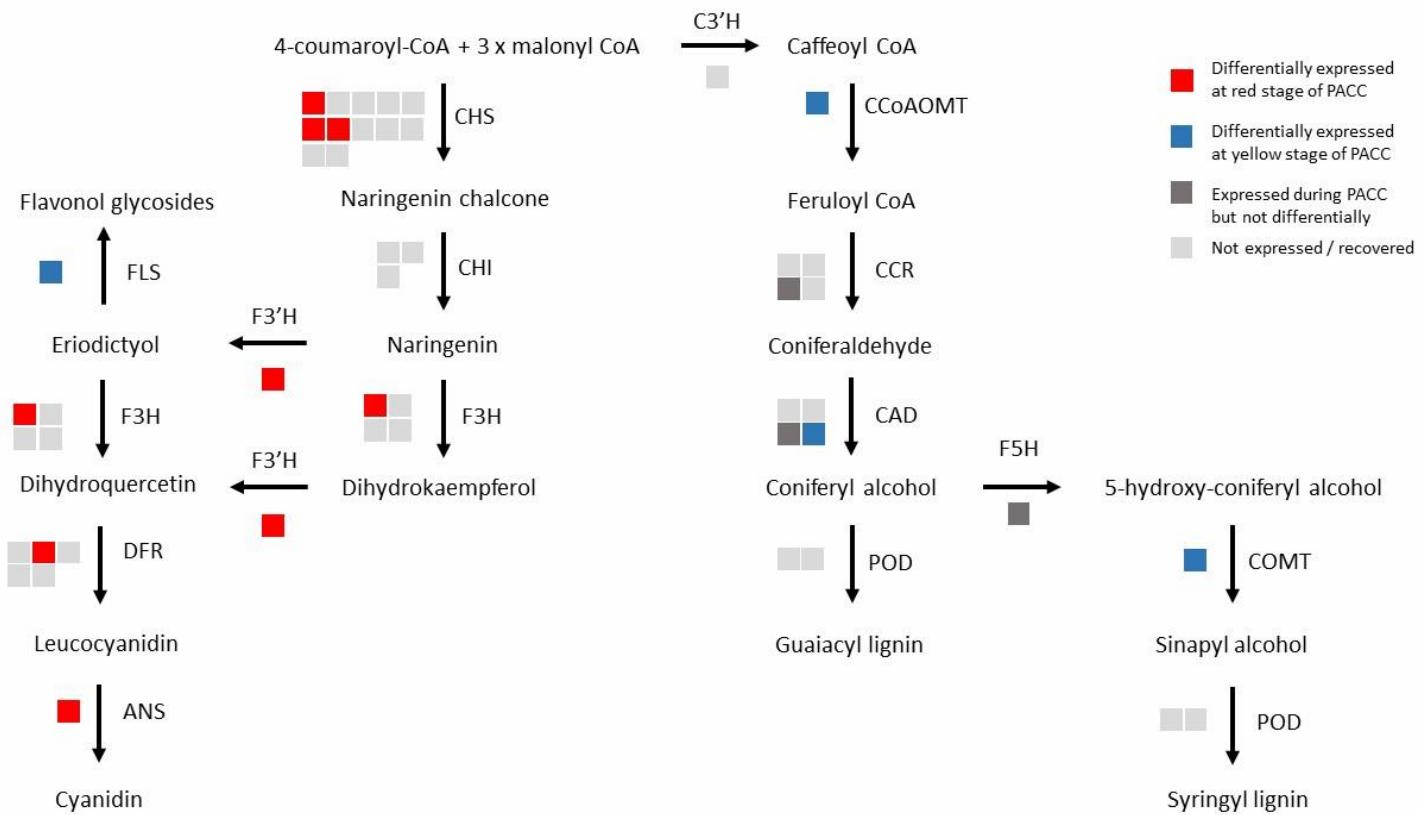
Although the cyanidin pathway is upregulated during PACC in both *Lotus* species, there are differences in the composition of genes expressed, specifically those genes that are a part of a gene family. For example, *CHALCONE SYNTHASE (CHS)* is active in both species but the gene family members expressed in *L. filicaulis* and *L. sessilifolius* are disjoint sets. Similar differences are observed in the expression of *DFR*. However, it is notable that of the four F3H duplicates known for *L. japonicus*, only one is implicated in PACC for the two *Lotus* species studied. The expression of transcription factors and regulators contribute to numerous other differences between PACC in *L. filicaulis* and *L. sessilifolius* (Supplementary Tables S11-S18, S27-S34). Finally, I bring attention to the presence of *FLAVONOL SYNTHASE (FLS)* in *L. sessilifolius* (Table 6, Figure 5). I detect an upregulation of FLS during the yellow stage of PACC in this species, and an absence of activity of this gene in *L. filicaulis*. The ecological and evolutionary implications of this are discussed below.



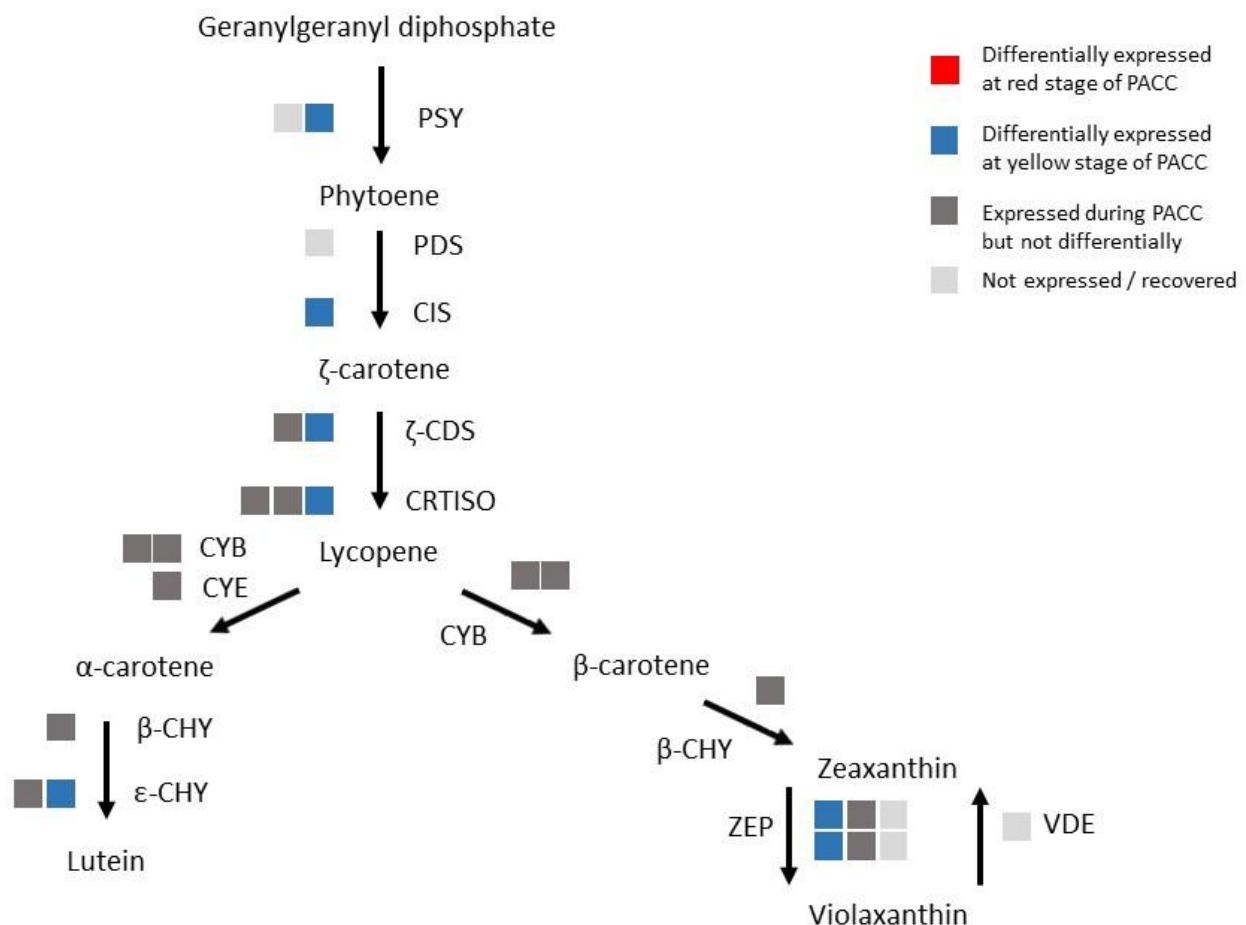
**Figure 3** Left panel: Standardization of flower colour using photographs of four distinct stages: anthesis ('Yellow', stage 0), onset of keel colouration ('Blush', stage 1), systemic orange across the corolla ('Orange', stage 2), and full colouration ('Red', stage 3). Graph: timing of stages in the hand pollinated treatment (orange line, colour stage = 0.739(Days) – 0.121) and control (unpollinated) (turquoise line, colour stage = 0.358(Days) – 0.059). Intercepts were not fixed (anthesis) to maintain zero-mean residuals. Each point represents the mean number of days to one of four stages (mean of three replicates per treatment per individual plant). The size of points is proportional to the amount of over-plotted data.



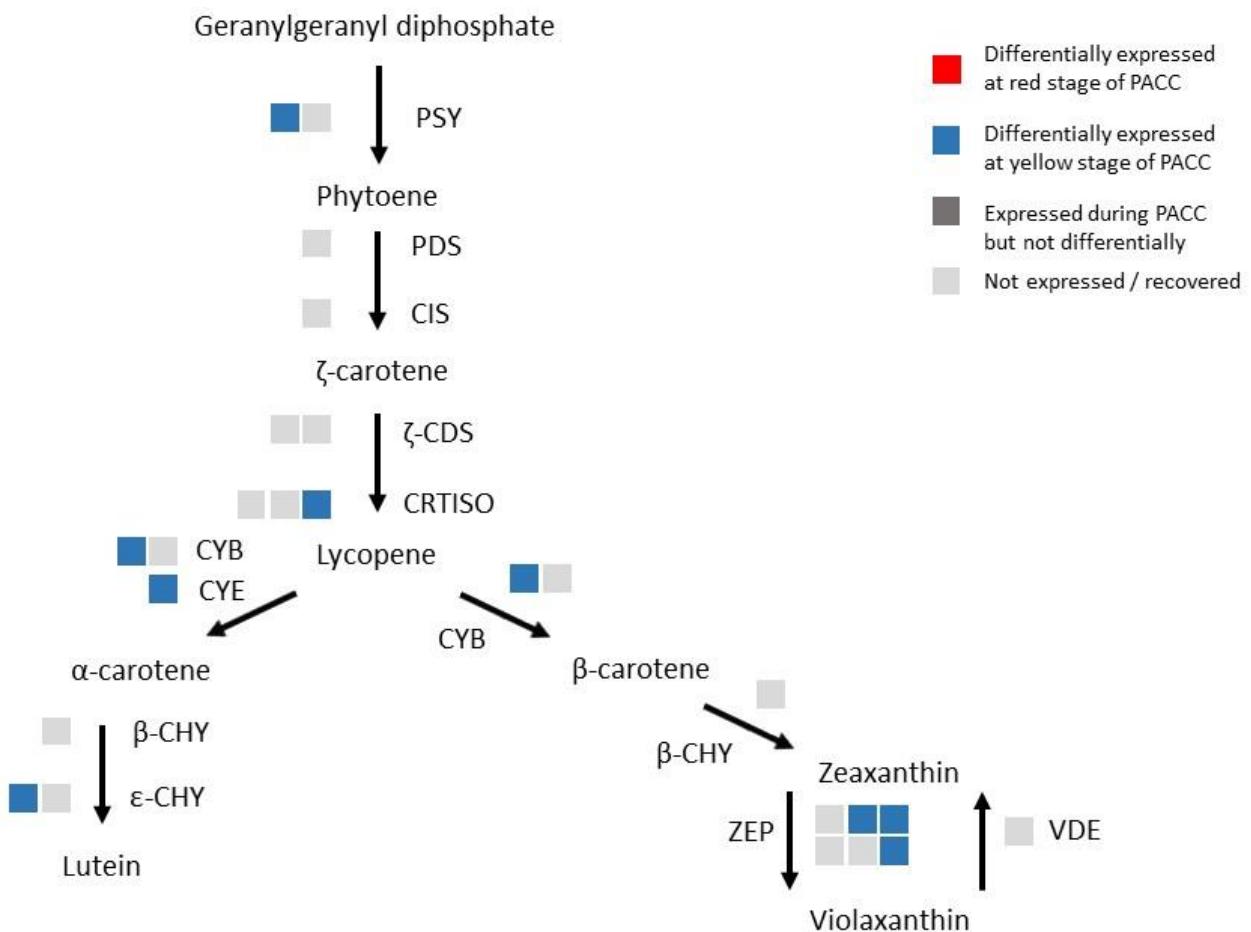
**Figure 4** Graphical representation of the differential expression of genes (Trinity contigs) involved in flavonoid and lignin biosynthesis throughout PACC in *Lotus filicaulis*. The boxes represent genes as annotated in the *Lotus japonicus* genome; light grey boxes represent genes that were not found in the *L. filicualis* transcriptome (gene may be absent or may have been undetected). Expression of putative orthologs in *L. filicualis* are indicated by colour (red: upregulated at red stage; blue: upregulated at yellow stage; dark grey: not differentially expressed). 4coumaroyl-CoA and malonyl CoA are derivatives of phenylalanine and acetatepyruvate, respectively. In the flavonoid pathway CHS = chalcone synthase, CHI = chalcone isomerase, F3'H = flavonoid 3'-hydroxylase, F3H = flavnone 3hydroxylase, DFR is dihydroflavonol 4-reductase, and ANS is anthocyanidin synthase. In the lignin pathway C3'H = coumaroyl shikimate 3'-hydroxylase, CCoAOMT = caffeoyl-CoA O-methyltransferase, CCR = cinnamoyl-CoA reductase, CAD = cinnamyl alcohol dehydrogenase, POD = peroxidase, F5H = ferulate 5hydroxylase, and COMT = caffeic acid O-methyltransferase.



**Figure 5** Graphical representation of the differential expression of genes (Trinity contigs) involved in flavonoid and lignin biosynthesis throughout PACC in *Lotus sessilifolius*. Conventions and acronyms are as defined in the legend to Figure 4.



**Figure 6** Graphical representation of the differential expression of genes (Trinity contigs) involved in carotenoid biosynthesis throughout PACC in *Lotus filicaulis*. The boxes represent genes as annotated in the *Lotus japonicus* genome; light grey boxes represent genes that were not found in the *L. filicualis* transcriptome (gene may be absent or may have been undetected). Expression of putative orthologs in *L. filicaulis* are indicated by colour (red: upregulated at red stage; blue: upregulated at yellow stage; dark grey: not differentially expressed). Geranylgeranyl diphosphate is a derivative of pyruvate and glyceraldehyde 3phosphate. In the carotenoid pathway PSY = phytoene synthase, PDS = phytoene desaturase, CIS = carotene isomerase, ζ-CDS = ζ-carotene desaturase, CRTISO = prolycopene isomerase, CYB = β-lycopene cyclase, CYE = ε-lycopene cyclase, β-CHY = β-carotene hydroxylase, ε-CHY = ε-carotene hydroxylase, ZEP = zeaxanthin epoxidase, and VDE = violaxanthin de-epoxidase.



**Figure 7** Graphical representation of the differential expression of genes (Trinity contigs) involved in carotenoid biosynthesis throughout PACC in *Lotus sessilifolius*. Conventions and acronyms are as defined in the legend to Figure 6.

**Table 3** Comparison of protocols used to detect differential expression of RNA in *L. filicaulis*.

| Analysis type<br>(mapping reference)             | Reads mapped<br>yellow stage | Reads mapped<br>red stage | DE genes<br>yellow stage<br>↑ | DE<br>genes<br>Red<br>stage ↑ | Enriched GO<br>terms yellow<br>stage | Enriched<br>GO terms<br>red stage |
|--|------------------------------|---------------------------|-------------------------------|-------------------------------|--------------------------------------|-----------------------------------|
| TopHat ( <i>L.<br/>japonicus</i><br>genome)      | 8,538,623                    | 7,243,178                 | 1419                          | 962                           | 107                                  | 84                                |
| STAR<br>( <i>L. japonicus</i> genome)            | 4,334,187                    | 3,566,554                 | 1398                          | 958                           | 128                                  | 97                                |
| Trinity<br>( <i>L. filicaulis</i> transcriptome) | 14,025,763                   | 13,167,862                | 3287                          | 1244                          | 112                                  | 44                                |

**Table 4** Comparison of protocols used to detect differential expression of RNA in *L. sessilifolius*.

| Analysis type<br>(mapping reference)                | Reads mapped<br>yellow stage | Reads mapped<br>red stage | DE genes<br>yellow stage<br>↑ | DE<br>genes<br>Red<br>stage ↑ | Enriched GO<br>terms yellow<br>stage | Enriched<br>GO terms<br>red stage |
|---|------------------------------|---------------------------|-------------------------------|-------------------------------|--------------------------------------|-----------------------------------|
| TopHat ( <i>L. japonicus</i><br>genome)             | 1,333,258                    | 1,855,091                 | 394                           | 632                           | 122                                  | 95                                |
| STAR<br>( <i>L. japonicus</i> genome)               | 1,272,184                    | 1,976,009                 | 610                           | 954                           | 151                                  | 120                               |
| Trinity<br>( <i>L. sessilifolius</i> transcriptome) | 13,085,868                   | 23,373,724                | 1175                          | 1468                          | 100                                  | 57                                |

**Table 5** Selected genes for enzymes involved in flavonoid biosynthesis in *L. filicaulis*. The first column lists differentially expressed (FDR<0.05) Trinity contigs. The second column lists the gene names produced by BLASTing and aligning contigs to the *Glycine max* CDS library, followed by the function of the enzyme which it codes for. The fourth and fifth columns list raw counts per gene at each colour stage, followed by the normalized log<sub>2</sub> fold change. Sequences for the Trinity contigs in this table are listed in Appendix 1.

| Trinity contig | Glycine max homolog  | Function  | Counts per gene<br>(Yellow stage) | Counts per gene (Red stage) | Normalized Log <sub>2</sub> FC<br>(Red/Yellow) |
|----------------|--|---|-----------------------------------|-----------------------------|--|
| TR19889 c3_g1  | Glyma.01g43880.1<br><i>CHALCONE SYNTHASE (CHS)</i>               | Produces naringenin chalcone 1385 from phenylalanine and acetate-pyruvate derivatives.      | 19064                             |                             | -4.1392  |
| TR48518 c0_g1  | Glyma.11G011500.1<br><i>CHALCONE SYNTHASE (CHS)</i>              | Produces naringenin chalcone 77 from phenylalanine and acetate-pyruvate derivatives.        | 430                               |                             | -2.8356  |
| TR19889 c2_g1  | Glyma.01G228700.1<br><i>CHALCONE SYNTHASE (CHS)</i>              | Produces naringenin chalcone 17 from phenylalanine and acetate-pyruvate derivatives.        | 207                               |                             | -3.9514  |
| TR52191 c0_g1  | Glyma.06G202300.1<br><i>FLAVONOID 3'-HYDROXYLASE (F3'H)</i>      | Catalyses naringenin to eriodictyol   | 1586                              | 15638                       | -3.6580  |
| TR38960 c0_g1  | Glyma02g05450.1<br><i>FLAVANONE 3HYDROXYLASE (F3H)</i>           | Catalyses naringenin to dihydroskaempferol and eriodictyol to dihydroquercetin              | 1830                              | 6123                        | -2.0988  |
| TR29586 c0_g1  | chr5.CM0077.210.r2.m<br><i>DIHYDROFLAVONOL 4-REDUCTASE (DFR)</i> | Catalyses dihydroquercetin to 148 leucocyanidin and dihydroskaempferol to leucopelargonidin | 10163                             |                             | -6.4567  |

| Trinity contig | Glycine max homolog  | Function  | Counts per gene<br>(Yellow stage) | Counts per gene (Red stage) | Normalized Log <sub>2</sub> FC<br>(Red/Yellow) |
|----------------|--|---|-----------------------------------|-----------------------------|--|
| TR50049 c0_g1  | <i>ANTHOCYANIDIN SYNTHASE (ANS)</i>                                | Catalyses leucocyanidin to cyanidin and leucopelargonidin to pelargonidin | 1010                              | 14783                       | -4.2278  |
| TR23981 c0_g1  | Glyma17g34530.2<br><i>MORE AXILLARY BRANCHES (MAX1)</i>            | Positive regulator of several flavonoid biosynthesis enzymes              | 45                                | 2987                        | -6.3605  |
| TR11002 c0_g1  | Glyma14g06750.1<br><i>PRODUCTION OF ANTHOCYANIN PIGMENT (PAPI)</i> | Encodes MYB75 transcription factor involved in anthocyanin metabolism     | 475                               | 54                          | 2.7778   |

**Table 6** Selected genes for enzymes involved in flavonoid biosynthesis in *L. sessilifolius*. See Table 5 for column descriptions. Sequences for the Trinity contigs in this table are listed in Appendix 2.

| Trinity contig            | Glycine max homolog   | Function  | Counts per gene (Yellow stage) | Counts per gene (Red stage) | Normalized Log <sub>2</sub> FC (Red/Yellow) |
|---------------------------|---|---|--------------------------------|-----------------------------|---|
| TRINITY_DN5<br>4168_c0_g2 | Glyma.11G011500.1<br><i>CHALCONE SYNTHASE (CHS)</i>         | Produces naringenin chalcone from phenylalanine and acetate-pyruvate derivatives. | 65                             | 4382                        | -6.1743                                     |
| TRINITY_DN5<br>4168_c0_g1 | Glyma.11G011500.1<br><i>CHALCONE SYNTHASE (CHS)</i>         | Produces naringenin chalcone from phenylalanine and acetate-pyruvate derivatives. | 17                             | 1134                        | -6.1510                                     |
| TRINITY_DN5<br>2652_c1_g1 | Glyma.19G105100.1<br><i>CHALCONE SYNTHASE (CHS)</i>         | Produces naringenin chalcone from phenylalanine and acetate-pyruvate derivatives. | 145                            | 660                         | -2.2875                                     |
| TRINITY_DN5<br>2780_c0_g1 | Glyma.06G202300.1<br><i>FLAVONOID 3'-HYDROXYLASE (F3'H)</i> | Catalyses naringenin to 7 eriodictyol   | 7                              | 2073                        | -8.2859                                     |
| TRINITY_DN5<br>2390_c0_g1 | Glyma.02G048400.1<br><i>FLAVANONE 3HYDROXYLASE (F3H)</i>    | Catalyses naringenin to dihydrokaempferol and eriodictyol to dihydroquercetin     | 276                            | 1893                        | -2.8794                                     |

| Trinity contig | Glycine max homolog                      | Function   | Counts per gene<br>(Yellow stage) | Counts per gene (Red stage) | Normalized Log <sub>2</sub> FC<br>(Red/Yellow) |
|----------------|--|--|-----------------------------------|-----------------------------|--|
| TRINITY_DN5    | Glyma.17G252200.1                        | Catalyses  | 19                                | 434                         | -4.6063  |
| 2789_c0_g1     | <i>DIHYDROFLAVONOL 4-REDUCTASE (DFR)</i> | dihydroquercetin to leucocyanidin and dihydrokaempferol to leucopelargonidin |                                   |                             |  |
| TRINITY_DN5    | Glyma.11G027700.1                        | Catalyses  | 30                                | 3761                        | -7.0659  |
| 3094_c0_g1     | <i>ANTHOCYANIDIN SYNTHASE (ANS)</i>      | leucocyanidin to cyanidin and leucopelargonidin to pelargonidin              |                                   |                             |  |
| TRINITY_DN3    | Glyma.13G082300.1                        | Mediates production of   | 1601                              | 112                         | 3.7338   |
| 9800_c0_g1     | <i>FLAVONOL SYNTHASE (FLS)</i>           | isorhamnetin, quercetin, and kaempferol from dihydroflavonol substrates      |                                   |                             |  |

## 4 Discussion

### 4.1 The significance of pollination as a trigger for PACC

I present the first experimental evidence that PACC is accelerated by pollination in *L. filicaulis* (Figure 2.1); these measurements support those made of *Acmispon glaber* (Jones & Cruzan, 1999). This is strongly suggestive that PACC is a trait that has evolved via interaction with pollinators rather than an incidental characteristic of the aging process with no fitness consequences. This is unsurprising as many floral traits are indeed driven by pollinators (Schiestl & Johnson, 2013) and it is consistent with this trait being a widespread evolutionary convergence (Weiss, 1995). Viewing PACC as an evolved adaptive trait raises the question of the precise adaptive function that it confers. Brito et al. (2015) suggest that the retention of flowers is to increase attraction of insect pollinators at long range, and then at short range increase foraging efficiency by signalling flower quality to pollinators.

The demonstration of a pollination trigger for PACC also raises the question of what the precise nature of the trigger is. Pollination involves both physical movement of the petals of the flower (thigmotaxis), deposition of pollen on the stigma, subsequent growth of pollen tubes, and fertilization of the ovules. All these processes have physiological consequences and could be transduced into signals leading to PACC. Further experimental work is required to dissect the events of pollination that lead to the acceleration of PACC. Even without pollination, colour change eventually occurs. This suggests that there is an additional, late-acting or ‘failsafe’ trigger for the onset of PACC, one that presumably responds to late acting autogamy or to some aspect of floral aging.

### 4.2 *De novo* assembly is preferred for RNA-seq data without a reference genome

The two RNA-seq aligners used in our study, STAR and TopHat, performed similarly in aligning sequenced reads to the genome of a sister species. Our results are consistent with a recently published comparison of 11 commonly used alignment protocols (Engström et al., 2013). STAR 1-pass uses gene annotation information to identify exon splice junctions before aligning to the reference genome. TopHat also uses an annotation file to identify junctions, and furthermore, uses this information to align reads directly against annotated transcripts. Therefore, both

alignment protocols require a well-annotated genome, which currently does not exist for *L. filicaulis* or *L. sessilifolius*. Aligning *L. filicaulis* and *L. sessilifolius* reads to the genome of *L. japonicus* led to a substantial loss of data as the majority of reads remained unmapped. However, the number of *L. filicaulis* reads mapped to *L. japonicus* outperformed the number of mapped *L. sessilifolius* reads when considering the same protocol. This underscores the importance of phylogenetic relatedness when using a model genome to study a congener.

Our results strongly support the use of a *de novo* assembly of RNA-seq reads into a transcriptome (then mapping reads back onto the transcriptome) as opposed to attempts at cross-species mapping in *Lotus*. Contigs may then be identified by reciprocal BLAST (with putative genes checked by amino acid alignment) against the most closely related species with an annotated genome.

#### **4.3 PACC is associated with the wholesale upregulation of the flavonoid pathway**

Ojeda et al. (2013) previously studied gene expression of some flavonoid biosynthesis genes involved in PACC in *L. filicaulis* and *L. sessilifolius* by PCR. Our results support and extend their findings. With the exception of chalcone isomerase, at least one copy of each gene in the flavonoid biosynthetic pathway leading to cyanidin production is differentially expressed (increased) at the red stage of PACC (Figures 3.2 and 3.3). An alternative scenario might be that most of these genes might already be expressed at the yellow stage with the exception of one critical enzyme without which pigment cannot be made. Under this scheme the upregulation of a single enzyme would be enough to initiate colour change. Our results make it clear that this is not the case. Instead nearly the entire pathway is upregulated. Except for chalcone isomerase, all genes in the pathway have relatively low expression at anthesis and increase dramatically at the PACC stage (Tables 3.3 and 3.4; Figures 3.2 and 3.3). This implies that the genes in the pathway are co-regulated as a unit during PACC, possibly by a master regulator (see discussion below under MAX1).

#### **4.4 PACC is associated with highly specific upregulation of members of gene families**

Many of the enzymes involved are coded for by multiple genes in gene families. An example is DFR which has numerous copies (DFR1-DFR5) in *Lotus japonicus* (Shimada et al., 2005). However, during PACC only one paralog is upregulated. Other examples are obvious from

inspection of Figures 3.2 and 3.3. In these cases, the upregulation of specific copies, rather than all copies of a particular enzyme gene, seems to drive PACC. Some gene copies are apparently not expressed at either stage, but may be involved in tissue-specific expression elsewhere. It is worth noting that different gene copies within the *DFR* and *CHS* families are expressed between *L. filicaulis* and *L. sessilifolius*. This is also the case for several gene families of the carotenoid pathway (Figures 3.4 and 3.5). This lends support to the convergent evolution of PACC between *L. filicaulis* and *L. sessilifolius*, as hypothesized by Ojeda et al (2013; Figure 2). However, it remains possible that, regardless of differences in the upregulation of gene copies, some general aspect of the flavonoid pathway (e.g. how it is regulated) is a developmental pre-pattern for PACC that is common to *Lotus*. In this case, the apparent convergent evolution of PACC could be considered a “latent homology” (Nagy et al., 2014).

Because anthocyanins are highly versatile and serve a wide range of functions (Kong, 2003), it may be that gene duplication is key to the evolution of novel adaptations involving anthocyanins, including PACC. This may be especially likely for the putatively tetraploid *L. sessilifolius*, and although *L. filicaulis* is a diploid, it has had numerous whole genome duplication events in its history, like many plants. Neo-functionalization, where directional selection leads to novel function following a gene duplication event, may explain why only certain copies of flavonoid biosynthesis genes were actively transcribed during PACC. Alternatively the association of PACC with duplicated genes might be explained by escape from adaptive conflict (Des Marais & Rausher, 2008). In this scenario a single copy gene is adapted to simultaneously perform its ancestral function while performing some novel function, but is constrained in its ability to improve due to antagonistic pleiotropy. Following gene duplication both ancestral and novel functions have the potential to be better adapted. Marais and Rausher (2008) demonstrated that adaptive changes in the *DFR* gene of pre-duplication and post-duplication plant species are best explained as escape from adaptive conflict. In order to determine if PACC is a result of gene duplication, evidence for the ancestral and novel functions of flavonoid biosynthesis genes would need to be studied in *L. filicaulis*, *L. sessilifolius* and their relatives. In the case of *L. japonicus*, various duplicates of DFR differ in their responses to stress and organ specificity (Yoshida et al., 2010), but whether any such gene copies are functionally divergent in our study species, and whether any of these copies are flower-specific remains unstudied.

#### 4.5 Upregulation of flavonoid biosynthesis may be antagonistic to other phenylpropanoid pathways

Expression of genes in the lignin biosynthetic pathway (LBP) suggests that a shift from the lignin branch of the phenylpropanoid pathway to the flavonoid branch may occur over the course of PACC (Figures 3.2 and 3.3). Transcriptome and metabolite profiling of *Fragaria x ananassa* (Duchesne) has demonstrated a metabolic interaction between flavonoid and lignin biosynthesis in strawberry fruit (Ring et al., 2013). Lignin biosynthesis competes for the same upstream substrate (coumaroyl-CoA) as flavonoid biosynthesis and, as would be expected under constant substrate availability, a shift from lignin production to flavonoid production can be measured in anthocyanin-rich tissues (Ring et al., 2013). Therefore it is plausible that in *L. filicaulis* and *L. sessilifolius* a drop in substrate availability may result in the downregulation of LBP genes observed during PACC.

Alternatively, expression of LBP genes may be reduced following flowering, regardless of PACC, due simply to developmental changes associated with aging.

#### 4.6 Carotenoid biosynthesis is downregulated during PACC

Carotenoids are widespread in the yellow to orange flowers of numerous insect-pollinated taxa, and likely play a role in the detectability of flowers by pollinators (Tananka et al. 2008, Cronk and Ojeda 2008). Consequently, the biosynthesis of carotenoids is generally well understood (Zhu et al. 2010). I detected the upregulation of the genes controlling the core carotenoid pathway at the yellow stage of PACC in both *Lotus* species – this is expected given the definitively yellow appearance of the flowers of both species at anthesis. To the extent that flavonoid biosynthesis may be crucial to the red stage of PACC, the coincident downregulation of carotenoid biosynthesis genes may be important in accentuating colour change. Alternatively, the downregulation of carotenoids may represent a senescence-related process separate from flavonoid production.

#### 4.7 Is *MAX1* a master regulator of PACC?

PACC in *Lotus filicaulis* and *Lotus sessilifolius* involves a wholesale upregulation of the cyanidin branch of the anthocyanin biosynthetic pathway (ABP). Given the number of genes

involved it is unlikely that each gene is regulated entirely independently. Instead it is possible that triggering of a top-level regulator then initiates a regulatory cascade activating the entire pathway in concert. An obvious such potential regulator would be *PRODUCTION OF ANTHOCYANIN PIGMENT 1* (*PAP1*), a gene known to be a powerful regulator of anthocyanin production in *Arabidopsis* (Borevitz et al., 2000). Perhaps strangely, the *Lotus* homologue of *PAP1* is actually downregulated during PACC in *L. filicaulis* (Table 5) and therefore does not seem to be involved. However, of the other known regulators that are differentially expressed during PACC in our study, one, *MORE AXILLARY BRANCHES* (*MAX1*), is a potential candidate as a top level regulator. *MAX1* is strongly upregulated at the red stage of PACC in *L. filicaulis* (Table 5). The sequence recovered for *MAX1* in *L. filicaulis* lacks a DNA-binding domain and is therefore likely to encode a Cytochrome P450 rather than a transcription factor (Figure S27). Indeed, in *Arabidopsis*, *MAX1* encodes CYP711A1, and is known to be a positive regulator of the flavonoid pathway (Lazar & Goodman, 2006). *Arabidopsis* mutants *max1*, have been demonstrated to have 11 down-regulated flavonoid biosynthesis genes including F3H, F3'H, DFR, ANS, and UFGT (Lazar & Goodman, 2006). However, beyond what is known of Cytochrome P450s in flavonoid biosynthesis in general (Ayabe & Akashi, 2006), the exact biochemical mechanism in ABP of CYP711A1 remains unstudied. Interestingly *MAX1* was first characterized for its effect on axillary bud behaviour. However the link between ABP and axillary bud formation (if any) remains obscure, although regulation of auxin transport through inhibition of PIN-function by flavonoids in the bud is one possibility that has been suggested (Lazar & Goodman, 2006). It is known that fertilized ovules are a source of auxin (Sundberg & Østergaard, 2009), and that auxin can control anthocyanin biosynthesis (Liu et al., 2014). Counter to that, it has been shown that the MAX pathway acts as an entirely independent regulator of auxin transport (Bennett et al. 2006). The versatility of *MAX1* is further confirmed by the involvement of *MAX1* in the strigolactone signalling pathway (Challis et al., 2013).

#### 4.8 The role of *FLS* in PACC in *Lotus sessilifolius*

*FLS* forms flavonols (quercetin, kaempferol, and isorhamnetin) from dihydroflavonols, the same substrate used by *DFR* to produce cyanidin. *FLS* has been shown to be antagonistic to anthocyanin accumulation, which increases in *FLS* knockouts (Lee et al., 2016). Ojeda et al. (2013) found that pre-colour change (yellow flowered) *Lotus* species from the Canary Islands

contain comparatively more flavonols (mostly isorhamnetin) than anthocyanins. Flavonols are generally colourless to the human eye but absorb strongly in the UV. Despite this Ojeda et al. (2013) demonstrated a reduction in UV reflectance of the petals following PACC (Ojeda et al. 2013). Whatever the mechanism, a shift from flavonol to cyanidin production may affect the detectability of the flowers by pollinators. It is of interest that *L. sessilifolius* and *L. filicaulis* differ markedly in the behaviour of *FLS*. The reduction of flavonol production with floral age in *L. sessilifolius* (a Canary Island species) is the basis of the suggestion made by Ojeda et al. (2013) that an *FLS* mediated switch to anthocyanins from flavonols may be occurring. However, the absence of *FLS* expression in *L. filicaulis* indicates that a different mechanism prevails in that species, although I cannot rule out the possibility that RNA levels were too low to be detected. The differential expression of *FLS* at the yellow stage of *L. sessilifolius* supports the notion that there is an *FLS*-mediated switch from flavonols to anthocyanins (which may therefore be the basis of an ecological function of PACC to signal floral viability to insect pollinators). It also implies that, in this species at least, anthocyanin biosynthesis is antagonistic to the production of flavonols. Furthermore, Ojeda et al. (2013) demonstrated that the transition from bee- to birdpollinated *Lotus* spp. involves the increased biosynthesis of anthocyanins at the expense of flavonol production.

#### 4.9 The role of red in PACC

Red anthocyanin pigmentation of flowers has long been associated with bird pollination. For instance, the scarlet colouration of bird pollinated Lamiaceae species has been attributed to both cyanidin and pelargonidin (Saito & Harborne, 1992). It is thought that in bird pollination syndromes red serves not only as a bird-attractant but also a bee deterrent (Rodríguez-Gironés & Santamaría, 2004), though red-coloured objects should not be considered as invisible to bees (Chittka & Waser, 1997; Martínez-Harms et al., 2010). It is possible that in plants exhibiting ornithophily, red acts as a common signal of low nutritional status to bees. In bird pollinated plants highly dilute nectar is also thought to be an adaptation to deter bees (Bolten & Feinsinger, 1978) as this is difficult for bees to process, and energetically expensive to convert to concentrated storage forms (i.e. honey). There is an intriguing parallel here between PACC and bird pollination; in both cases red pigmentation is potentially signalling aspects of nutritional

status to bees (Chittka & Waser, 1997; Martínez-Harms et al., 2010). Red and related colours that are common in PACC may therefore constitute a preferred signal of foraging unsuitability, so incurring an adaptive advantage to the plant by optimizing pollinator foraging on young, newly opened, yellow flowers.

## 5 Conclusion

In this thesis I 1) present an analysis of the transcriptomics of post-anthesis colour change in *Lotus filicaulis* and *Lotus sessilifolius* and 2) demonstrate that PACC is mediated by pollination in *L. filicaulis*.

I present the first evidence that PACC is mediated by pollination in *L. filicaulis*. This is the foundation for understanding the ecological function of PACC in *Lotus*. Further work will need to address the physiological basis of the trigger(s) of PACC, e.g. is PACC triggered by the deposition of pollen on the stigma, or some other physical cue?

This is the first transcriptome-wide view of how gene expression is modulated through PACC for any species; the contigs listed in Appendices 1 and 2 are the first coding sequences available for these *Lotus* species. These sequences are hypotheses, which allows for both verification of this thesis and further work – especially if gene expression in other *Lotus* species is to be studied. Thus, this is an important contribution to better understand the evolution of pollination in *Lotus*.

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## Appendix 1

### Sequences for Trinity contigs listed in Table 5

```
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>TR52191|c0\_g1 [organism=Lotus filicaulis] F3'H  
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>TR38960|c0\_g1 [organism=Lotus filicaulis] F3H  
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>TR29586|c0\_g1\_i1 [organism=Lotus filicaulis] DFR  
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>TR23981|c0\_g1 [organism=Lotus filicaulis] MAX1

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## Appendix 2

### Sequences for Trinity contigs listed in Table 6

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## Appendix 3

### Supplementary Tables S1-S54

**Table S1.** Tophat Results: Significantly enriched GO terms corresponding to the yellow stage of a *L. filicaulis* flower.

| Enriched GO Term: Description                                  | Number of associated genes | FDR      |
|--|----------------------------|----------|
| GO:0015979photosynthesis                                       | 42                         | 1.70E-05 |
| GO:0009628response to abiotic stimulus                         | 179                        | 1.70E-05 |
| GO:0042221response to chemical stimulus                        | 243                        | 2.50E-05 |
| GO:0050896response to stimulus                                 | 376                        | 0.00014  |
| GO:0010033response to organic substance                        | 159                        | 0.00014  |
| GO:0009725response to hormone stimulus                         | 124                        | 0.00014  |
| GO:0044281small molecule metabolic process                     | 207                        | 0.00018  |
| GO:0044283small molecule biosynthetic process                  | 107                        | 0.00024  |
| GO:0009719response to endogenous stimulus                      | 131                        | 0.00024  |
| GO:0019684photosynthesis, light reaction                       | 26                         | 0.00024  |
| GO:0046394carboxylic acid biosynthetic process                 | 69                         | 0.0024   |
| GO:0016053organic acid biosynthetic process                    | 69                         | 0.0024   |
| GO:0006091generation of precursor metabolites and energy       | 44                         | 0.0037   |
| GO:0009416response to light stimulus                           | 81                         | 0.0041   |
| GO:0008152metabolic process                                    | 796                        | 0.0041   |
| GO:0005976polysaccharide metabolic process                     | 40                         | 0.0041   |
| GO:0009314response to radiation                                | 81                         | 0.0044   |
| GO:0010218response to far red light                            | 15                         | 0.0044   |
| GO:0043436oxoacid metabolic process                            | 106                        | 0.0044   |
| GO:0006950response to stress                                   | 233                        | 0.0044   |
| GO:0010608posttranscriptional regulation of gene expression    | 25                         | 0.0044   |
| GO:0006082organic acid metabolic process                       | 106                        | 0.0044   |
| GO:0019752carboxylic acid metabolic process                    | 106                        | 0.0044   |
| GO:0009409response to cold                                     | 52                         | 0.0047   |
| GO:0009607response to biotic stimulus                          | 102                        | 0.0056   |
| GO:0042180cellular ketone metabolic process                    | 108                        | 0.0056   |
| GO:0044264cellular polysaccharide metabolic process            | 35                         | 0.0064   |
| GO:0009637response to blue light                               | 16                         | 0.008    |
| GO:0006519cellular amino acid and derivative metabolic process | 87                         | 0.008    |

| Enriched GO Term: Description                              | Number of associated genes | FDR    |
|--|----------------------------|--------|
| GO:0032501multicellular organismal process                 | 207                        | 0.0086 |
| GO:0016072rRNA metabolic process                           | 16                         | 0.011  |
| GO:0009639response to red or far red light                 | 29                         | 0.011  |
| GO:0051707response to other organism                       | 96                         | 0.011  |
| GO:0008610lipid biosynthetic process                       | 58                         | 0.011  |
| GO:0006073cellular glucan metabolic process                | 29                         | 0.011  |
| GO:0019748secondary metabolic process                      | 58                         | 0.011  |
| GO:0016441posttranscriptional gene silencing               | 17                         | 0.012  |
| GO:0009733response to auxin stimulus                       | 44                         | 0.012  |
| GO:0033692cellular polysaccharide biosynthetic process     | 24                         | 0.012  |
| GO:0044042glucan metabolic process                         | 29                         | 0.012  |
| GO:0051704multi-organism process                           | 115                        | 0.012  |
| GO:0043648dicarboxylic acid metabolic process              | 22                         | 0.014  |
| GO:0007275multicellular organismal development             | 197                        | 0.014  |
| GO:0000271polysaccharide biosynthetic process              | 24                         | 0.014  |
| GO:0055114oxidation reduction                              | 133                        | 0.015  |
| GO:0005975carbohydrate metabolic process                   | 114                        | 0.016  |
| GO:0008652cellular amino acid biosynthetic process         | 33                         | 0.018  |
| GO:0009791post-embryonic development                       | 119                        | 0.02   |
| GO:0070887cellular response to chemical stimulus           | 80                         | 0.023  |
| GO:0006720isoprenoid metabolic process                     | 25                         | 0.024  |
| GO:0009617response to bacterium                            | 55                         | 0.024  |
| GO:0006520cellular amino acid metabolic process            | 55                         | 0.024  |
| GO:0009250glucan biosynthetic process                      | 18                         | 0.024  |
| GO:0006364rRNA processing                                  | 14                         | 0.024  |
| GO:0042214terpene metabolic process                        | 13                         | 0.026  |
| GO:0009415response to water                                | 41                         | 0.028  |
| GO:0016246RNA interference                                 | 12                         | 0.028  |
| GO:0009765photosynthesis, light harvesting                 | 10                         | 0.029  |
| GO:0009414response to water deprivation                    | 40                         | 0.031  |
| GO:0009308amine metabolic process                          | 64                         | 0.037  |
| GO:0032502developmental process                            | 204                        | 0.038  |
| GO:0006629lipid metabolic process                          | 91                         | 0.044  |
| GO:0006721terpenoid metabolic process                      | 20                         | 0.044  |
| GO:0030422production of siRNA involved in RNA interference | 11                         | 0.046  |
| GO:0009309amine biosynthetic process                       | 34                         | 0.048  |

| Enriched GO Term: Description                      | Number of associated genes | FDR      |
|--|----------------------------|----------|
| GO:0044106cellular amine metabolic process         | 57                         | 0.048    |
| GO:0003824catalytic activity                       | 688                        | 0.002    |
| GO:0009507chloroplast                              | 311                        | 3.60E-35 |
| GO:0044435plastid part                             | 232                        | 3.60E-35 |
| GO:0044434chloroplast part                         | 228                        | 1.00E-34 |
| GO:0009536plastid                                  | 319                        | 1.90E-33 |
| GO:0009532plastid stroma                           | 145                        | 7.50E-26 |
| GO:0009570chloroplast stroma                       | 139                        | 2.80E-24 |
| GO:0044464cell part                                | 910                        | 2.10E-19 |
| GO:0043227membrane-bounded organelle               | 630                        | 2.10E-19 |
| GO:0044444cytoplasmic part                         | 570                        | 2.10E-19 |
| GO:0005623cell                                     | 910                        | 2.10E-19 |
| GO:0043231intracellular membrane-bounded organelle | 628                        | 2.10E-19 |
| GO:0009526plastid envelope                         | 123                        | 6.00E-19 |
| GO:0043226organelle                                | 656                        | 1.00E-18 |
| GO:0043229intracellular organelle                  | 655                        | 1.20E-18 |
| GO:0005737cytoplasm                                | 598                        | 1.80E-18 |
| GO:0044422organelle part                           | 370                        | 4.00E-18 |
| GO:0044446intracellular organelle part             | 368                        | 4.50E-18 |
| GO:0009941chloroplast envelope                     | 118                        | 5.70E-18 |
| GO:0009579thylakoid                                | 106                        | 6.70E-17 |
| GO:0031976plastid thylakoid                        | 92                         | 1.30E-16 |
| GO:0009534chloroplast thylakoid                    | 92                         | 1.30E-16 |
| GO:0031984organelle subcompartment                 | 92                         | 1.50E-16 |
| GO:0044436thylakoid part                           | 86                         | 2.70E-16 |
| GO:0044424intracellular part                       | 720                        | 3.50E-16 |
| GO:0055035plastid thylakoid membrane               | 78                         | 2.10E-15 |
| GO:0042651thylakoid membrane                       | 80                         | 2.80E-15 |
| GO:0005622intracellular                            | 727                        | 3.50E-15 |
| GO:0009535chloroplast thylakoid membrane           | 77                         | 4.20E-15 |
| GO:0031975envelope                                 | 136                        | 5.50E-15 |
| GO:0031967organelle envelope                       | 134                        | 1.90E-14 |
| GO:0034357photosynthetic membrane                  | 80                         | 3.50E-14 |
| GO:0048046apoplast                                 | 71                         | 3.00E-08 |
| GO:0005576extracellular region                     | 92                         | 1.30E-07 |
| GO:0010287plastoglobule                            | 20                         | 6.20E-06 |

| Enriched GO Term: Description                          | Number of associated genes | FDR      |
|--|----------------------------|----------|
| GO:0030312external encapsulating structure             | 87                         | 6.30E-06 |
| GO:0016020membrane                                     | 441                        | 8.20E-06 |
| GO:0005618cell wall                                    | 85                         | 1.40E-05 |
| GO:0031090organelle membrane                           | 148                        | 2.70E-05 |
| GO:0031977thylakoid lumen                              | 20                         | 4.60E-05 |
| GO:0005886plasma membrane                              | 234                        | 7.00E-05 |
| GO:0043232intracellular non-membrane-bounded organelle | 121                        | 7.10E-05 |
| GO:0043228non-membrane-bounded organelle               | 121                        | 7.10E-05 |
| GO:0010319stromule                                     | 15                         | 0.00015  |
| GO:0009505plant-type cell wall                         | 40                         | 0.00029  |
| GO:0031974membrane-enclosed lumen                      | 80                         | 0.00042  |
| GO:0070013intracellular organelle lumen                | 79                         | 0.00042  |
| GO:0043233organelle lumen                              | 79                         | 0.00044  |
| GO:0005730nucleolus                                    | 48                         | 0.0026   |
| GO:0009295nucleoid                                     | 10                         | 0.01     |
| GO:0044459plasma membrane part                         | 104                        | 0.012    |
| GO:0031978plastid thylakoid lumen                      | 11                         | 0.015    |
| GO:0009543chloroplast thylakoid lumen                  | 11                         | 0.015    |
| GO:0009521photosystem                                  | 13                         | 0.016    |
| GO:0042646plastid nucleoid                             | 8                          | 0.025    |
| GO:0009506plasmodesma                                  | 91                         | 0.036    |
| GO:0055044symplast                                     | 91                         | 0.036    |
| GO:0030054cell junction                                | 91                         | 0.038    |
| GO:0005911cell-cell junction                           | 91                         | 0.038    |
| GO:0000229cytoplasmic chromosome                       | 6                          | 0.043    |
| GO:0009508plastid chromosome                           | 6                          | 0.043    |
| GO:0046658anchored to plasma membrane                  | 12                         | 0.044    |

**Table S2.** Tophat Results: Significantly enriched GO terms corresponding to the red stage of a *L. filicaulis* flower.

| Enriched GO Term: Description                                 | Number of associated genes | FDR     |
|---|----------------------------|---------|
| GO:0050896response to stimulus                                | 266                        | 0.00056 |
| GO:0055085transmembrane transport                             | 70                         | 0.00056 |
| GO:0005975carbohydrate metabolic process                      | 92                         | 0.0012  |
| GO:0051179localization  | 152                        | 0.0014  |
| GO:0009605response to external stimulus                       | 56                         | 0.0015  |
| GO:0042221response to chemical stimulus                       | 164                        | 0.0015  |
| GO:0009991response to extracellular stimulus                  | 27                         | 0.0016  |
| GO:0051234establishment of localization                       | 145                        | 0.0016  |
| GO:0006810transport   | 144                        | 0.0016  |
| GO:0009932cell tip growth                                     | 23                         | 0.0017  |
| GO:0071704organic substance metabolic process                 | 139                        | 0.0017  |
| GO:0009813flavonoid biosynthetic process                      | 19                         | 0.0029  |
| GO:0035295tube development                                    | 24                         | 0.0029  |
| GO:0048868pollen tube development                             | 24                         | 0.0029  |
| GO:0031667response to nutrient levels                         | 23                         | 0.0029  |
| GO:0031668cellular response to extracellular stimulus         | 25                         | 0.0029  |
| GO:0071496cellular response to external stimulus              | 25                         | 0.0031  |
| GO:0006575cellular amino acid derivative metabolic process    | 41                         | 0.0048  |
| GO:0042398cellular amino acid derivative biosynthetic process | 32                         | 0.0049  |
| GO:0009725response to hormone stimulus                        | 82                         | 0.0051  |
| GO:0010033response to organic substance                       | 105                        | 0.0059  |
| GO:0009812flavonoid metabolic process                         | 20                         | 0.0074  |
| GO:0006811ion transport                                       | 53                         | 0.0074  |
| GO:0009719response to endogenous stimulus                     | 87                         | 0.0074  |
| GO:0031669cellular response to nutrient levels                | 21                         | 0.0077  |
| GO:0009699phenylpropanoid biosynthetic process                | 22                         | 0.01    |
| GO:0009860pollen tube growth                                  | 18                         | 0.011   |
| GO:0042594response to starvation                              | 19                         | 0.011   |
| GO:0009826unidimensional cell growth                          | 28                         | 0.011   |

| Enriched GO Term: Description                                  | Number of associated genes | FDR     |
|--|----------------------------|---------|
| GO:0060560developmental growth involved in morphogenesis       | 28                         | 0.011   |
| GO:0016036cellular response to phosphate starvation            | 14                         | 0.011   |
| GO:0009737response to abscisic acid stimulus                   | 43                         | 0.014   |
| GO:0032989cellular component morphogenesis                     | 36                         | 0.016   |
| GO:0048588developmental cell growth                            | 21                         | 0.016   |
| GO:0006725cellular aromatic compound metabolic process         | 93                         | 0.016   |
| GO:0015698inorganic anion transport                            | 14                         | 0.016   |
| GO:0044282small molecule catabolic process                     | 37                         | 0.016   |
| GO:0006576cellular biogenic amine metabolic process            | 13                         | 0.017   |
| GO:0044262cellular carbohydrate metabolic process              | 59                         | 0.018   |
| GO:0009267cellular response to starvation                      | 18                         | 0.019   |
| GO:0019438aromatic compound biosynthetic process               | 70                         | 0.019   |
| GO:0051704multi-organism process                               | 81                         | 0.022   |
| GO:0048610reproductive cellular process                        | 18                         | 0.026   |
| GO:0009856pollination  | 25                         | 0.03    |
| GO:0016137glycoside metabolic process                          | 16                         | 0.033   |
| GO:0009718anthocyanin biosynthetic process                     | 11                         | 0.034   |
| GO:0008361regulation of cell size                              | 34                         | 0.037   |
| GO:0010200response to chitin                                   | 17                         | 0.037   |
| GO:0032502developmental process                                | 144                        | 0.038   |
| GO:0006519cellular amino acid and derivative metabolic process | 59                         | 0.038   |
| GO:0019748secondary metabolic process                          | 40                         | 0.038   |
| GO:0000902cell morphogenesis                                   | 31                         | 0.043   |
| GO:0032501multicellular organismal process                     | 140                        | 0.044   |
| GO:0006595polyamine metabolic process                          | 7                          | 0.046   |
| GO:0044248cellular catabolic process                           | 61                         | 0.046   |
| GO:0022892substrate-specific transporter activity              | 72                         | 0.00021 |
| GO:0005215transporter activity                                 | 90                         | 0.00021 |
| GO:0022857transmembrane transporter activity                   | 77                         | 0.00021 |
| GO:0015291secondary active transmembrane transporter activity  | 30                         | 0.0005  |

| Enriched GO Term: Description                                   | Number of associated genes | FDR     |
|---|----------------------------|---------|
| GO:0022891substrate-specific transmembrane transporter activity | 65                         | 0.00072 |
| GO:0022804active transmembrane transporter activity             | 45                         | 0.00072 |
| GO:0016791phosphatase activity                                  | 27                         | 0.0017  |
| GO:0016798hydrolase activity, acting on glycosyl bonds          | 44                         | 0.0017  |
| GO:0004553hydrolase activity, hydrolyzing O-glycosyl compounds  | 42                         | 0.0017  |
| GO:0015075ion transmembrane transporter activity                | 50                         | 0.0017  |
| GO:0003779actin binding   | 13                         | 0.0047  |
| GO:0015103inorganic anion transmembrane transporter activity    | 15                         | 0.0047  |
| GO:0015297antiporter activity                                   | 19                         | 0.0053  |
| GO:0042578phosphoric ester hydrolase activity                   | 29                         | 0.0058  |
| GO:0008509anion transmembrane transporter activity              | 18                         | 0.024   |
| GO:0016020membrane  | 305                        | 0.0014  |
| GO:0005886plasma membrane                                       | 164                        | 0.0033  |

**Table S3.** Yellow Stage TopHat Results: Genes for enzymes annotated to GO:0009813 (Flavonoid biosynthetic process, FDR=1). The first column lists differentially expressed (FDR<0.005) assembled RNA-seq reads that have mapped to the *Lotus japonicus* genome. The second column is their soybean (*Glycine max*) BLAST homolog, followed by expression at two stages of flower development.

| <i>L. japonicus</i> ID | <i>G. max</i> BLAST ID | <i>G. max</i> description  | Counts per gene<br>(Yellow) | Counts per gene<br>(Red) | Log <sub>2</sub> FC |
|------------------------|------------------------|--|-----------------------------|--------------------------|---------------------|
| chr2.CM0018.1300.r2.m  | Glyma.01G228700.1      | Naringenin-chalcone synthase                                     | 143                         | 11                       | 2.87090             |
| chr5.CM1077.590.r2.m   | Glyma.03G187000.1      | UDP-glucosyl transferase 73C                                     | 44                          | 1                        | 4.52042             |
| chr1.CM0122.1600.r2.m  | Glyma.03G222100.1      | Nitrogen regulatory protein P-II                                 | 134                         | 18                       | 2.07132             |
| chr1.CM0012.990.r2.m   | Glyma.04G254200.1      | B3 DNA binding domain // Auxin response factor                   | 119                         | 12                       | 2.48162             |
| chr1.CM0064.910.r2.m   | Glyma.05G076300.1      | NA   | 1067                        | 169                      | 1.83858             |
| chr3.LjT40P18.80.r2.m  | Glyma.07G021600.1      | Shikimate O-hydroxycinnamoyltransferase                          | 350                         | 25                       | 2.98347             |
| chr3.CM0452.240.r2.d   | Glyma.07G021600.1      | Shikimate O-hydroxycinnamoyltransferase                          | 8669                        | 1189                     | 2.04670             |
| chr3.LjT10E18.60.r2.m  | Glyma.10G210600.1      | B3 DNA binding domain // Auxin response factor                   | 230                         | 26                       | 2.32171             |
| chr3.CM0136.10.r2.m    | Glyma.11G145500.1      | AUX/IAA family // B3 DNA binding domain // Auxin response factor | 28                          | 2                        | 2.93242             |
| chr4.CM0432.2880.r2.m  | Glyma.13G173300.1      | O-METHYLTRANSFERASE  | 2443                        | 223                      | 2.63371             |
| chr3.CM0106.330.r2.m   | Glyma.13G344700.1      | B-box zinc finger  | 712                         | 37                       | 3.44375             |
| chr1.LjT35H06.80.r2.m  | NA                     | NA   | 51                          | 0                        | 8.32466             |

**Table S4.** Red Stage TopHat Results: Genes for enzymes annotated to GO:0009813 (Flavonoid biosynthetic process, FDR = 0.0029) . The first column lists differentially expressed (FDR<0.005) assembled RNA-seq reads that have mapped to the *Lotus japonicus* genome. The second column is their soybean (*Glycine max*) BLAST homolog, followed by expression at two stages of flower development.

| <i>L. japonicus</i> ID | <i>G. max</i> BLAST ID | <i>G. max</i> description                                      | Counts per gene (Yellow) | Counts per gene (Red) | Log <sub>2</sub> FC |
|------------------------|------------------------|--|--------------------------|-----------------------|---------------------|
| chr2.CM0608.560.r2.m   | Glyma.01G048200<br>.1  | RIBONUCLEASE T2  | 872                      | 8349                  | -4.07828            |
| chr2.CM0124.30.r2.m    | Glyma.01G088300<br>.1  | STEROL REGULATORY ELEMENT-BINDING PROTEIN                      | 8                        | 26                    | -2.49628            |
| chr3.LjB14006.120.r2.a | Glyma.01G166200<br>.1  | naringenin 3-dioxygenase                                       | 228                      | 542                   | -2.06782            |
| chr4.CM0119.240.r2.m   | Glyma.01G166200<br>.1  | naringenin 3-dioxygenase                                       | 1043                     | 3498                  | -2.56494            |
| chr1.CM0284.250.r2.m   | Glyma.01G228700<br>.1  | Naringenin-chalcone synthase                                   | 7                        | 144                   | -5.15029            |
| chr2.CM0018.760.r2.m   | Glyma.01G228700<br>.1  | Naringenin-chalcone synthase                                   | 115                      | 905                   | -3.79376            |
| chr2.CM0018.730.r2.m   | Glyma.01G228700<br>.1  | Naringenin-chalcone synthase                                   | 48                       | 170                   | -2.63974            |
| chr2.CM0018.1200.r2.m  | Glyma.01G228700<br>.1  | Naringenin-chalcone synthase                                   | 3                        | 61                    | -5.09247            |
| chr5.CM0077.110.r2.m   | Glyma.02G158700<br>.1  | bifunctional dihydroflavonol 4-reductase/flavanone 4-reductase | 32                       | 929                   | -5.67183            |
| chr5.CM0077.210.r2.m   | Glyma.02G158700<br>.1  | bifunctional dihydroflavonol 4-reductase/flavanone 4-reductase | 150                      | 10354                 | -6.92689            |
| chr1.CM0105.1870.r2.m  | Glyma.03G113400<br>.1  | Proton-exporting ATPase  | 304                      | 1049                  | -2.60557            |
| chr1.CM0133.560.r2.m   | Glyma.04G052100<br>.1  | Cytochrome P450 CYP3/CYP5/CYP6/CYP9 subfamilies                | 40                       | 2793                  | -6.9393             |
| chr1.LjT39K18.30.r2.m  | Glyma.04G147700<br>.1  | HEME OXYGENASE   | 636                      | 1935                  | -2.42427            |
| chr2.CM0191.680.r2.m   | Glyma.04G227700<br>.1  | Quercetin 3-O-methyltransferase                                | 4                        | 21                    | -3.16141            |
| chr1.CM0410.430.r2.m   | Glyma.08G247100<br>.1  | Transferase family   | 2403                     | 7930                  | -2.54173            |
| chr6.CM0013.1540.r2.a  | Glyma.09G038900<br>.1  | MYB-LIKE DNA-BINDING PROTEIN MYB                               | 7                        | 125                   | -4.94628            |

| <i>L. japonicus</i> ID | <i>G. max</i> BLAST ID | <i>G. max</i> description                                      | Counts per gene<br>(Yellow) | Counts per gene<br>(Red) | Log <sub>2</sub> FC |
|------------------------|------------------------|--|-----------------------------|--------------------------|---------------------|
| chr2.CM0021.2820.r2.m  | Glyma.11G027700        | Leucocyanidin oxygenase  | 1001                        | 15407                    | -4.76318            |
|                        | .1                     |  |                             |                          |                     |
| chr5.CM0077.120.r2.m   | Glyma.14G072700        | bifunctional dihydroflavonol 4-reductase/flavanone 4-reductase | 11                          | 61                       | -3.27199            |
|                        | .1                     |  |                             |                          |                     |
| chr5.CM0180.690.r2.m   | Glyma.20G241700        | chalcone isomerase   | 529                         | 1254                     | -2.06419            |
|                        | .1                     |  |                             |                          |                     |

**Table S5.** Yellow Stage TopHat Results: Genes for enzymes annotated to GO:0009809 (Lignin biosynthetic process, FDR=0.24). The first column lists differentially expressed (FDR<0.005) assembled RNA-seq reads that have mapped to the *Lotus japonicus* genome. The second column is their soybean (*Glycine max*) BLAST homolog, followed by expression at two stages of flower development.

| <i>L. japonicus</i> ID | <i>G. max</i> BLAST ID | <i>G. max</i> description               | Counts per gene<br>(Yellow) | Counts per gene<br>(Red) | Log <sub>2</sub> FC |
|------------------------|------------------------|---|-----------------------------|--------------------------|---------------------|
| chr6.LjT35D18.30.r2.m  | Glyma.03G223000.1      | Methionine adenosyltransferase          | 17391                       | 2734                     | 1.849893            |
|                        |                        |   |                             |                          | 404                 |
| chr3.LjT40P18.80.r2.m  | Glyma.07G021600.1      | Shikimate O-hydroxycinnamoyltransferase | 350                         | 25                       | 2.983475            |
|                        |                        |   |                             |                          | 586                 |
| chr3.CM0452.240.r2.d   | Glyma.07G021600.1      | Shikimate O-hydroxycinnamoyltransferase | 8669                        | 1189                     | 2.046705            |
|                        |                        |   |                             |                          | 844                 |
| chr3.LjT45M09.50.r2.d  | Glyma.07G023700.1      | cinnamoyl-CoA reductase                 | 1692                        | 266                      | 1.849549            |
|                        |                        |   |                             |                          | 234                 |
| chr4.CM0387.960.r2.m   | Glyma.07G258700.1      | beta-glucosidase                        | 131                         | 1                        | 6.090954            |
|                        |                        |   |                             |                          | 877                 |
| chr6.CM0385.240.r2.d   | Glyma.09G038500.1      | CHITINASE                               | 1204                        | 8                        | 6.398266            |
|                        |                        |   |                             |                          | 711                 |
| chr5.CM0200.3250.r2.m  | Glyma.10G262400.1      | cinnamyl-alcohol dehydrogenase          | 4676                        | 705                      | 1.910116            |
|                        |                        |   |                             |                          | 099                 |

\*Lignin biosynthetic process was not found to be enriched at the red stage of floral colour change in *L. filicaulis*, given the TopHat results.

**Table S6.** STAR Results: Significantly enriched GO terms corresponding to the yellow stage of a *L. filicaulis* flower.

| Enriched GO Term: Description                               | Number of associated genes | FDR      |
|---|----------------------------|----------|
| GO:0009628response to abiotic stimulus                      | 184                        | 1.00E-06 |
| GO:0050896response to stimulus                              | 379                        | 1.80E-05 |
| GO:0042221response to chemical stimulus                     | 241                        | 1.80E-05 |
| GO:0044281small molecule metabolic process                  | 206                        | 0.00017  |
| GO:0009416response to light stimulus                        | 87                         | 0.00024  |
| GO:0015979photosynthesis                                    | 37                         | 0.00024  |
| GO:0009314response to radiation                             | 87                         | 0.00032  |
| GO:0010033response to organic substance                     | 153                        | 0.00059  |
| GO:0044283small molecule biosynthetic process               | 103                        | 0.00086  |
| GO:0006950response to stress                                | 237                        | 0.00086  |
| GO:0005976polysaccharide metabolic process                  | 42                         | 0.00089  |
| GO:0008610lipid biosynthetic process                        | 63                         | 0.00097  |
| GO:0009725response to hormone stimulus                      | 117                        | 0.00097  |
| GO:0008152metabolic process                                 | 789                        | 0.0018   |
| GO:0044264cellular polysaccharide metabolic process         | 37                         | 0.0018   |
| GO:0009607response to biotic stimulus                       | 104                        | 0.0018   |
| GO:0046394carboxylic acid biosynthetic process              | 68                         | 0.0018   |
| GO:0016053organic acid biosynthetic process                 | 68                         | 0.0018   |
| GO:0009719response to endogenous stimulus                   | 123                        | 0.0021   |
| GO:0019684photosynthesis, light reaction                    | 23                         | 0.0023   |
| GO:0009637response to blue light                            | 17                         | 0.0027   |
| GO:0033692cellular polysaccharide biosynthetic process      | 26                         | 0.0027   |
| GO:0000271polysaccharide biosynthetic process               | 26                         | 0.0028   |
| GO:0051707response to other organism                        | 98                         | 0.0028   |
| GO:0010218response to far red light                         | 15                         | 0.0028   |
| GO:0009409response to cold                                  | 52                         | 0.0028   |
| GO:0043436oxoacid metabolic process                         | 105                        | 0.0028   |
| GO:0032501multicellular organismal process                  | 208                        | 0.0028   |
| GO:0010608posttranscriptional regulation of gene expression | 25                         | 0.0028   |
| GO:0006082organic acid metabolic process                    | 105                        | 0.0028   |
| GO:0019752carboxylic acid metabolic process                 | 105                        | 0.0028   |
| GO:0007275multicellular organismal development              | 200                        | 0.0033   |
| GO:0042214terpene metabolic process                         | 15                         | 0.0035   |
| GO:0005975carbohydrate metabolic process                    | 117                        | 0.0037   |
| GO:0019748secondary metabolic process                       | 59                         | 0.0045   |

| Enriched GO Term: Description                                  | Number of associated genes | FDR    |
|--|----------------------------|--------|
| GO:0009791post-embryonic development                           | 122                        | 0.0047 |
| GO:0006720isoprenoid metabolic process                         | 27                         | 0.0051 |
| GO:0042180cellular ketone metabolic process                    | 106                        | 0.0051 |
| GO:0055114oxidation reduction                                  | 134                        | 0.0067 |
| GO:0006073cellular glucan metabolic process                    | 29                         | 0.0075 |
| GO:0032502developmental process                                | 208                        | 0.0085 |
| GO:0044042glucan metabolic process                             | 29                         | 0.0095 |
| GO:0071554cell wall organization or biogenesis                 | 45                         | 0.011  |
| GO:0006091generation of precursor metabolites and energy       | 40                         | 0.012  |
| GO:0051704multi-organism process                               | 113                        | 0.014  |
| GO:0009639response to red or far red light                     | 28                         | 0.014  |
| GO:0044255cellular lipid metabolic process                     | 70                         | 0.015  |
| GO:0006629lipid metabolic process                              | 93                         | 0.016  |
| GO:0071555cell wall organization                               | 32                         | 0.017  |
| GO:0006519cellular amino acid and derivative metabolic process | 83                         | 0.017  |
| GO:0048856anatomical structure development                     | 168                        | 0.018  |
| GO:0044262cellular carbohydrate metabolic process              | 80                         | 0.018  |
| GO:0006721terpenoid metabolic process                          | 21                         | 0.018  |
| GO:0009266response to temperature stimulus                     | 62                         | 0.019  |
| GO:0009250glucan biosynthetic process                          | 18                         | 0.019  |
| GO:0016441posttranscriptional gene silencing                   | 16                         | 0.019  |
| GO:0005982starch metabolic process                             | 14                         | 0.025  |
| GO:0008299isoprenoid biosynthetic process                      | 22                         | 0.025  |
| GO:0009617response to bacterium                                | 54                         | 0.025  |
| GO:0048731system development                                   | 105                        | 0.027  |
| GO:0048513organ development                                    | 105                        | 0.027  |
| GO:0034637cellular carbohydrate biosynthetic process           | 37                         | 0.03   |
| GO:0016051carbohydrate biosynthetic process                    | 38                         | 0.031  |
| GO:0071669plant-type cell wall organization or biogenesis      | 24                         | 0.031  |
| GO:0009414response to water deprivation                        | 39                         | 0.038  |
| GO:0010154fruit development                                    | 53                         | 0.038  |
| GO:0006633fatty acid biosynthetic process                      | 31                         | 0.039  |
| GO:0009658chloroplast organization                             | 20                         | 0.04   |
| GO:0016072rRNA metabolic process                               | 14                         | 0.041  |
| GO:0010035response to inorganic substance                      | 75                         | 0.044  |
| GO:0032787monocarboxylic acid metabolic process                | 56                         | 0.048  |

| Enriched GO Term: Description                      | Number of associated genes | FDR      |
|--|----------------------------|----------|
| GO:0003824catalytic activity                       | 689                        | 0.00013  |
| GO:0009507chloroplast                              | 312                        | 1.80E-36 |
| GO:0044435plastid part                             | 232                        | 2.90E-36 |
| GO:0044434chloroplast part                         | 228                        | 1.10E-35 |
| GO:0009536plastid                                  | 321                        | 2.20E-35 |
| GO:0009532plastid stroma                           | 148                        | 7.90E-28 |
| GO:0009570chloroplast stroma                       | 142                        | 3.30E-26 |
| GO:0044464cell part                                | 920                        | 2.20E-24 |
| GO:0005623cell                                     | 920                        | 2.20E-24 |
| GO:0043227membrane-bounded organelle               | 639                        | 3.00E-23 |
| GO:0044444cytoplasmic part                         | 579                        | 3.00E-23 |
| GO:0043231intracellular membrane-bounded organelle | 637                        | 3.50E-23 |
| GO:0005737cytoplasm                                | 608                        | 3.10E-22 |
| GO:0043226organelle                                | 662                        | 7.90E-22 |
| GO:0043229intracellular organelle                  | 661                        | 9.00E-22 |
| GO:0044424intracellular part                       | 732                        | 2.60E-20 |
| GO:0044422organelle part                           | 371                        | 1.90E-19 |
| GO:0009579thylakoid                                | 111                        | 2.10E-19 |
| GO:0044446intracellular organelle part             | 369                        | 2.10E-19 |
| GO:0009526plastid envelope                         | 122                        | 3.10E-19 |
| GO:0005622intracellular                            | 738                        | 4.60E-19 |
| GO:0031976plastid thylakoid                        | 96                         | 8.40E-19 |
| GO:0009534chloroplast thylakoid                    | 96                         | 8.40E-19 |
| GO:0031984organelle subcompartment                 | 96                         | 1.00E-18 |
| GO:0009941chloroplast envelope                     | 117                        | 3.60E-18 |
| GO:0044436thylakoid part                           | 89                         | 4.60E-18 |
| GO:0055035plastid thylakoid membrane               | 82                         | 1.30E-17 |
| GO:0042651thylakoid membrane                       | 84                         | 1.80E-17 |
| GO:0009535chloroplast thylakoid membrane           | 81                         | 2.80E-17 |
| GO:0034357photosynthetic membrane                  | 84                         | 3.10E-16 |
| GO:0031975envelope                                 | 136                        | 1.70E-15 |
| GO:0031967organelle envelope                       | 134                        | 6.10E-15 |
| GO:0048046apoplast                                 | 75                         | 6.60E-10 |
| GO:0005576extracellular region                     | 97                         | 2.20E-09 |
| GO:0016020membrane                                 | 443                        | 8.30E-07 |
| GO:0030312external encapsulating structure         | 88                         | 1.90E-06 |

| Enriched GO Term: Description                          | Number of associated genes | FDR      |
|--|----------------------------|----------|
| GO:0031090organelle membrane                           | 152                        | 2.10E-06 |
| GO:0031977thylakoid lumen                              | 22                         | 3.50E-06 |
| GO:0005618cell wall                                    | 86                         | 4.40E-06 |
| GO:0010287plastoglobule                                | 19                         | 1.60E-05 |
| GO:0010319stromule                                     | 16                         | 3.60E-05 |
| GO:0005886plasma membrane                              | 232                        | 4.80E-05 |
| GO:0009505plant-type cell wall                         | 41                         | 0.0001   |
| GO:0043232intracellular non-membrane-bounded organelle | 118                        | 0.00013  |
| GO:0043228non-membrane-bounded organelle               | 118                        | 0.00013  |
| GO:0031974membrane-enclosed lumen                      | 81                         | 0.00016  |
| GO:0070013intracellular organelle lumen                | 80                         | 0.00016  |
| GO:0043233organelle lumen                              | 80                         | 0.00016  |
| GO:0031978plastid thylakoid lumen                      | 13                         | 0.0014   |
| GO:0009543chloroplast thylakoid lumen                  | 13                         | 0.0014   |
| GO:0005730nucleolus                                    | 46                         | 0.0056   |
| GO:0009295nucleoid                                     | 10                         | 0.0089   |
| GO:0044459plasma membrane part                         | 103                        | 0.01     |
| GO:0042646plastid nucleoid                             | 8                          | 0.023    |
| GO:0009506plasmodesma                                  | 90                         | 0.035    |
| GO:0055044symplast                                     | 90                         | 0.035    |
| GO:0030054cell junction                                | 90                         | 0.037    |
| GO:0005911cell-cell junction                           | 90                         | 0.037    |
| GO:0000229cytoplasmic chromosome                       | 6                          | 0.041    |
| GO:0009508plastid chromosome                           | 6                          | 0.041    |

**Table S7.** STAR Results: Significantly enriched GO terms corresponding to the red stage of a *L. filicaulis* flower.

| Enriched GO Term: Description                                 | Number of associated genes | FDR      |
|---|----------------------------|----------|
| GO:0050896response to stimulus                                | 278                        | 3.30E-06 |
| GO:0042221response to chemical stimulus                       | 175                        | 2.40E-05 |
| GO:0009605response to external stimulus                       | 60                         | 0.00014  |
| GO:0005975carbohydrate metabolic process                      | 95                         | 0.00014  |
| GO:0009991response to extracellular stimulus                  | 29                         | 0.00025  |
| GO:0009725response to hormone stimulus                        | 89                         | 0.00033  |
| GO:0009813flavonoid biosynthetic process                      | 21                         | 0.00042  |
| GO:0010033response to organic substance                       | 111                        | 0.00054  |
| GO:0031668cellular response to extracellular stimulus         | 27                         | 0.00054  |
| GO:0051179localization  | 151                        | 0.00054  |
| GO:0009719response to endogenous stimulus                     | 93                         | 0.00054  |
| GO:0071496cellular response to external stimulus              | 27                         | 0.00054  |
| GO:0071704organic substance metabolic process                 | 141                        | 0.00054  |
| GO:0031667response to nutrient levels                         | 24                         | 0.0009   |
| GO:0055085transmembrane transport                             | 66                         | 0.0009   |
| GO:0051234establishment of localization                       | 144                        | 0.00093  |
| GO:0042398cellular amino acid derivative biosynthetic process | 34                         | 0.00093  |
| GO:0006575cellular amino acid derivative metabolic process    | 43                         | 0.00094  |
| GO:0006810transport   | 143                        | 0.00094  |
| GO:0009812flavonoid metabolic process                         | 22                         | 0.00099  |
| GO:0009699phenylpropanoid biosynthetic process                | 24                         | 0.0017   |
| GO:0009932cell tip growth                                     | 22                         | 0.0023   |
| GO:0031669cellular response to nutrient levels                | 22                         | 0.0029   |
| GO:0006725cellular aromatic compound metabolic process        | 97                         | 0.0035   |
| GO:0042594response to starvation                              | 20                         | 0.0041   |
| GO:0035295tube development                                    | 23                         | 0.0041   |
| GO:0048868pollen tube development                             | 23                         | 0.0041   |
| GO:0009718anthocyanin biosynthetic process                    | 13                         | 0.0041   |
| GO:0044282small molecule catabolic process                    | 39                         | 0.0048   |

| Enriched GO Term: Description                                  | Number of associated genes | FDR    |
|--|----------------------------|--------|
| GO:0019438aromatic compound biosynthetic process               | 73                         | 0.0051 |
| GO:0009737response to abscisic acid stimulus                   | 44                         | 0.0067 |
| GO:0044262cellular carbohydrate metabolic process              | 61                         | 0.0067 |
| GO:0006519cellular amino acid and derivative metabolic process | 63                         | 0.0067 |
| GO:0032989cellular component morphogenesis                     | 37                         | 0.0071 |
| GO:0009267cellular response to starvation                      | 19                         | 0.0077 |
| GO:0019748secondary metabolic process                          | 43                         | 0.0077 |
| GO:0006950response to stress                                   | 161                        | 0.0079 |
| GO:0016036cellular response to phosphate starvation            | 14                         | 0.0086 |
| GO:0046283anthocyanin metabolic process                        | 14                         | 0.012  |
| GO:0016137glycoside metabolic process                          | 17                         | 0.013  |
| GO:0006811ion transport  | 51                         | 0.013  |
| GO:0015698inorganic anion transport                            | 14                         | 0.014  |
| GO:0009698phenylpropanoid metabolic process                    | 27                         | 0.014  |
| GO:0006576cellular biogenic amine metabolic process            | 13                         | 0.014  |
| GO:0009826unidimensional cell growth                           | 27                         | 0.015  |
| GO:0060560developmental growth involved in morphogenesis       | 27                         | 0.015  |
| GO:0009628response to abiotic stimulus                         | 109                        | 0.015  |
| GO:0009415response to water                                    | 31                         | 0.017  |
| GO:0009860pollen tube growth                                   | 17                         | 0.018  |
| GO:0000902cell morphogenesis                                   | 32                         | 0.022  |
| GO:0048588developmental cell growth                            | 20                         | 0.024  |
| GO:0009714chalcone metabolic process                           | 7                          | 0.024  |
| GO:0042181ketone biosynthetic process                          | 7                          | 0.024  |
| GO:0009715chalcone biosynthetic process                        | 7                          | 0.024  |
| GO:0044281small molecule metabolic process                     | 131                        | 0.025  |
| GO:0044248cellular catabolic process                           | 62                         | 0.026  |
| GO:0006012galactose metabolic process                          | 6                          | 0.027  |
| GO:0006796phosphate metabolic process                          | 93                         | 0.029  |
| GO:0006793phosphorus metabolic process                         | 93                         | 0.032  |

| Enriched GO Term: Description   | Number of associated genes | FDR     |
|---|----------------------------|---------|
| GO:0006066alcohol metabolic process   | 38                         | 0.032   |
| GO:0034656nucleobase, nucleoside and nucleotide catabolic process               | 15                         | 0.032   |
| GO:0034655nucleobase, nucleoside, nucleotide and nucleic acid catabolic process | 15                         | 0.032   |
| GO:0006595polyamine metabolic process   | 7                          | 0.04    |
| GO:0044283small molecule biosynthetic process                                   | 65                         | 0.04    |
| GO:0006855multidrug transport   | 11                         | 0.042   |
| GO:0009856pollination   | 24                         | 0.042   |
| GO:0048610reproductive cellular process   | 17                         | 0.042   |
| GO:0051704multi-organism process  | 78                         | 0.045   |
| GO:0008361regulation of cell size   | 33                         | 0.046   |
| GO:0009611response to wounding  | 26                         | 0.046   |
| GO:0015893drug transport  | 11                         | 0.046   |
| GO:0046395carboxylic acid catabolic process                                     | 15                         | 0.047   |
| GO:0016054organic acid catabolic process  | 15                         | 0.047   |
| GO:0000904cell morphogenesis involved in differentiation                        | 21                         | 0.048   |
| GO:0044270cellular nitrogen compound catabolic process                          | 17                         | 0.048   |
| GO:0005215transporter activity  | 89                         | 0.00069 |
| GO:0022857transmembrane transporter activity                                    | 75                         | 0.00092 |
| GO:0022892substrate-specific transporter activity                               | 69                         | 0.0011  |
| GO:0015291secondary active transmembrane transporter activity                   | 29                         | 0.0012  |
| GO:0016798hydrolase activity, acting on glycosyl bonds                          | 44                         | 0.0026  |
| GO:0004553hydrolase activity, hydrolyzing O-glycosyl compounds                  | 42                         | 0.0026  |
| GO:0022891substrate-specific transmembrane transporter activity                 | 62                         | 0.0034  |
| GO:0016791phosphatase activity  | 26                         | 0.0035  |
| GO:0022804active transmembrane transporter activity                             | 42                         | 0.0035  |
| GO:0003824catalytic activity  | 466                        | 0.0035  |
| GO:0015103inorganic anion transmembrane transporter activity                    | 15                         | 0.0046  |

| Enriched GO Term: Description                      | Number of associated genes | FDR    |
|--|----------------------------|--------|
| GO:0042578phosphoric ester hydrolase activity      | 29                         | 0.0065 |
| GO:0015075ion transmembrane transporter activity   | 46                         | 0.012  |
| GO:0015297antiporter activity                      | 18                         | 0.013  |
| GO:0003779actin binding                            | 12                         | 0.013  |
| GO:0016210naringenin-chalcone synthase activity    | 7                          | 0.02   |
| GO:0008509anion transmembrane transporter activity | 18                         | 0.02   |
| GO:0015238drug transmembrane transporter activity  | 11                         | 0.044  |
| GO:0016787hydrolase activity                       | 183                        | 0.047  |
| GO:0016020membrane                                 | 302                        | 0.0026 |
| GO:0005886plasma membrane                          | 159                        | 0.016  |

**Table S8.** Yellow Stage STAR Results: Genes for enzymes annotated to GO:0009813 (Flavonoid biosynthetic process, FDR=1). The first column lists differentially expressed (FDR<0.005) assembled RNA-seq reads that have mapped to the *Lotus japonicus* genome. The second column is their soybean (*Glycine max*) BLAST homolog, followed by expression at two stages of flower development.

| <i>L. japonicus</i> ID | <i>G. max</i> BLAST ID | <i>G. max</i> description                      | Counts per gene<br>(Yellow) | Counts per gene<br>(Red) | Log <sub>2</sub> FC |
|------------------------|------------------------|--|-----------------------------|--------------------------|---------------------|
| chr2.CM0018.1300.r2.m  | Glyma.01G228700.1      | Naringenin-chalcone synthase.                  | 237.49                      | 21.67                    | 2.60689             |
| chr5.CM1077.590.r2.m   | Glyma.03G187000.1      | UDP-glucosyl transferase 73C                   | 81.84                       | 1.67                     | 4.479036            |
| chr1.CM0122.1600.r2.m  | Glyma.03G222100.1      | Nitrogen regulatory protein P-II               | 118                         | 15                       | 2.151554            |
| chr1.CM0012.990.r2.m   | Glyma.04G254200.1      | B3 DNA binding domain // Auxin response factor | 111                         | 12                       | 2.383216            |
| chr1.CM0064.910.r2.m   | Glyma.05G076300.1      | NA   | 725                         | 113                      | 1.863352            |
| chr3.LjT40P18.80.r2.m  | Glyma.07G021600.1      | Shikimate O-hydroxycinnamoyltransferase.       | 329                         | 23                       | 3.015946            |
| chr3.CM0452.240.r2.d   | Glyma.07G021600.1      | Shikimate O-hydroxycinnamoyltransferase.       | 8006                        | 1066                     | 2.091311            |
| chr3.LjT10E18.60.r2.m  | Glyma.10G210600.1      | B3 DNA binding domain // Auxin response factor | 192                         | 19                       | 2.513908            |
| chr4.CM0432.2880.r2.m  | Glyma.13G173300.1      | O-METHYLTRANSFERASE                            | 1884                        | 169                      | 2.660585            |
| chr3.CM0106.330.r2.m   | Glyma.13G344700.1      | B-box zinc finger                              | 603                         | 34                       | 3.327629            |
| chr1.LjT35H06.80.r2.m  | NA                     | NA   | 40                          | 0                        | 7.976076            |

**Table S9.** Red Stage STAR Results: Genes for enzymes annotated to GO:0009813 (Flavonoid biosynthetic process, FDR = 0.00042). The first column lists differentially expressed (FDR<0.005) assembled RNA-seq reads that have mapped to the *Lotus japonicus* genome. The second column is their soybean (*Glycine max*) BLAST homolog, followed by expression at two stages of flower development.

| <i>L. japonicus</i> ID | <i>G. max</i> BLAST ID | <i>G. max</i> description                                      | Counts per gene (Yellow) | Counts per gene (Red) | Log <sub>2</sub> FC |
|------------------------|------------------------|--|--------------------------|-----------------------|---------------------|
| chr2.CM0608.560.r2.m   | Glyma.01G048200.1      | RIBONUCLEASE T2  | 792                      | 7451                  | -4.05106            |
| chr3.LjB14006.120.r2.a | Glyma.01G166200.1      | naringenin 3-dioxygenase                                       | 263.79                   | 764.34                | -2.3498             |
| chr4.CM0119.240.r2.m   | Glyma.01G166200.1      | naringenin 3-dioxygenase                                       | 2001.21                  | 6829.66               | -2.58854            |
| chr3.CM0590.770.r2.d   | Glyma.01G228700.1      | Naringenin-chalcone synthase                                   | 21.17                    | 687.21                | -5.83859            |
| chr1.CM0284.250.r2.m   | Glyma.01G228700.1      | Naringenin-chalcone synthase                                   | 11.33                    | 274.05                | -5.43578            |
| chr2.CM0018.760.r2.m   | Glyma.01G228700.1      | Naringenin-chalcone synthase                                   | 327.66                   | 1550.24               | -3.05735            |
| chr2.CM0018.730.r2.m   | Glyma.01G228700.1      | Naringenin-chalcone synthase                                   | 86.27                    | 323.5                 | -2.72878            |
| chr3.CM0590.840.r2.m   | Glyma.01G228700.1      | Naringenin-chalcone synthase                                   | 21.17                    | 687.21                | -5.83859            |
| chr2.CM0018.1190.r2.m  | Glyma.01G228700.1      | Naringenin-chalcone synthase                                   | 176.4                    | 432.25                | -2.11192            |
| chr2.CM0018.1200.r2.m  | Glyma.01G228700.1      | Naringenin-chalcone synthase.                                  | 5.93                     | 97.27                 | -4.79591            |
| chr5.CM0077.110.r2.m   | Glyma.02G158700.1      | bifunctional dihydroflavonol 4-reductase/flavanone 4-reductase | 29                       | 799                   | -5.59379            |
| chr5.CM0077.210.r2.m   | Glyma.02G158700.1      | bifunctional dihydroflavonol 4-reductase/flavanone 4-reductase | 140                      | 9266                  | -6.86429            |
| chr1.CM0105.1870.r2.m  | Glyma.03G113400.1      | Proton-exporting ATPase.                                       | 285                      | 949                   | -2.55225            |
| chr1.CM0133.560.r2.m   | Glyma.04G052100.1      | Cytochrome P450 CYP3/CYP5/CYP6/CYP9 subfamilies                | 40                       | 2680                  | -6.87787            |
| chr1.LjT39K18.30.r2.m  | Glyma.04G147700.1      | HEME OXYGENASE   | 546                      | 1688                  | -2.44547            |
| chr2.CM0191.680.r2.m   | Glyma.04G227700.1      | Quercetin 3-O-methyltransferase.                               | 3                        | 20                    | -3.48622            |

| <i>L. japonicus</i> ID | <i>G. max</i> BLAST ID | <i>G. max</i> description                                      | Counts per gene<br>(Yellow) | Counts per gene<br>(Red) | Log <sub>2</sub> FC |
|------------------------|------------------------|--|-----------------------------|--------------------------|---------------------|
| chr1.CM0410.430.r2.m   | Glyma.08G247100.1      | Transferase family   | 2201                        | 7151                     | -2.51737            |
| chr6.CM0013.1540.r2.a  | Glyma.09G038900.1      | MYB-LIKE DNA-BINDING PROTEIN MYB                               | 7                           | 114                      | -4.81165            |
| chr2.CM0021.2820.r2.m  | Glyma.11G027700.1      | Leucocyanidin oxygenase.                                       | 929                         | 14061                    | -4.73711            |
| chr5.CM0077.120.r2.m   | Glyma.14G072700.1      | bifunctional dihydroflavonol 4-reductase/flavanone 4-reductase | 14                          | 60                       | -2.90284            |
| chr5.CM0180.690.r2.m   | Glyma.20G241700.1      | chalcone isomerase   | 484                         | 1165                     | -2.08436            |

**Table S10.** Yellow Stage STAR Results: Genes for enzymes annotated to GO:0009809 (Lignin biosynthetic process, FDR=0.41). The first column lists differentially expressed (FDR<0.005) assembled RNA-seq reads that have mapped to the *Lotus japonicus* genome. The second column is their soybean (*Glycine max*) BLAST homolog, followed by expression at two stages of flower development.

| <i>L. japonicus</i> ID | <i>G. max</i> BLAST ID | <i>G. max</i> description                | Counts per gene<br>(Yellow) | Counts per gene<br>(Red) | Log <sub>2</sub> FC |
|------------------------|------------------------|--|-----------------------------|--------------------------|---------------------|
| chr6.LjT35D18.30.r2.m  | Glyma.03G223000.1      | Methionine adenosyltransferase           | 16493                       | 2557                     | 1.87182             |
| chr3.LjT40P18.80.r2.m  | Glyma.07G021600.1      | cinnamoyl-CoA reductase                  | 329                         | 23                       | 3.01594             |
| chr3.CM0452.240.r2.d   | Glyma.07G021600.1      | cinnamoyl-CoA reductase                  | 8006                        | 1066                     | 2.09131             |
| chr3.LjT45M09.50.r2.d  | Glyma.07G023700.1      | Shikimate O-hydroxycinnamoyltransferase. | 1426                        | 220                      | 1.87849             |
| chr4.CM0387.960.r2.m   | Glyma.07G258700.1      | beta-glucosidase                         | 106                         | 1                        | 5.78762             |
| chr6.CM0385.240.r2.d   | Glyma.09G038500.1      | CHITINASE                                | 1058                        | 11                       | 5.75861             |
| chr5.CM0200.3250.r2.m  | Glyma.10G262400.1      | cinnamyl-alcohol dehydrogenase           | 3805                        | 583                      | 1.88869             |

\*Lignin biosynthetic process was not found to be expressed at the red stage of floral colour change in *L. filicaulis*, given the STAR results.

**Table S11.** Trinity Results: Significantly enriched GO terms corresponding to the yellow stage of a *L. filicaulis* flower.

| Enriched GO category:Description                        | Number of associated genes | FDR      |
|---|----------------------------|----------|
| GO:0009790embryonic development                         | 162                        | 1.60E-16 |
| GO:0032501multicellular organismal process              | 557                        | 6.60E-16 |
| GO:0032502developmental process                         | 566                        | 1.30E-15 |
| GO:0007275multicellular organismal development          | 532                        | 5.60E-15 |
| GO:0009628response to abiotic stimulus                  | 399                        | 1.90E-10 |
| GO:0009791post-embryonic development                    | 314                        | 2.80E-10 |
| GO:0009987cellular process                              | 1908                       | 1.10E-09 |
| GO:0044237cellular metabolic process                    | 1498                       | 1.10E-09 |
| GO:0048856anatomical structure development              | 434                        | 1.20E-09 |
| GO:0000003reproduction                                  | 319                        | 1.30E-09 |
| GO:0050896response to stimulus                          | 859                        | 1.70E-09 |
| GO:0008152metabolic process                             | 1871                       | 1.80E-09 |
| GO:0006396RNA processing                                | 140                        | 2.40E-09 |
| GO:0044281small molecule metabolic process              | 462                        | 5.30E-08 |
| GO:0009793embryonic development ending in seed dormancy | 101                        | 7.00E-08 |
| GO:0009058biosynthetic process                          | 821                        | 4.60E-07 |
| GO:0022414reproductive process                          | 296                        | 1.00E-06 |
| GO:0010467gene expression                               | 548                        | 1.30E-06 |
| GO:0044238primary metabolic process                     | 1517                       | 1.40E-06 |
| GO:0003006reproductive developmental process            | 260                        | 2.30E-06 |
| GO:0010154fruit development                             | 136                        | 2.30E-06 |
| GO:0040007growth  | 142                        | 2.70E-06 |
| GO:0044085cellular component biogenesis                 | 161                        | 4.10E-06 |
| GO:0042254ribosome biogenesis                           | 46                         | 4.40E-06 |
| GO:0009653anatomical structure morphogenesis            | 203                        | 5.10E-06 |
| GO:0048316seed development                              | 126                        | 6.00E-06 |
| GO:0065007biological regulation                         | 739                        | 6.10E-06 |
| GO:0022613ribonucleoprotein complex biogenesis          | 49                         | 7.00E-06 |
| GO:0044249cellular biosynthetic process                 | 783                        | 7.90E-06 |
| GO:0009416response to light stimulus                    | 178                        | 1.10E-05 |
| GO:0048731system development                            | 254                        | 1.10E-05 |
| GO:0009314response to radiation                         | 180                        | 1.30E-05 |
| GO:0008610lipid biosynthetic process                    | 133                        | 1.40E-05 |
| GO:0034470ncRNA processing                              | 58                         | 2.60E-05 |
| GO:0034660ncRNA metabolic process                       | 73                         | 3.50E-05 |
| GO:0048608reproductive structure development            | 226                        | 3.60E-05 |
| GO:0050789regulation of biological process              | 641                        | 4.00E-05 |
| GO:0006950response to stress                            | 519                        | 4.90E-05 |

| Enriched GO category:Description                         | Number of associated genes | FDR      |
|--|----------------------------|----------|
| GO:0048518positive regulation of biological process      | 128                        | 4.90E-05 |
| GO:0042221response to chemical stimulus                  | 496                        | 5.30E-05 |
| GO:0009607response to biotic stimulus                    | 224                        | 6.20E-05 |
| GO:0048513organ development                              | 247                        | 8.00E-05 |
| GO:0009887organ morphogenesis                            | 73                         | 8.80E-05 |
| GO:0015979photosynthesis                                 | 67                         | 9.30E-05 |
| GO:0009409response to cold                               | 110                        | 9.60E-05 |
| GO:0044283small molecule biosynthetic process            | 211                        | 0.00023  |
| GO:0005976polysaccharide metabolic process               | 80                         | 0.00025  |
| GO:0051707response to other organism                     | 210                        | 0.00026  |
| GO:0051704multi-organism process                         | 255                        | 0.00026  |
| GO:0006412translation                                    | 130                        | 0.00026  |
| GO:0008219cell death                                     | 64                         | 0.00029  |
| GO:0016265death  | 64                         | 0.00029  |
| GO:0009266response to temperature stimulus               | 140                        | 0.0003   |
| GO:0043436oxoacid metabolic process                      | 225                        | 0.0003   |
| GO:0019752carboxylic acid metabolic process              | 225                        | 0.0003   |
| GO:0006082organic acid metabolic process                 | 225                        | 0.0003   |
| GO:0010033response to organic substance                  | 317                        | 0.00031  |
| GO:0016043cellular component organization                | 331                        | 0.00035  |
| GO:0040008regulation of growth                           | 46                         | 0.00038  |
| GO:0007166cell surface receptor linked signaling pathway | 33                         | 0.00043  |
| GO:0016072rRNA metabolic process                         | 32                         | 0.00044  |
| GO:0048869cellular developmental process                 | 165                        | 0.0006   |
| GO:0042180cellular ketone metabolic process              | 228                        | 0.00078  |
| GO:0009451RNA modification                               | 33                         | 0.00082  |
| GO:0000271polysaccharide biosynthetic process            | 49                         | 0.00093  |
| GO:0044264cellular polysaccharide metabolic process      | 69                         | 0.00095  |
| GO:0006364rRNA processing                                | 29                         | 0.00097  |
| GO:0006629lipid metabolic process                        | 205                        | 0.00099  |
| GO:0033692cellular polysaccharide biosynthetic process   | 48                         | 0.00099  |
| GO:0009725response to hormone stimulus                   | 237                        | 0.0011   |
| GO:0009719response to endogenous stimulus                | 256                        | 0.0011   |
| GO:0019748secondary metabolic process                    | 120                        | 0.0016   |
| GO:0023033signaling pathway                              | 67                         | 0.002    |
| GO:0044255cellular lipid metabolic process               | 150                        | 0.002    |
| GO:0009657plastid organization                           | 55                         | 0.0029   |
| GO:0008380RNA splicing                                   | 37                         | 0.0032   |

| Enriched GO category:Description                                   | Number of associated genes | FDR    |
|--|----------------------------|--------|
| GO:0009640photomorphogenesis                                       | 28                         | 0.0037 |
| GO:0046394carboxylic acid biosynthetic process                     | 131                        | 0.0037 |
| GO:0016053organic acid biosynthetic process                        | 131                        | 0.0037 |
| GO:0010608posttranscriptional regulation of gene expression        | 44                         | 0.0037 |
| GO:0019684photosynthesis, light reaction                           | 39                         | 0.0041 |
| GO:0006996organelle organization                                   | 204                        | 0.0042 |
| GO:0006457protein folding  | 51                         | 0.0045 |
| GO:0010025wax biosynthetic process                                 | 20                         | 0.0046 |
| GO:0044267cellular protein metabolic process                       | 474                        | 0.0053 |
| GO:0050794regulation of cellular process                           | 534                        | 0.0058 |
| GO:0006519cellular amino acid and derivative metabolic process     | 176                        | 0.0062 |
| GO:0019538protein metabolic process                                | 556                        | 0.0062 |
| GO:0048646anatomical structure formation involved in morphogenesis | 57                         | 0.0062 |
| GO:0023052signaling  | 255                        | 0.0067 |
| GO:0009733response to auxin stimulus                               | 86                         | 0.0069 |
| GO:0009637response to blue light                                   | 27                         | 0.0069 |
| GO:0012501programmed cell death                                    | 51                         | 0.0074 |
| GO:0006779porphyrin biosynthetic process                           | 29                         | 0.0078 |
| GO:0006631fatty acid metabolic process                             | 80                         | 0.0096 |
| GO:0005982starch metabolic process                                 | 26                         | 0.01   |
| GO:0065008regulation of biological quality                         | 214                        | 0.011  |
| GO:0032787monocarboxylic acid metabolic process                    | 120                        | 0.011  |
| GO:0009639response to red or far red light                         | 53                         | 0.011  |
| GO:0046148pigment biosynthetic process                             | 51                         | 0.012  |
| GO:0019953sexual reproduction                                      | 38                         | 0.013  |
| GO:0023034intracellular signaling pathway                          | 33                         | 0.014  |
| GO:0030154cell differentiation                                     | 120                        | 0.015  |
| GO:0033014tetrapyrrole biosynthetic process                        | 30                         | 0.015  |
| GO:0000375RNA splicing, via transesterification reactions          | 23                         | 0.016  |
| GO:0007389pattern specification process                            | 53                         | 0.018  |
| GO:0003002regionalization  | 47                         | 0.02   |
| GO:0008283cell proliferation                                       | 32                         | 0.02   |
| GO:0009606tropism  | 27                         | 0.02   |
| GO:0006633fatty acid biosynthetic process                          | 62                         | 0.02   |
| GO:0023060signal transmission                                      | 200                        | 0.023  |
| GO:0006778porphyrin metabolic process                              | 34                         | 0.024  |

| Enriched GO category:Description   | Number of associated genes | FDR      |
|--|----------------------------|----------|
| GO:0023046signaling process  | 200                        | 0.025    |
| GO:0043067regulation of programmed cell death  | 24                         | 0.026    |
| GO:0042440pigment metabolic process  | 57                         | 0.027    |
| GO:0016071mRNA metabolic process   | 40                         | 0.031    |
| GO:0000377RNA splicing, via transesterification reactions with bulged adenosine as nucleophile | 22                         | 0.031    |
| GO:0033013tetrapyrrole metabolic process   | 35                         | 0.032    |
| GO:0006073cellular glucan metabolic process  | 50                         | 0.041    |
| GO:0022621shoot system development   | 94                         | 0.043    |
| GO:0048367shoot development  | 94                         | 0.043    |
| GO:0032989cellular component morphogenesis   | 91                         | 0.044    |
| GO:0006875cellular metal ion homeostasis   | 16                         | 0.044    |
| GO:0048569post-embryonic organ development   | 58                         | 0.048    |
| GO:0003824catalytic activity   | 1566                       | 6.40E-06 |
| GO:0009055electron carrier activity  | 60                         | 2.10E-05 |
| GO:0005198structural molecule activity   | 108                        | 0.0096   |
| GO:0016740transferase activity   | 596                        | 0.0096   |
| GO:0004872receptor activity  | 38                         | 0.019    |
| GO:0005623cell   | 2324                       | 2.10E-90 |
| GO:0044464cell part  | 2324                       | 2.10E-90 |
| GO:0043226organelle  | 1659                       | 5.60E-66 |
| GO:0043229intracellular organelle  | 1656                       | 9.90E-66 |
| GO:0044424intracellular part   | 1838                       | 1.20E-65 |
| GO:0005622intracellular  | 1866                       | 2.20E-65 |
| GO:0043227membrane-bounded organelle   | 1581                       | 1.60E-64 |
| GO:0043231intracellular membrane-bounded organelle   | 1576                       | 3.10E-64 |
| GO:0005737cytoplasm  | 1511                       | 1.00E-62 |
| GO:0044444cytoplasmic part   | 1406                       | 5.50E-57 |
| GO:0009536plastid  | 696                        | 8.80E-55 |
| GO:0009507chloroplast  | 659                        | 4.80E-53 |
| GO:0044435plastid part   | 414                        | 1.10E-32 |
| GO:0044434chloroplast part   | 398                        | 9.00E-30 |
| GO:0044446intracellular organelle part   | 814                        | 1.90E-28 |
| GO:0044422organelle part   | 817                        | 2.40E-28 |
| GO:0012505endomembrane system  | 148                        | 1.80E-23 |
| GO:0031976plastid thylakoid  | 188                        | 2.40E-23 |
| GO:0009534chloroplast thylakoid  | 188                        | 2.40E-23 |
| GO:0031984organelle subcompartment   | 188                        | 3.10E-23 |

| Enriched GO category:Description                        | Number of associated genes | FDR      |
|---|----------------------------|----------|
| GO:0044436 thylakoid part                               | 176                        | 4.20E-23 |
| GO:0009579 thylakoid                                    | 212                        | 3.90E-22 |
| GO:0042651 thylakoid membrane                           | 164                        | 1.00E-21 |
| GO:0055035 plastid thylakoid membrane                   | 159                        | 1.00E-21 |
| GO:0009535 chloroplast thylakoid membrane               | 158                        | 1.50E-21 |
| GO:0034357 photosynthetic membrane                      | 165                        | 3.20E-20 |
| GO:0005739 mitochondrion                                | 261                        | 4.30E-13 |
| GO:0009532 plastid stroma                               | 206                        | 2.00E-12 |
| GO:0031975 envelope                                     | 243                        | 9.20E-12 |
| GO:0009526 plastid envelope                             | 193                        | 1.60E-11 |
| GO:0031967 organelle envelope                           | 240                        | 2.40E-11 |
| GO:0009570 chloroplast stroma                           | 192                        | 2.40E-10 |
| GO:0009941 chloroplast envelope                         | 183                        | 2.90E-10 |
| GO:0031974 membrane-enclosed lumen                      | 197                        | 1.00E-09 |
| GO:0070013 intracellular organelle lumen                | 194                        | 1.20E-09 |
| GO:0043233 organelle lumen                              | 194                        | 1.40E-09 |
| GO:0043232 intracellular non-membrane-bounded organelle | 280                        | 3.80E-09 |
| GO:0043228 non-membrane-bounded organelle               | 280                        | 3.80E-09 |
| GO:0031090 organelle membrane                           | 332                        | 1.60E-08 |
| GO:0009505 plant-type cell wall                         | 92                         | 1.00E-07 |
| GO:0010287 plastoglobule                                | 36                         | 1.10E-06 |
| GO:0016020 membrane                                     | 971                        | 1.40E-06 |
| GO:0030529 ribonucleoprotein complex                    | 142                        | 2.30E-06 |
| GO:0044428 nuclear part                                 | 183                        | 4.20E-06 |
| GO:0031977 thylakoid lumen                              | 37                         | 8.10E-06 |
| GO:0005634 nucleus                                      | 524                        | 9.50E-06 |
| GO:0032991 macromolecular complex                       | 360                        | 5.30E-05 |
| GO:0031978 plastid thylakoid lumen                      | 26                         | 0.0001   |
| GO:0009543 chloroplast thylakoid lumen                  | 26                         | 0.0001   |
| GO:0030312 external encapsulating structure             | 164                        | 0.00011  |
| GO:0031224 intrinsic to membrane                        | 250                        | 0.00016  |
| GO:0005618 cell wall                                    | 159                        | 0.00039  |
| GO:0005730 nucleolus                                    | 100                        | 0.00051  |
| GO:0005576 extracellular region                         | 155                        | 0.00074  |
| GO:0005840 ribosome                                     | 104                        | 0.00097  |
| GO:0031981 nuclear lumen                                | 134                        | 0.0017   |
| GO:0031225 anchored to membrane                         | 44                         | 0.0063   |
| GO:0009706 chloroplast inner membrane                   | 22                         | 0.013    |

| Enriched GO category:Description      | Number of associated genes | FDR   |
|---------------------------------------|----------------------------|-------|
| GO:0009295nucleoid                    | 17                         | 0.015 |
| GO:0042170plastid membrane            | 34                         | 0.016 |
| GO:0005794Golgi apparatus             | 162                        | 0.017 |
| GO:0016021integral to membrane        | 204                        | 0.02  |
| GO:0009528plastid inner membrane      | 22                         | 0.022 |
| GO:0010319stromule                    | 19                         | 0.022 |
| GO:0042646plastid nucleoid            | 14                         | 0.023 |
| GO:0031969chloroplast membrane        | 30                         | 0.03  |
| GO:0005887integral to plasma membrane | 13                         | 0.035 |
| GO:0005874microtubule                 | 24                         | 0.043 |
| GO:0009521photosystem                 | 22                         | 0.043 |
| GO:0044425membrane part               | 429                        | 0.048 |

**Table 12.** Trinity Results: Significantly enriched GO terms corresponding to the red stage of a *L. filicaulis* flower.

| Enriched GO category:Description                               | Number of associated genes | FDR      |
|--|----------------------------|----------|
| GO:0050896 response to stimulus                                | 426                        | 2.60E-23 |
| GO:0006950 response to stress                                  | 280                        | 1.20E-17 |
| GO:0023052 signaling   | 168                        | 1.60E-17 |
| GO:0065007 biological regulation                               | 368                        | 1.60E-17 |
| GO:0032502 developmental process                               | 256                        | 2.80E-16 |
| GO:0050794 regulation of cellular process                      | 289                        | 1.80E-15 |
| GO:0007242 intracellular signaling cascade                     | 111                        | 8.50E-15 |
| GO:0032501 multicellular organismal process                    | 245                        | 8.50E-15 |
| GO:0050789 regulation of biological process                    | 318                        | 1.10E-14 |
| GO:0009607 response to biotic stimulus                         | 134                        | 1.40E-14 |
| GO:0023060 signal transmission                                 | 133                        | 2.90E-14 |
| GO:0023046 signaling process                                   | 133                        | 3.00E-14 |
| GO:0051707 response to other organism                          | 127                        | 9.80E-14 |
| GO:0007275 multicellular organismal development                | 232                        | 1.70E-13 |
| GO:0007165 signal transduction                                 | 128                        | 2.30E-13 |
| GO:0009987 cellular process                                    | 780                        | 9.60E-13 |
| GO:0048856 anatomical structure development                    | 202                        | 2.50E-12 |
| GO:0042221 response to chemical stimulus                       | 246                        | 2.90E-12 |
| GO:0051179 localization  | 226                        | 8.80E-12 |
| GO:0006575 cellular amino acid derivative metabolic process    | 74                         | 1.00E-11 |
| GO:0023034 intracellular signaling pathway                     | 34                         | 1.00E-11 |
| GO:0051704 multi-organism process                              | 141                        | 1.10E-11 |
| GO:0042398 cellular amino acid derivative biosynthetic process | 60                         | 1.10E-11 |
| GO:0009699 phenylpropanoid biosynthetic process                | 45                         | 2.50E-11 |
| GO:0023033 signaling pathway                                   | 51                         | 2.70E-11 |
| GO:0010033 response to organic substance                       | 167                        | 4.30E-11 |
| GO:0009719 response to endogenous stimulus                     | 142                        | 4.60E-11 |
| GO:0009790 embryonic development                               | 69                         | 4.70E-11 |
| GO:0009751 response to salicylic acid stimulus                 | 42                         | 5.60E-11 |
| GO:0009653 anatomical structure morphogenesis                  | 106                        | 3.60E-10 |
| GO:0051234 establishment of localization                       | 211                        | 4.40E-10 |
| GO:0009605 response to external stimulus                       | 86                         | 8.00E-10 |
| GO:0006810 transport   | 208                        | 1.20E-09 |
| GO:0019748 secondary metabolic process                         | 74                         | 1.30E-09 |
| GO:0044238 primary metabolic process                           | 626                        | 2.30E-09 |
| GO:0009698 phenylpropanoid metabolic process                   | 50                         | 3.50E-09 |
| GO:0007166 cell surface receptor linked signaling pathway      | 26                         | 4.60E-09 |

| Enriched GO category:Description                               | Number of associated genes | FDR      |
|--|----------------------------|----------|
| GO:0012501programmed cell death                                | 39                         | 5.00E-09 |
| GO:0006519cellular amino acid and derivative metabolic process | 101                        | 5.00E-09 |
| GO:0008219cell death   | 42                         | 6.30E-09 |
| GO:0016265death  | 42                         | 6.30E-09 |
| GO:0009813flavonoid biosynthetic process                       | 32                         | 1.00E-08 |
| GO:0048518positive regulation of biological process            | 70                         | 1.40E-08 |
| GO:0048731system development                                   | 123                        | 2.10E-08 |
| GO:0009725response to hormone stimulus                         | 122                        | 4.40E-08 |
| GO:0009056catabolic process                                    | 109                        | 5.80E-08 |
| GO:0009624response to nematode                                 | 32                         | 6.70E-08 |
| GO:0000003reproduction   | 136                        | 7.10E-08 |
| GO:0048513organ development                                    | 120                        | 1.20E-07 |
| GO:0009628response to abiotic stimulus                         | 163                        | 2.00E-07 |
| GO:0009812flavonoid metabolic process                          | 33                         | 2.30E-07 |
| GO:0044237cellular metabolic process                           | 589                        | 2.80E-07 |
| GO:0009723response to ethylene stimulus                        | 34                         | 4.50E-07 |
| GO:0009753response to jasmonic acid stimulus                   | 43                         | 4.50E-07 |
| GO:0022414reproductive process                                 | 131                        | 5.00E-07 |
| GO:0009620response to fungus                                   | 48                         | 7.50E-07 |
| GO:0040007growth   | 67                         | 1.10E-06 |
| GO:0031323regulation of cellular metabolic process             | 169                        | 2.40E-06 |
| GO:0007610behavior   | 15                         | 2.60E-06 |
| GO:0002376immune system process                                | 55                         | 2.90E-06 |
| GO:0009058biosynthetic process                                 | 332                        | 4.50E-06 |
| GO:0042493response to drug                                     | 21                         | 4.90E-06 |
| GO:0044283small molecule biosynthetic process                  | 99                         | 6.50E-06 |
| GO:0061024membrane organization                                | 27                         | 7.00E-06 |
| GO:0016044cellular membrane organization                       | 27                         | 7.00E-06 |
| GO:0003006reproductive developmental process                   | 111                        | 1.40E-05 |
| GO:0080090regulation of primary metabolic process              | 159                        | 1.40E-05 |
| GO:0006955immune response                                      | 52                         | 2.70E-05 |
| GO:0006952defense response                                     | 100                        | 2.80E-05 |
| GO:0031326regulation of cellular biosynthetic process          | 152                        | 2.90E-05 |
| GO:0050832defense response to fungus                           | 36                         | 3.10E-05 |
| GO:0009889regulation of biosynthetic process                   | 152                        | 3.20E-05 |
| GO:0048869cellular developmental process                       | 78                         | 3.70E-05 |
| GO:0044281small molecule metabolic process                     | 183                        | 3.80E-05 |
| GO:0015893drug transport                                       | 19                         | 4.20E-05 |

| Enriched GO category:Description  | Number of associated genes | FDR      |
|---|----------------------------|----------|
| GO:0044248cellular catabolic process  | 89                         | 5.60E-05 |
| GO:0044249cellular biosynthetic process   | 314                        | 7.90E-05 |
| GO:0006873cellular ion homeostasis  | 20                         | 0.0001   |
| GO:0055082cellular chemical homeostasis   | 20                         | 0.0001   |
| GO:0048608reproductive structure development  | 97                         | 0.00011  |
| GO:0008152metabolic process   | 710                        | 0.00011  |
| GO:0051171regulation of nitrogen compound metabolic process                                   | 145                        | 0.00011  |
| GO:0019222regulation of metabolic process   | 173                        | 0.00012  |
| GO:0009308amine metabolic process   | 67                         | 0.00012  |
| GO:0045449regulation of transcription   | 138                        | 0.00014  |
| GO:0010556regulation of macromolecule biosynthetic process                                    | 142                        | 0.00014  |
| GO:0009793embryonic development ending in seed dormancy                                       | 40                         | 0.00018  |
| GO:0019219regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process | 142                        | 0.00018  |
| GO:0009887organ morphogenesis   | 34                         | 0.0002   |
| GO:0048646anatomical structure formation involved in morphogenesis                            | 31                         | 0.0002   |
| GO:0009611response to wounding  | 39                         | 0.0002   |
| GO:0009737response to abscisic acid stimulus  | 58                         | 0.00021  |
| GO:0009408response to heat  | 32                         | 0.00023  |
| GO:0016042lipid catabolic process   | 19                         | 0.00023  |
| GO:0016043cellular component organization   | 141                        | 0.00023  |
| GO:0031667response to nutrient levels   | 28                         | 0.00024  |
| GO:0045087innate immune response  | 45                         | 0.00027  |
| GO:0043067regulation of programmed cell death   | 16                         | 0.00027  |
| GO:0044242cellular lipid catabolic process  | 16                         | 0.00027  |
| GO:0007568aging   | 23                         | 0.00031  |
| GO:0009991response to extracellular stimulus  | 31                         | 0.00033  |
| GO:0019953sexual reproduction   | 22                         | 0.00039  |
| GO:0009266response to temperature stimulus  | 62                         | 0.00042  |
| GO:0006350transcription   | 145                        | 0.00053  |
| GO:0009791post-embryonic development  | 113                        | 0.00054  |
| GO:0043069negative regulation of programmed cell death  | 11                         | 0.00056  |
| GO:0000278mitotic cell cycle  | 17                         | 0.00062  |
| GO:0060255regulation of macromolecule metabolic process                                       | 151                        | 0.00069  |
| GO:0009863salicylic acid mediated signaling pathway   | 14                         | 0.00076  |
| GO:0040008regulation of growth  | 22                         | 0.00078  |
| GO:0016192vesicle-mediated transport  | 36                         | 0.00084  |
| GO:0048522positive regulation of cellular process   | 42                         | 0.00085  |

| Enriched GO category:Description                           | Number of associated genes | FDR     |
|--|----------------------------|---------|
| GO:0042594response to starvation                           | 24                         | 0.00088 |
| GO:0046942carboxylic acid transport                        | 20                         | 0.00088 |
| GO:0010468regulation of gene expression                    | 146                        | 0.00089 |
| GO:0006855multidrug transport                              | 16                         | 0.0009  |
| GO:0010646regulation of cell communication                 | 25                         | 0.0009  |
| GO:0006928cellular component movement                      | 15                         | 0.0011  |
| GO:0060548negative regulation of cell death                | 11                         | 0.0011  |
| GO:0071554cell wall organization or biogenesis             | 43                         | 0.0011  |
| GO:0006022aminoglycan metabolic process                    | 8                          | 0.0013  |
| GO:0009966regulation of signal transduction                | 24                         | 0.0013  |
| GO:0023051regulation of signaling process                  | 24                         | 0.0013  |
| GO:0010941regulation of cell death                         | 16                         | 0.0014  |
| GO:0015849organic acid transport                           | 20                         | 0.0014  |
| GO:0006979response to oxidative stress                     | 50                         | 0.0015  |
| GO:0071446cellular response to salicylic acid stimulus     | 14                         | 0.0015  |
| GO:0071495cellular response to endogenous stimulus         | 54                         | 0.0019  |
| GO:0015837amine transport                                  | 15                         | 0.0021  |
| GO:0006629lipid metabolic process                          | 86                         | 0.0027  |
| GO:0009733response to auxin stimulus                       | 40                         | 0.0029  |
| GO:0019932second-messenger-mediated signaling              | 10                         | 0.003   |
| GO:0048878chemical homeostasis                             | 29                         | 0.003   |
| GO:0009415response to water                                | 39                         | 0.0031  |
| GO:0009414response to water deprivation                    | 38                         | 0.0037  |
| GO:0048583regulation of response to stimulus               | 38                         | 0.0037  |
| GO:0006865amino acid transport                             | 14                         | 0.004   |
| GO:0009814defense response, incompatible interaction       | 30                         | 0.004   |
| GO:0006796phosphate metabolic process                      | 120                        | 0.004   |
| GO:0009739response to gibberellin stimulus                 | 20                         | 0.0044  |
| GO:0006793phosphorus metabolic process                     | 120                        | 0.0045  |
| GO:0000272polysaccharide catabolic process                 | 13                         | 0.0047  |
| GO:0044106cellular amine metabolic process                 | 54                         | 0.0047  |
| GO:0065008regulation of biological quality                 | 92                         | 0.0049  |
| GO:0071310cellular response to organic substance           | 63                         | 0.0049  |
| GO:0009888tissue development                               | 50                         | 0.0049  |
| GO:0009409response to cold                                 | 44                         | 0.0052  |
| GO:0022603regulation of anatomical structure morphogenesis | 14                         | 0.0058  |
| GO:0042439ethanolamine and derivative metabolic process    | 7                          | 0.006   |
| GO:0007267cell-cell signaling                              | 7                          | 0.006   |

| Enriched GO category:Description                       | Number of associated genes | FDR    |
|--|----------------------------|--------|
| GO:0048523negative regulation of cellular process      | 44                         | 0.006  |
| GO:0048316seed development                             | 47                         | 0.0065 |
| GO:0007010cytoskeleton organization                    | 26                         | 0.0065 |
| GO:0043687post-translational protein modification      | 127                        | 0.0066 |
| GO:0006576cellular biogenic amine metabolic process    | 15                         | 0.0066 |
| GO:0006464protein modification process                 | 137                        | 0.0066 |
| GO:0030036actin cytoskeleton organization              | 18                         | 0.0071 |
| GO:0009057macromolecule catabolic process              | 48                         | 0.0071 |
| GO:0030029actin filament-based process                 | 19                         | 0.0072 |
| GO:0010118stomatal movement                            | 16                         | 0.0072 |
| GO:0009642response to light intensity                  | 23                         | 0.008  |
| GO:0030154cell differentiation                         | 53                         | 0.0083 |
| GO:0005975carbohydrate metabolic process               | 100                        | 0.009  |
| GO:0070887cellular response to chemical stimulus       | 71                         | 0.0093 |
| GO:0042538hyperosmotic salinity response               | 13                         | 0.01   |
| GO:0009873ethylene mediated signaling pathway          | 13                         | 0.01   |
| GO:0009416response to light stimulus                   | 66                         | 0.011  |
| GO:0009755hormone-mediated signaling pathway           | 43                         | 0.012  |
| GO:0030003cellular cation homeostasis                  | 13                         | 0.013  |
| GO:0009612response to mechanical stimulus              | 7                          | 0.014  |
| GO:0000902cell morphogenesis                           | 38                         | 0.014  |
| GO:0007626locomotory behavior                          | 8                          | 0.014  |
| GO:0006972hyperosmotic response                        | 14                         | 0.014  |
| GO:0006811ion transport                                | 60                         | 0.015  |
| GO:0010154fruit development                            | 48                         | 0.016  |
| GO:0016567protein ubiquitination                       | 20                         | 0.016  |
| GO:0009314response to radiation                        | 66                         | 0.016  |
| GO:0043436oxoacid metabolic process                    | 87                         | 0.016  |
| GO:0010054trichoblast differentiation                  | 14                         | 0.016  |
| GO:0006082organic acid metabolic process               | 87                         | 0.016  |
| GO:0019752carboxylic acid metabolic process            | 87                         | 0.016  |
| GO:0016310phosphorylation                              | 102                        | 0.017  |
| GO:0048585negative regulation of response to stimulus  | 16                         | 0.018  |
| GO:0032870cellular response to hormone stimulus        | 45                         | 0.018  |
| GO:0022610biological adhesion                          | 7                          | 0.019  |
| GO:0007155cell adhesion                                | 7                          | 0.019  |
| GO:0051094positive regulation of developmental process | 14                         | 0.019  |
| GO:0046395carboxylic acid catabolic process            | 18                         | 0.02   |

| Enriched GO category:Description                                | Number of associated genes | FDR   |
|---|----------------------------|-------|
| GO:0016054organic acid catabolic process                        | 18                         | 0.02  |
| GO:0044255cellular lipid metabolic process                      | 60                         | 0.021 |
| GO:0048468cell development                                      | 35                         | 0.022 |
| GO:0043412macromolecule modification                            | 140                        | 0.023 |
| GO:0071555cell wall organization                                | 27                         | 0.023 |
| GO:0006026aminoglycan catabolic process                         | 6                          | 0.024 |
| GO:0006030chitin metabolic process                              | 6                          | 0.024 |
| GO:0006032chitin catabolic process                              | 6                          | 0.024 |
| GO:0019722calcium-mediated signaling                            | 7                          | 0.026 |
| GO:0032446protein modification by small protein conjugation     | 20                         | 0.027 |
| GO:0010119regulation of stomatal movement                       | 11                         | 0.027 |
| GO:0015692lead ion transport                                    | 5                          | 0.028 |
| GO:0009963positive regulation of flavonoid biosynthetic process | 5                          | 0.028 |
| GO:0007584response to nutrient                                  | 5                          | 0.028 |
| GO:0048645organ formation                                       | 10                         | 0.028 |
| GO:0032989cellular component morphogenesis                      | 40                         | 0.028 |
| GO:0070882cellular cell wall organization or biogenesis         | 17                         | 0.029 |
| GO:0010053root epidermal cell differentiation                   | 16                         | 0.03  |
| GO:0050801ion homeostasis                                       | 21                         | 0.031 |
| GO:0016049cell growth   | 38                         | 0.032 |
| GO:0009867jasmonic acid mediated signaling pathway              | 13                         | 0.032 |
| GO:0007264small GTPase mediated signal transduction             | 17                         | 0.033 |
| GO:0006754ATP biosynthetic process                              | 9                          | 0.033 |
| GO:0019725cellular homeostasis                                  | 24                         | 0.033 |
| GO:0048519negative regulation of biological process             | 55                         | 0.034 |
| GO:0032535regulation of cellular component size                 | 42                         | 0.035 |
| GO:0090066regulation of anatomical structure size               | 42                         | 0.035 |
| GO:0042180cellular ketone metabolic process                     | 87                         | 0.035 |
| GO:0006644phospholipid metabolic process                        | 18                         | 0.035 |
| GO:0048532anatomical structure arrangement                      | 12                         | 0.035 |
| GO:0007389pattern specification process                         | 23                         | 0.035 |
| GO:0071395cellular response to jasmonic acid stimulus           | 13                         | 0.037 |
| GO:0023057negative regulation of signaling process              | 11                         | 0.037 |
| GO:0009968negative regulation of signal transduction            | 11                         | 0.037 |
| GO:0010648negative regulation of cell communication             | 11                         | 0.037 |
| GO:0008361regulation of cell size                               | 39                         | 0.038 |
| GO:0023056positive regulation of signaling process              | 8                          | 0.038 |
| GO:0009967positive regulation of signal transduction            | 8                          | 0.038 |

| Enriched GO category:Description                                       | Number of associated genes | FDR      |
|--|----------------------------|----------|
| GO:0002218activation of innate immune response                         | 10                         | 0.039    |
| GO:0002253activation of immune response                                | 10                         | 0.039    |
| GO:0006839mitochondrial transport                                      | 9                          | 0.039    |
| GO:0048584positive regulation of response to stimulus                  | 16                         | 0.041    |
| GO:0070647protein modification by small protein conjugation or removal | 21                         | 0.044    |
| GO:0022403cell cycle phase   | 17                         | 0.045    |
| GO:0006970response to osmotic stress                                   | 53                         | 0.046    |
| GO:0000087M phase of mitotic cell cycle                                | 10                         | 0.046    |
| GO:0007067mitosis  | 10                         | 0.046    |
| GO:0009055electron carrier activity                                    | 56                         | 1.10E-19 |
| GO:0003824catalytic activity   | 688                        | 7.80E-17 |
| GO:0030528transcription regulator activity                             | 119                        | 1.40E-14 |
| GO:0022804active transmembrane transporter activity                    | 76                         | 2.40E-11 |
| GO:0005215transporter activity   | 135                        | 3.30E-11 |
| GO:0003700transcription factor activity                                | 97                         | 4.90E-10 |
| GO:0022857transmembrane transporter activity                           | 107                        | 3.90E-08 |
| GO:0015291secondary active transmembrane transporter activity          | 45                         | 3.90E-08 |
| GO:0015294solute:cation symporter activity                             | 19                         | 1.30E-07 |
| GO:0043169cation binding   | 268                        | 1.60E-07 |
| GO:0022892substrate-specific transporter activity                      | 97                         | 2.30E-07 |
| GO:0042578phosphoric ester hydrolase activity                          | 47                         | 3.20E-07 |
| GO:0015293symporter activity   | 19                         | 3.70E-07 |
| GO:0004091carboxylesterase activity                                    | 36                         | 1.40E-06 |
| GO:0016787hydrolase activity   | 266                        | 1.80E-06 |
| GO:0015295solute:hydrogen symporter activity                           | 14                         | 7.40E-06 |
| GO:0016298lipase activity  | 24                         | 9.40E-06 |
| GO:0022891substrate-specific transmembrane transporter activity        | 85                         | 1.20E-05 |
| GO:0004497monooxygenase activity                                       | 26                         | 2.30E-05 |
| GO:0005509calcium ion binding  | 40                         | 2.50E-05 |
| GO:0000287magnesium ion binding  | 25                         | 2.70E-05 |
| GO:0016791phosphatase activity   | 36                         | 2.90E-05 |
| GO:0004722protein serine/threonine phosphatase activity                | 18                         | 5.20E-05 |
| GO:0008324cation transmembrane transporter activity                    | 51                         | 6.40E-05 |
| GO:0016563transcription activator activity                             | 11                         | 8.70E-05 |
| GO:0019787small conjugating protein ligase activity                    | 31                         | 8.80E-05 |
| GO:0015075ion transmembrane transporter activity                       | 64                         | 8.80E-05 |
| GO:0016788hydrolase activity, acting on ester bonds                    | 105                        | 0.00011  |
| GO:0046872metal ion binding  | 247                        | 0.00012  |

| Enriched GO category:Description  | Number of associated genes | FDR      |
|---|----------------------------|----------|
| GO:0051119sugar transmembrane transporter activity  | 14                         | 0.00013  |
| GO:0016874ligase activity   | 51                         | 0.00014  |
| GO:0016301kinase activity   | 122                        | 0.00015  |
| GO:0016879ligase activity, forming carbon-nitrogen bonds  | 41                         | 0.00018  |
| GO:0016638oxidoreductase activity, acting on the CH-NH <sub>2</sub> group of donors             | 10                         | 0.0002   |
| GO:0016881acid-amino acid ligase activity   | 34                         | 0.00023  |
| GO:0046943carboxylic acid transmembrane transporter activity                                    | 19                         | 0.00024  |
| GO:0004842ubiquitin-protein ligase activity   | 29                         | 0.00034  |
| GO:0005342organic acid transmembrane transporter activity                                       | 19                         | 0.00044  |
| GO:0005506iron ion binding  | 59                         | 0.00044  |
| GO:0008134transcription factor binding  | 15                         | 0.0006   |
| GO:0016798hydrolase activity, acting on glycosyl bonds  | 53                         | 0.0006   |
| GO:0004553hydrolase activity, hydrolyzing O-glycosyl compounds                                  | 50                         | 0.00087  |
| GO:0015297antiporter activity   | 23                         | 0.0013   |
| GO:0004672protein kinase activity   | 100                        | 0.0014   |
| GO:0015144carbohydrate transmembrane transporter activity                                       | 14                         | 0.0014   |
| GO:0016740transferase activity  | 242                        | 0.0014   |
| GO:0016773phosphotransferase activity, alcohol group as acceptor                                | 113                        | 0.0014   |
| GO:0004721phosphoprotein phosphatase activity   | 19                         | 0.0018   |
| GO:0015662ATPase activity, coupled to transmembrane movement of ions, phosphorylative mechanism | 9                          | 0.0028   |
| GO:0008194UDP-glycosyltransferase activity  | 24                         | 0.0047   |
| GO:0005275amine transmembrane transporter activity  | 14                         | 0.0052   |
| GO:0015171amino acid transmembrane transporter activity   | 13                         | 0.0065   |
| GO:0015179L-amino acid transmembrane transporter activity                                       | 6                          | 0.0078   |
| GO:0015197peptide transporter activity  | 6                          | 0.0078   |
| GO:0004674protein serine/threonine kinase activity  | 80                         | 0.0092   |
| GO:0008081phosphoric diester hydrolase activity   | 11                         | 0.0095   |
| GO:0015300solute:solute antiporter activity   | 13                         | 0.011    |
| GO:0015238drug transmembrane transporter activity   | 13                         | 0.017    |
| GO:0030246carbohydrate binding  | 27                         | 0.017    |
| GO:0004568chitinase activity  | 6                          | 0.019    |
| GO:0030234enzyme regulator activity   | 27                         | 0.029    |
| GO:0008415acyltransferase activity  | 22                         | 0.03     |
| GO:0015399primary active transmembrane transporter activity                                     | 22                         | 0.033    |
| GO:0005337nucleoside transmembrane transporter activity   | 5                          | 0.037    |
| GO:0012505endomembrane system   | 126                        | 5.00E-52 |
| GO:0044464cell part   | 882                        | 5.80E-40 |

| Enriched GO category:Description                       | Number of associated genes | FDR      |
|--|----------------------------|----------|
| GO:0005623cell   | 882                        | 5.80E-40 |
| GO:0031224intrinsic to membrane                        | 166                        | 3.80E-20 |
| GO:0016021integral to membrane                         | 141                        | 7.10E-16 |
| GO:0009505plant-type cell wall                         | 47                         | 1.70E-07 |
| GO:0005622intracellular                                | 600                        | 5.40E-07 |
| GO:0044424intracellular part                           | 578                        | 1.30E-05 |
| GO:0005887integral to plasma membrane                  | 13                         | 1.80E-05 |
| GO:0044425membrane part                                | 201                        | 4.00E-05 |
| GO:0005739mitochondrion                                | 94                         | 0.00012  |
| GO:0031410cytoplasmic vesicle                          | 23                         | 0.00012  |
| GO:0008287protein serine/threonine phosphatase complex | 10                         | 0.00013  |
| GO:0031982vesicle                                      | 25                         | 0.00014  |
| GO:0031988membrane-bounded vesicle                     | 23                         | 0.00078  |
| GO:0016023cytoplasmic membrane-bounded vesicle         | 21                         | 0.00078  |
| GO:0043226organelle                                    | 482                        | 0.0073   |
| GO:0043229intracellular organelle                      | 481                        | 0.0077   |
| GO:0043227membrane-bounded organelle                   | 450                        | 0.023    |
| GO:0043231intracellular membrane-bounded organelle     | 449                        | 0.023    |
| GO:0005634nucleus                                      | 197                        | 0.023    |
| GO:0045177apical part of cell                          | 7                          | 0.036    |
| GO:0005856cytoskeleton                                 | 30                         | 0.043    |

**Table S13.** Red Stage Trinity Results: Genes for enzymes annotated to the over-represented GO:0009813 (Flavonoid biosynthetic process, FDR=1.0e-08). The first two columns list differentially expressed (FDR<0.05) Trinity contigs and gene names as annotated by BLASTing the *Lotus japonicus* genome. The third column is their soybean BLAST homolog, followed by expression at two stages of flower development.

| Trinity contig | <i>L. japonicus</i> ID | <i>G. max</i> BLAST ID | Gene description  | Counts per gene (Yellow) | Counts per gene (Red) | Log <sub>2</sub> FC |
|----------------|------------------------|------------------------|---|--------------------------|-----------------------|---------------------|
| TR29586 c1_g3  | chr5.CM0077.790.nc     | Glyma.17G252200.1      | bifunctional dihydroflavonol 4-reductase/flavanone 4-reductase (DFR1) | 0                        | 78.33                 | -9.4766             |
| TR29586 c0_g1  | chr5.CM0077.690.nc     | Glyma.14G072700.1      | bifunctional dihydroflavonol 4-reductase/flavanone 4-reductase (DFR3) | 148                      | 10163                 | -6.4567             |
| TR17543 c0_g1  | chr5.CM0077.790.nc     | Glyma.14G072700.1      | bifunctional dihydroflavonol 4-reductase/flavanone 4-reductase (DFR5) | 0                        | 28                    | -8.0022             |
| TR29586 c1_g4  | chr5.CM0077.790.nc     | Glyma.17G252200.1      | bifunctional dihydroflavonol 4-reductase/flavanone 4-reductase (DFR5) | 0                        | 65.1                  | -9.2140             |
| TR29586 c2_g1  | chr5.CM0077.790.nc     | Glyma.17G252200.1      | bifunctional dihydroflavonol 4-reductase/flavanone 4-reductase (DFR5) | 9                        | 195                   | -4.7724             |
| TR29586 c1_g1  | chr5.CM0077.790.nc     | Glyma.17G252200.1      | bifunctional dihydroflavonol 4-reductase/flavanone 4-reductase (DFR5) | 6                        | 38.56                 | -3.0276             |
| TR52576 c0_g1  | chr5.CM0077.780.nc     | Glyma.17G252200.1      | bifunctional dihydroflavonol 4-reductase/flavanone 4-reductase (DFR5) | 7                        | 33                    | -2.5697             |
| TR43444 c0_g2  | chr1.CM0133.170.nc     | Glyma.17G227500.1      | Cytochrome P450 CYP3/CYP5/CYP6/CYP 9 subfamilies                      | 6                        | 98.36                 | -4.3545             |
| TR43444 c0_g1  | chr1.CM0133.170.nc     | Glyma.17G227500.1      | Cytochrome P450 CYP3/CYP5/CYP6/CYP 9 subfamilies                      | 0                        | 13.64                 | -7.0078             |

| Trinity contig | <i>L. japonicus</i> ID   | <i>G. max</i> BLAST ID | Gene description  | Counts per gene (Yellow) | Counts per gene (Red) | Log <sub>2</sub> FC |
|----------------|--------------------------|------------------------|---|--------------------------|-----------------------|---------------------|
| TR23263 c0_g1  | LjSGA_060583.1           | Glyma.17G227500.1      | Cytochrome P450<br>CYP3/CYP5/CYP6/CYP<br>9 subfamilies                    | 13                       | 182                   | -4.1492             |
| TR23981 c0_g1  | chr1.CM0133.170.nc       | Glyma.04G052100.1      | Cytochrome P450<br>CYP3/CYP5/CYP6/CYP<br>9 subfamilies (putative<br>MAX1) | 45                       | 2897                  | -6.3605             |
| TR38960 c0_g1  | chr4.CM0119.240.nc       | Glyma.02G048400.1      | Flavanone 3-<br>dioxygenase (F3H1)  | 1830                     | 6123                  | -<br>2.0988<br>4    |
| TR61355 c0_g2  | LjSGA_043399.2           | Glyma.20G009300.2      | Flavanone 3-<br>dioxygenase (F3H1)  | 117.49                   | 453.22                | -2.3081             |
| TR13112 c0_g1  | chr2.CM0124.280.nd       | Glyma.02G124700.1      | Flavanone 3-<br>dioxygenase (F3H2)  | 6                        | 33                    | -2.7873             |
| TR53383 c0_g1  | chr4.CM0387.340.nc       | Glyma.08G092800.1      | Flavanone 3-<br>dioxygenase (F3H5)  | 1                        | 50                    | -5.8140             |
| TR50359 c0_g2  | LjSGA_025000.1           | Glyma.12G235300.1      | Flavanone 3-<br>dioxygenase (F3H8)  | 4                        | 40                    | -3.6326             |
| TR46116 c0_g1* | chr2.CM0124.160.nd       | Glyma.02G124700.1      | Flavanone 3-<br>dioxygenase *   | 1                        | 21                    | -4.5668             |
| TR52191 c0_g1  | chr4.CM0429.360.nc       | Glyma.06G202300.1      | Flavonoid 3'-<br>monooxygenase<br>(F3'H1)                                 | 1586                     | 15638                 | -3.6580             |
| TR38633 c0_g1  | chr6.CM0055.290.nc       | Glyma.09G146300.1      | Kinesin motor domain<br>// PPR repeat //<br>Trehalose-<br>phosphatase     | 1219                     | 3586                  | -1.9130             |
| TR50049 c0_g2  | chr2.CM0304.350.nc       | Glyma.11G027700.1      | Leucocyanidin<br>oxygenase (ANS1)   | 0                        | 165.97                | -10.565             |
| TR50049 c0_g1  | chr2.CM0304.350.nc       | Glyma.11G027700.1      | Leucocyanidin<br>oxygenase (ANS1)   | 1010                     | 14783.                | -4.2278<br>03       |
| TR42413 c0_g1  | chr2.LjT36E17.150.n<br>d | Glyma.14G032400.1      | MATE EFFLUX FAMILY<br>PROTEIN   | 0                        | 53                    | -8.9201             |
| TR32220 c0_g1  | LjT02E24.110.nc          | Glyma.09G038900.1      | MYB-LIKE DNA-<br>BINDING PROTEIN<br>MYB                                   | 7                        | 111                   | -<br>4.3163<br>8    |

| Trinity contig | <i>L. japonicus</i> ID | <i>G. max</i> BLAST ID | Gene description                        | Counts per gene (Yellow) | Counts per gene (Red) | Log <sub>2</sub> FC |
|----------------|------------------------|------------------------|---|--------------------------|-----------------------|---------------------|
| TR19889 c3_g1  | chr1.CM0591.390.nd     | Glyma.11G011500.1      | Naringenin-chalcone synthase (CHS1)     | 1385                     | 19064                 | -4.1392             |
| TR48518 c0_g1  | chr2.CM0018.700.nc     | Glyma.11G011500.1      | Naringenin-chalcone synthase (CHS2)     | 77                       | 430                   | -2.8356             |
| TR19889 c2_g1  | chr2.CM0018.710.nc     | Glyma.01G228700.1      | Naringenin-chalcone synthase (CHS9)     | 17                       | 207                   | -3.9514             |
| TR51814 c0_g1  | chr1.CM0105.880.nc     | Glyma.03G262600.1      | Proton-exporting ATPase.                | 272                      | 951                   | -2.1617             |
| TR1381 c0_g1   | chr1.CM0105.600.nc     | Glyma.07G048700.1      | Quercetin 3-O-methyltransferase (3-OMT) | 4                        | 126                   | -5.2853             |
| TR57984 c0_g1  | chr2.CM0641.550.nc     | Glyma.01G048400.1      | Ribonuclease T(2)                       | 383                      | 12922                 | -5.4323             |
| TR57984 c0_g2  | chr2.CM0641.560.nc     | Glyma.01G048200.1      | RIBONUCLEASE T2                         | 850                      | 8077                  | -3.6046             |
| TR45786 c0_g1  | chr3.LjT46L11.20.nc    | Glyma.12G238900.1      | Thaumatin family                        | 1                        | 261                   | -8.1955             |
| TR14130 c0_g1  | chr1.CM0063.200.nc     | Glyma.08G010500.1      | Transferase family                      | 1                        | 29                    | -5.0304             |
| TR45798 c0_g1  | chr1.CM0371.40.nc      | Glyma.16G039500.1      | Transferase family                      | 21                       | 432                   | -4.7098             |
| TR33284 c0_g1  | chr1.CM0410.130.nc     | Glyma.18G268100.1      | Transferase family                      | 2499                     | 8136                  | -2.0594             |
|                |                        |                        |   |                          |                       | 4                   |
| TR34067 c0_g2  | LjSGA_053613.2         | Glyma.10G062200.1      | UDP-glucosyl transferase 73C (UGT)      | 0                        | 16.8                  | -7.2859             |
| TR48968 c0_g1  | chr1.CM0012.680.nd     | Glyma.19G187000.1      | UDP-glucosyl transferase 73C (UGT)      | 164                      | 5940                  | -5.5340             |

**Table S14.** Yellow Stage Trinity Results: Genes for enzymes annotated to GO:0009813 (Flavonoid biosynthetic process, FDR=1). The first two columns list differentially expressed (FDR<0.05) Trinity contigs and gene names as annotated by BLASTing the *Lotus japonicus* genome. The third column is their soybean BLAST equivalent, followed by expression at two stages of flower development.

| Trinity contig | <i>L. japonicus</i> ID | <i>G. max</i> BLAST ID | Gene description  | Counts per gene (Yellow) | Counts per gene (Red) | Log <sub>2</sub> FC |
|----------------|------------------------|------------------------|---|--------------------------|-----------------------|---------------------|
| TR27395 c0_g1  | chr1.CM0122.1600.r2.m  | Glyma.03G222100.2      | Nitrogen regulatory protein P-II                                      | 119                      | 14                    | 2.7213              |
| TR4932 c0_g1   | chr1.CM0476.410.r2.a   | Glyma.04G227700.1      | Quercetin 3-O-methyltransferase (3-OMT)                               | 5616                     | 1315                  | 1.7378              |
| TR4801 c6_g1   | LjSGA_035363.1         | Glyma.05G052000.1      | Cytochrome P450   | 57                       | 0                     | 8.6691              |
| TR31484 c0_g1  | chr1.CM0064.910.r2.m   | Glyma.05G076300.1      | NA  | 1020                     | 163                   | 2.2883              |
| TR35194 c0_g1  | chr3.CM0452.240.r2.d   | Glyma.08G220200.1      | Shikimate O-hydroxycinnamoyltransferase (HCT)                         | 8894                     | 1190                  | 2.5452              |
| TR34244 c0_g1  | LjSGA_050386.1         | Glyma.09G186300.1      | Cytochrome P450 CYP2 subfamily  | 74                       | 14                    | 2.0370              |
| TR32422 c0_g1  | chr5.CM1125.790.r2.a   | Glyma.10G026000.1      | EGL3 (ENHANCER OF GLABRA3), DNA BINDING / TRANSCRIPTION FACTOR (EGL3) | 131.18                   | 0                     | 9.8676              |
| TR66030 c0_g1  | chr1.CM0012.990.r2.m   | Glyma.10G053500.1      | AUX/IAA family // B3 DNA binding domain // Auxin response factor      | 169                      | 23                    | 2.5151              |
| TR34067 c0_g1  | chr5.CM1077.590.r2.m   | Glyma.10G062200.1      | UDP-glucosyl transferase 73C (UGT73C)                                 | 170                      | 0.2                   | 10.2433             |
| TR34449 c0_g1  | chr3.LjT10E18.60.r2.m  | Glyma.12G076200.1      | AUX/IAA family // B3 DNA binding domain // Auxin response factor      | 265                      | 29                    | 2.8306              |
| TR46051 c0_g2  | LjSGA_013806.1         | Glyma.12G109800.1      | Quercetin 3-O-methyltransferase (3-OMT)                               | 4923.93                  | 244.53                | 3.9718              |

| Trinity contig | <i>L. japonicus</i> ID | <i>G. max</i> BLAST ID | Gene description                               | Counts per gene (Yellow) | Counts per gene (Red) | Log <sub>2</sub> FC |
|----------------|------------------------|------------------------|--|--------------------------|-----------------------|---------------------|
| TR28709 c0_g1  | chr4.CM0432.2880.r2.m  | Glyma.13G173300.1      | O-METHYLTRANSFERASE (OMT)                      | 2026.03                  | 168.18                | 3.2347              |
| TR28709 c0_g2  | chr4.CM0432.2880.r2.m  | Glyma.13G173300.1      | O-METHYLTRANSFERASE (OMT)                      | 231.97                   | 25.82                 | 2.7958              |
| TR39181 c0_g1  | chr3.CM0106.330.r2.m   | Glyma.13G344700.1      | B-box zinc finger                              | 625                      | 32                    | 3.9265              |
| TR30560 c0_g1  | chr3.LjT40P18.80.r2.m  | Glyma.13G371000.1      | Shikimate O-hydroxycinnamoyltransferase (HCT)  | 259.39                   | 14.83                 | 3.7436              |
| TR30560 c0_g2  | chr3.LjT40P18.80.r2.m  | Glyma.13G371000.1      | Shikimate O-hydroxycinnamoyltransferase (HCT)  | 80.61                    | 7.17                  | 3.1560              |
| TR3240 c0_g1   | LjSGA_097658.1         | Glyma.15G154100.1      | Flavanone 3-hydroxylase (F3H)                  | 1520                     | 83                    | 3.8365              |
| TR11418 c0_g1  | LjSGA_027597.1         | Glyma.15G221300.1      | Flavonol 3-O-glucosyltransferase (UGT)         | 1126                     | 23                    | 5.2502              |
| TR24510 c0_g2  | LjSGA_071841.2         | Glyma.15G221300.1      | Flavonol 3-O-glucosyltransferase (UGT)         | 134                      | 0                     | 9.9003              |
| TR20072 c0_g1  | chr6.CM0013.1540.r2.a  | Glyma.16G073000.1      | myb proto-oncogene protein, plant              | 315                      | 0                     | 11.1325             |
| TR4801 c4_g1   | LjSGA_035363.1         | Glyma.17G134100.1      | Cytochrome P450 CYP2 subfamily                 | 77                       | 0                     | 9.1021              |
| TR26322 c0_g1  | chr1.CM0410.460.r2.m   | Glyma.18G268100.1      | Transferase family                             | 534                      | 104                   | 2.0025              |
| TR50011 c0_g1  | chr1.CM0104.1140.r2.m  | Glyma.19G105100.1      | Naringenin-chalcone synthase (CHS)             | 24259                    | 5817                  | 1.7036              |
| TR19685 c0_g1  | LjSGA_045968.2         | Glyma.20G180000.1      | B3 DNA binding domain // Auxin response factor | 26.01                    | 0                     | 7.5409              |

**Table S15.** Similarly Expressed Trinity Results: Genes for enzymes annotated to GO:0009813 (Flavonoid biosynthetic process, FDR=1). The first two columns list Trinity contigs expresand gene names as annotated by BLASTing the *Lotus japonicus* genome. The third column is their soybean BLAST homolog, followed by expression at two stages of flower development.

| Trinity contig | <i>L. japonicus</i> ID | <i>G. max</i> BLAST ID | Gene description                                 | Counts per gene (Yellow) | Counts per gene (Red) | Log <sub>2</sub> FC |
|----------------|------------------------|------------------------|--|--------------------------|-----------------------|---------------------|
| TR43354 c0_g1  | chr5.CM0180.690.r2.m   | Glyma.20G241700.1      | chalcone isomerase ( <i>CHI</i> )                | 61.37                    | 168.88                | -1.6533             |
| TR20726 c0_g1  | LjSGA_018608.1         | Glyma.18G048300.1      | Flavanone 3-hydroxylase ( <i>F3H2</i> )          | 14.78                    | 10.7                  | 0.2720              |
| TR20726 c0_g2  | LjSGA_018608.1         | Glyma.18G048300.1      | Flavanone 3-hydroxylase ( <i>F3H2</i> )          | 4.18                     | 2.31                  | 0.6411              |
| TR9870 c1_g1   | LjSGA_041422.1         | Glyma.08G149800.1      | Flavanone 3-hydroxylase ( <i>F3H3</i> )          | 2.73                     | 0                     | 4.4836              |
| TR31785 c0_g1  | LjSGA_067411.1         | Glyma.02G136200.1      | Flavanone 3-dioxygenase ( <i>F3H4</i> )          | 1218.06                  | 1201.12               | 0.7287              |
| TR123 c0_g1    | chr2.CM0191.680.r2.m   | Glyma.04G227700.1      | Quercetin 3-O-methyltransferase ( <i>3-OMT</i> ) | 0.27                     | 1.84                  | -2.7781             |
| TR31193 c0_g1  | chr4.CM0227.690.r2.m   | Glyma.02G104700.1      | Flavonol 3-O-glucosyltransferase ( <i>UFGT</i> ) | 3.44                     | 1.08                  | 1.5431              |
| TR45767 c0_g1  | chr4.CM1616.680.r2.m   | Glyma.08G062000.1      | Anthocyanidin reductase ( <i>ANR</i> )           | 0.46                     | 2.7                   | -2.5202             |
| TR3325 c1_g1   | chr2.CM0021.2950.r2.m  | Glyma.01G215400.1      | 3,5-epimerase/4-reductase ( <i>GME</i> )         | 9.01                     | 12.07                 | -0.6159             |
| TR18411 c0_g1  | LjSGA_047295.1         | Glyma.01G096600.1      | transcription factor MYC2                        | 24.74                    | 7.74                  | 1.4663              |
| TR38249 c0_g1  | chr4.CM0007.1140.r2.m  | Glyma.05G127300.2      | 26S proteasome regulatory subunit N12            | 62.23                    | 63.42                 | -0.2245             |

| Trinity contig | <i>L. japonicus</i> ID | <i>G. max</i> BLAST ID | Gene description   | Counts per gene (Yellow) | Counts per gene (Red) | Log <sub>2</sub> FC |
|----------------|------------------------|------------------------|--|--------------------------|-----------------------|---------------------|
| TR34052 c0_g1  | chr2.CM0608.560.r2.m   | Glyma.02G107900.1      | RIBONUCLEASE T2  | 0                        | 5.01                  | -5.5424             |
| TR17827 c0_g1  | LjSGA_052002.1         | Glyma.19G187500.1      | UDP-glucosyl transferase 73C<br>( <i>UGT73C</i> )              | 3.21                     | 3.48                  | -0.3123             |
| TR62356 c1_g1  | chr5.CM0052.810.r2.m   | Glyma.02G081000.1      | Sterol 3-beta-glucosyltransferase                              | 13.47                    | 31.35                 | -1.4669             |
| TR62356 c1_g3  | chr5.CM0052.810.r2.m   | Glyma.10G224000.1      | SPHINGOMYELIN SYNTHETASE // SUBFAMILY NOT NAMED                | 13.29                    | 14.18                 | -0.3049             |
| TR35590 c0_g1  | chr2.CM0124.30.r2.m    | Glyma.02G100700.1      | STEROL REGULATORY ELEMENT-BINDING PROTEIN                      | 1.18                     | 1.53                  | -0.3171             |
| TR5549 c0_g1   | chr2.CM0124.30.r2.m    | Glyma.02G100700.1      | STEROL REGULATORY ELEMENT-BINDING PROTEIN                      | 0.42                     | 2.39                  | -2.5963             |
| TR26623 c0_g1  | chr2.CM0177.810.r2.m   | Glyma.09G204500.1      | transcription factor MYC2                                      | 24.75                    | 22.14                 | -0.0484             |
| TR61787 c0_g1  | LjSGA_091313.0.1       | Glyma.02G136000.1      | ATP-dependent Clp protease adaptor protein ClpS                | 5.78                     | 16.45                 | -1.5641             |
| TR32422 c0_g2  | chr5.CM1125.790.r2.a   | Glyma.10G026000.1      | EGL3 (ENHANCER OF GLABRA3), DNA BINDING / TRANSCRIPTION FACTOR | 49.62                    | 66.36                 | -0.6287             |

| Trinity contig | <i>L. japonicus</i> ID | <i>G. max</i> BLAST ID | Gene description   | Counts per gene (Yellow) | Counts per gene (Red) | Log <sub>2</sub> FC |
|----------------|------------------------|------------------------|--|--------------------------|-----------------------|---------------------|
| TR40782 c0_g1  | chr3.CM0136.10.r2.m    | Glyma.11G145500.1      | AUX/IAA family // B3 DNA binding domain // Auxin response factor | 5.23                     | 0                     | 4.8824              |
| TR7528 c0_g1   | chr3.CM0136.10.r2.m    | Glyma.11G145500.1      | AUX/IAA family // B3 DNA binding domain // Auxin response factor | 4.6                      | 0                     | 4.4836              |
| TR20842 c1_g1  | LjSGA_014979.1         | Glyma.18G104100.1      | Transferase family   | 1731.14                  | 1737.77               | 1.4098              |
| TR19685 c0_g2  | LjSGA_045968.2         | Glyma.20G180000.1      | B3 DNA binding domain // Auxin response factor                   | 6.87                     | 3.12                  | 0.9419              |
| TR14793 c0_g1  | LjSGA_111580.1         | Glyma.13G082700.1      | Thaumatin family   | 3.54                     | 0.6                   | 2.3293              |
| TR14793 c1_g1  | LjSGA_111580.1         | Glyma.14G163700.1      | Thaumatin family   | 4.99                     | 0                     | 3.9305              |
| TR47026 c0_g1  | chr1.CM1911.100.r2.m   | Glyma.03G122000.1      | coumaroylquinate(coumaroylshikimate) 3'-monooxygenase            | 38.65                    | 16.98                 | 0.9787              |
| TR47026 c0_g2  | chr1.CM1911.100.r2.m   | Glyma.03G122000.1      | coumaroylquinate(coumaroylshikimate) 3'-monooxygenase            | 182.35                   | 58.95                 | 1.4244              |
| TR38719 c0_g1  | LjSGA_024944.1         | Glyma.10G210600.1      | B3 DNA binding domain // Auxin response factor                   | 1.24                     | 0                     | 4.4836              |
| TR38719 c0_g2  | LjSGA_024944.1         | Glyma.10G210600.1      | B3 DNA binding domain // Auxin response factor                   | 13.28                    | 5.31                  | 1.1199              |
| TR52576 c1_g1  | chr5.CM0077.120.r2.m   | NA                     | NA   | 2.08                     | 1.34                  | 0.5912              |
| TR65913 c1_g1  | chr2.CM0191.680.r2.m   | NA                     | NA   | 1.2                      | 9.4                   | -2.7781             |

| Trinity contig | <i>L. japonicus</i> ID | <i>G. max</i> BLAST ID | Gene description | Counts per gene (Yellow) | Counts per gene (Red) | Log <sub>2</sub> FC |
|----------------|------------------------|------------------------|------------------|--------------------------|-----------------------|---------------------|
| TR38780 c0_g2  | chr5.CM0180.670.r2.m   | NA                     | NA               | 4.1                      | 8.72                  | -1.2662             |
| TR55653 c1_g1  | LjSGA_022904.1         | NA                     | NA               | 4.66                     | 2.09                  | 1.1443              |
| TR62356 c0_g1  | chr5.CM0052.810.r2.m   | NA                     | NA               | 13.82                    | 12.13                 | 0.0737              |
| TR42787 c0_g1  | chr1.CM0104.1140.r2.m  | NA                     | NA               | 2.72                     | 2.5                   | 0.0564              |
| TR17090 c0_g1  | LjSGA_020776.1.1       | NA                     | NA               | 2.29                     | 0                     | 4.4836              |
| TR41909 c0_g1  | LjSGA_020776.1.1       | NA                     | NA               | 6.82                     | 0                     | 3.9305              |
| TR31193 c1_g1  | chr4.CM0227.690.r2.m   | NA                     | NA               | 2.89                     | 0                     | 3.9305              |
| TR37424 c0_g1  | chr4.CM0227.690.r2.m   | NA                     | NA               | 2.8                      | 1.11                  | 1.1847              |
| TR13846 c0_g1  | chr3.CM0136.10.r2.m    | NA                     | NA               | 7.76                     | 0                     | 6.1747              |
| TR27061 c0_g1  | chr6.LjT09C09.100.r2.a | NA                     | NA               | 1.64                     | 1.03                  | 0.5912              |
| TR30767 c0_g1  | chr6.LjT09C09.100.r2.a | Glyma.20G207700.1      | NA               | 4.24                     | 0                     | 4.4836              |
| TR40275 c0_g1  | chr6.LjT09C09.100.r2.a | Glyma.02G004200.1      | NA               | 3.84                     | 0                     | 3.93057             |
| TR19889 c1_g1  | chr2.CM0018.760.r2.m   | NA                     | NA               | 52.86                    | 99.33                 | -0.8652             |
| TR25290 c0_g1  | chr5.CM0180.690.r2.m   | NA                     | NA               | 1.11                     | 3.35                  | -1.6119             |
| TR12068 c0_g1  | chr5.CM0052.810.r2.m   | NA                     | NA               | 0.6                      | 4.36                  | -2.7781             |
| TR48553 c0_g1  | chr5.CM1125.790.r2.a   | NA                     | NA               | 1.45                     | 3.57                  | -1.2976             |
| TR46080 c0_g1  | LjSGA_040150.1         | NA                     | NA               | 1.33                     | 3.88                  | -1.7486             |
| TR46080 c0_g2  | LjSGA_040150.1         | NA                     | NA               | 5.24                     | 5.38                  | -0.2436             |

| Trinity contig | <i>L. japonicus</i> ID | <i>G. max</i> BLAST ID | Gene description | Counts per gene (Yellow) | Counts per gene (Red) | Log <sub>2</sub> FC |
|----------------|------------------------|------------------------|------------------|--------------------------|-----------------------|---------------------|
| TR39677 c1_g1  | LjSGA_092302.1         | NA                     | NA               | 1.44                     | 1.4                   | -0.1316             |
| TR28458 c1_g1  | LjSGA_054002.1         | NA                     | NA               | 1.82                     | 0                     | 3.9305              |
| TR51898 c0_g1  | chr2.CM0124.30.r2.m    | NA                     | NA               | 0.46                     | 2.38                  | -2.4460             |
| TR3728 c0_g1   | chr1.CM0476.410.r2.a   | NA                     | NA               | 2.45                     | 3.5                   | -0.31712            |
| TR61647 c0_g1  | chr5.CM0200.3220.r2.m  | NA                     | NA               | 68.47                    | 112.77                | -0.9246             |
| TR5553 c0_g1   | chr3.CM0452.240.r2.d   | NA                     | NA               | 2.23                     | 3.14                  | -0.3171             |
| TR60161 c0_g1  | chr1.CM0104.1140.r2.m  | NA                     | NA               | 2.64                     | 5.23                  | -0.8954             |

**Table S16.** Yellow Stage Trinity Results: Genes for enzymes annotated to the over-represented GO:000 (Lignin biosynthetic process, FDR=). The first two columns list differentially expressed (FDR<0.05) Trinity contigs and gene names as annotated by BLASTing the *Lotus japonicus* genome. The third column is their soybean BLAST homolog, followed by expression at two stages of flower development.

| Trinity contig | <i>L. japonicus</i> ID | <i>G. max</i> BLAST ID | Gene description  | Counts per gene (Yellow) | Counts per gene (Red) | Log <sub>2</sub> FC |
|----------------|------------------------|------------------------|---|--------------------------|-----------------------|---------------------|
| TR4932 c0_g1   | chr1.CM0476.410.r2.a   | Glyma.04G227700.1      | Quercetin 3-O-methyltransferase (-3-OMT)                    | 5616                     | 1315                  | 1.7378              |
| TR4801 c6_g1   | LjSGA_035363.1         | Glyma.05G052000.1      | Cytochrome P450   | 57                       | 0                     | 8.6691              |
| TR37863 c1_g1  | LjSGA_024849.1         | Glyma.07G023700.1      | cinnamoyl-CoA reductase (CCR)                               | 24                       | 1                     | 4.0867              |
| TR47015 c0_g1  | LjSGA_040039.1         | Glyma.07G026300.1      | NAD DEPENDENT EPIMERASE/DEHYD RATASE // SUBFAMILY NOT NAMED | 239                      | 22                    | 3.0785              |
| TR25023 c0_g1  | LjSGA_027787.1         | Glyma.07G050600.1      | No apical meristem (NAM) protein                            | 69                       | 0                     | 8.9441              |
| TR8127 c0_g1   | chr4.CM0387.960.r2.m   | Glyma.07G258700.1      | beta-glucosidase  | 118                      | 1                     | 6.3777              |
| TR35194 c0_g1  | chr3.CM0452.240.r2.d   | Glyma.08G220200.1      | Shikimate O-hydroxycinnamoyltransferase (HCT)               | 8894                     | 1190                  | 2.5452              |
| TR5179 c0_g1   | chr1.CM0017.470.r2.a   | Glyma.08G362900.1      | Transaldolase.  | 4763                     | 939                   | 1.9860              |
| TR46051 c0_g2  | LjSGA_013806.1         | Glyma.12G109800.1      | caffeoyle-CoA O-methyltransferase (CCoAOMT)                 | 536                      | 10                    | 5.3722              |
| TR30560 c0_g1  | chr3.LjT40P18.80.r2.m  | Glyma.13G371000.1      | Quercetin 3-O-methyltransferase (-3-OMT)                    | 4923.93                  | 244.53                | 3.9718              |
| TR30560 c0_g2  | chr3.LjT40P18.80.r2.m  | Glyma.13G371000.1      | Shikimate O-hydroxycinnamoyltransferase (HCT)               | 259.39                   | 14.83                 | 3.7436              |
| TR34012 c0_g1  | chr3.LjT45M09.50.r2.d  | Glyma.15G003600.1      | Shikimate O-hydroxycinnamoyltransferase (HCT)               | 80.61                    | 7.17                  | 3.1560              |

| Trinity contig | <i>L. japonicus</i> ID | <i>G. max</i> BLAST ID | Gene description                     | Counts per gene (Yellow) | Counts per gene (Red) | Log <sub>2</sub> FC |
|----------------|------------------------|------------------------|--------------------------------------|--------------------------|-----------------------|---------------------|
| TR49520 c0_g1  | chr6.CM0385.240.r2.d   | Glyma.15G143600.1      | cinnamoyl-CoA reductase (CCR)        | 2562                     | 398                   | 2.3295              |
| TR36532 c0_g1  | chr6.LjT35D18.30.r2.m  | Glyma.15G190500.1      | CHITINASE                            | 1112                     | 7                     | 6.9328              |
| TR4801 c4_g1   | LjSGA_035363.1         | Glyma.17G134100.1      | Methionine adenosyltransferase (MAT) | 19644                    | 3001                  | 2.3539              |
| TR38742 c0_g1  | LjSGA_021886.2         | Glyma.17G171100.1      | Cytochrome P450 CYP2 subfamily       | 77                       | 0                     | 9.1021              |
| TR61337 c0_g1  | chr5.CM0200.3250.r2.m  | Glyma.20G128600.1      | cinnamyl-alcohol dehydrogenase (CAD) | 138.5                    | 20.92                 | 2.3536              |
| TR61337 c0_g3  | chr5.CM0200.3250.r2.m  | Glyma.20G128600.1      | cinnamyl-alcohol dehydrogenase (CAD) | 47.87                    | 0                     | 8.4218              |
| TR61337 c0_g2  | chr5.CM0200.3250.r2.m  | Glyma.20G128600.1      | cinnamyl-alcohol dehydrogenase (CAD) | 4056.51                  | 588.62                | 2.4273              |

**Table S17.** Red Stage Trinity Results: Genes for enzymes annotated to the over-represented GO:000 (Lignin biosynthetic process, FDR=). The first two columns list differentially expressed (FDR<0.05) Trinity contigs and gene names as annotated by BLASTing the *Lotus japonicus* genome. The third column is their soybean BLAST homolog, followed by expression at two stages of flower development.

| Trinity contig | <i>L. japonicus</i> ID | <i>G. max</i> BLAST ID | Gene description                             | Counts per gene (Yellow) | Counts per gene (Red) | Log <sub>2</sub> FC |
|----------------|------------------------|------------------------|--|--------------------------|-----------------------|---------------------|
| TR24303 c0_g1  | LjSGA_128268.1         | Glyma.01G177900.1      | coniferyl-alcohol glucosyltransferase (CAGT) | 60                       | 761                   | -4.0182             |
| TR13393 c0_g1  | LjSGA_030253.1         | Glyma.03G184000.1      | Methionine adenosyltransferase (MAT)         | 1894                     | 5353                  | -1.8553             |
| TR1381 c0_g1   | chr1.CM0105.600.nc     | Glyma.07G048700.1      | Quercetin 3-O-methyltransferase (3-OMT)      | 4                        | 126                   | -5.2853             |
| TR14130 c0_g1  | chr1.CM0063.200.nc     | Glyma.08G010500.1      | Transferase family                           | 1                        | 29                    | -5.0304             |
| TR4013 c1_g3   | LjSGA_011490.1         | Glyma.12G019700.1      | Cinnamyl-alcohol dehydrogenase (CAD)         | 8                        | 172                   | -4.7586             |
| TR45798 c0_g1  | chr1.CM0371.40.nc      | Glyma.16G039500.1      | Transferase family                           | 21                       | 432                   | -4.7098             |

**Table S18.** Similarly Expressed Trinity Results: Genes for enzymes annotated to GO:0009813 (Lignin process, FDR=1). The first two columns list Trinity contigs expresand gene names as annotated by BLASTing the *Lotus japonicus* genome. The third column is their soybean BLAST homolog, followed by expression at two stages of flower development.

| Trinity contig | <i>L. japonicus</i> ID | <i>G. max</i> BLAST ID | Gene description                     | Counts per gene<br>(Yellow) | Counts per gene<br>(Red) | Log <sub>2</sub> FC |
|----------------|------------------------|------------------------|--------------------------------------|-----------------------------|--------------------------|---------------------|
| TR4638 c1_g1   | chr2.CM0249.1510.r2.m  | Glyma.11G069600.1      | LACCASE                              | 0.49                        | 0.28                     | 0.5912              |
| TR13756 c0_g1  | chr6.CM0084.30.r2.m    | Glyma.18G065100.1      | LACCASE                              | 8.82                        | 0                        | 10.251              |
| TR2439 c0_g1   | chr1.CM2049.30.r2.m    | Glyma.18G193400.1      | LACCASE                              | 3.37                        | 0.6                      | 2.2824              |
| TR35330 c1_g1  | chr1.CM2049.30.r2.m    | Glyma.18G193200.1      | LACCASE                              | 3.99                        | 0.59                     | 2.5184              |
| TR48670 c0_g2  | chr1.CM2049.30.r2.m    | Glyma.07G142400.1      | LACCASE                              | 2.73                        | 0                        | 5.6687              |
| TR34461 c0_g1  | chr1.CM0147.370.r2.m   | Glyma.08G359100.1      | LACCASE                              | 7.9                         | 0                        | 6.4344              |
| TR34461 c1_g1  | chr1.CM0147.370.r2.m   | Glyma.18G177400.1      | LACCASE                              | 4.28                        | 0                        | 8.2967              |
| TR34461 c2_g1  | chr1.CM0147.370.r2.m   | Glyma.07G133900.1      | LACCASE                              | 12.03                       | 0                        | 5.6687              |
| TR13756 c0_g2  | chr6.CM0084.30.r2.m    | Glyma.18G065100.1      | LACCASE                              | 0                           | 1.77                     | -6.5269             |
| TR65076 c0_g1  | chr6.CM0084.30.r2.m    | Glyma.02G224800.1      | LACCASE                              | 0.59                        | 6.62                     | -3.6477             |
| TR62643 c0_g1  | chr3.CM0243.390.r2.m   | Glyma.07G054200.1      | LACCASE                              | 0.59                        | 2.87                     | -2.2060             |
| TR36532 c0_g1  | chr6.LjT35D18.30.r2.m  | Glyma.15G190500.1      | Methionine adenosyltransferase (MAT) | 1483.98                     | 252.36                   | 2.3539              |
| TR45709 c0_g1  | chr6.LjT35D18.30.r2.m  | Glyma.17G039000.1      | Methionine adenosyltransferase (MAT) | 112.08                      | 58.48                    | 0.7446              |
| TR64116 c0_g1  | LjSGA_030253.1         | Glyma.13G141600.1      | Methionine adenosyltransferase (MAT) | 40.05                       | 69.47                    | -0.6148             |
| TR13393 c0_g1  | LjSGA_030253.1         | Glyma.03G184000.1      | Methionine adenosyltransferase (MAT) | 101.29                      | 317.51                   | -1.8553             |

| Trinity contig | <i>L. japonicus</i> ID | <i>G. max</i> BLAST ID | Gene description  | Counts per gene (Yellow) | Counts per gene (Red) | Log <sub>2</sub> FC |
|----------------|------------------------|------------------------|---|--------------------------|-----------------------|---------------------|
| TR24590 c0_g1  | LjSGA_140570.1         | Glyma.13G141600.1      | Methionine adenosyltransferase ( <i>MAT</i> )                         | 21.61                    | 58.23                 | -1.6092             |
| TR13516 c0_g1  | LjSGA_035751.3         | Glyma.07G089700.1      | Cytochrome P450 CYP2 subfamily  | 25.96                    | 16.48                 | 0.4589              |
| TR15440 c0_g1  | LjSGA_035751.3         | Glyma.09G186400.1      | Cytochrome P450 CYP2 subfamily  | 6.11                     | 10.04                 | -0.7962             |
| TR37032 c0_g1  | LjSGA_021748.1.1       | Glyma.07G023700.1      | cinnamoyl-CoA reductase ( <i>CCR</i> )                                | 7.23                     | 1.2                   | 2.4086              |
| TR4013 c1_g1   | LjSGA_073761.1         | Glyma.12G019700.1      | Cinnamyl-alcohol dehydrogenase ( <i>CAD</i> )                         | 24.28                    | 21.27                 | 0.0141              |
| TR39650 c0_g1  | chr2.CM0249.890.r2.m   | Glyma.11G072000.1      | Peroxidase ( <i>POD</i> )   | 5.78                     | 1.47                  | 1.7657              |
| TR39650 c0_g2  | chr2.CM0249.890.r2.m   | Glyma.11G072000.1      | Peroxidase ( <i>POD</i> )   | 0.52                     | 0.01                  | 5.4510              |
| TR10667 c0_g1  | chr4.CM0131.580.r2.m   | Glyma.02G052700.1      | Peroxidase ( <i>POD</i> )   | 7.12                     | 0                     | 4.4836              |
| TR47026 c0_g1  | chr1.CM1911.100.r2.m   | Glyma.03G122000.1      | coumaroylquinate(coumaroylshikimate) 3'-monooxygenase ( <i>C3'H</i> ) | 38.65                    | 16.98                 | 0.9787              |
| TR47026 c0_g2  | chr1.CM1911.100.r2.m   | Glyma.03G122000.1      | coumaroylquinate(coumaroylshikimate) 3'-monooxygenase ( <i>C3'H</i> ) | 182.35                   | 58.95                 | 1.4244              |
| TR31562 c0_g1  | chr1.CM0017.470.r2.a   | Glyma.08G362900.1      | Transaldolase.  | 3.1                      | 0                     | 4.4836              |
|                |                        | Glyma.08G362900.1      | Transaldolase.  | 318.62                   | 69.82                 | 1.9860              |
| TR123 c0_g1    | chr2.CM0191.680.r2.m   | Glyma.04G227700.1      | Quercetin 3-O-methyltransferase ( <i>3-OMT</i> )                      | 0.27                     | 1.84                  | -2.7781             |

| Trinity contig | <i>L. japonicus</i> ID | <i>G. max</i> BLAST ID | Gene description                                     | Counts per gene<br>(Yellow) | Counts per gene<br>(Red) | Log <sub>2</sub> FC |
|----------------|------------------------|------------------------|--|-----------------------------|--------------------------|---------------------|
| TR39944 c0_g1  | chr3.CM0574.80.r2.d    | Glyma.19G182300.1      | Phenylalanine ammonia-lyase ( <i>PAL</i> )           | 1.33                        | 2.63                     | -1.0588             |
| TR49219 c1_g1  | chr3.CM0574.80.r2.d    | Glyma.03G181600.1      | Phenylalanine ammonia-lyase ( <i>PAL</i> )           | 0                           | 16.69                    | -7.1991             |
| TR36182 c0_g1  | chr2.CM0177.130.r2.d   | Glyma.01G020900.1      | L-THREONINE 3-DEHYDROGENASE ( <i>TDH</i> )           | 27.5                        | 43.99                    | -0.8770             |
| TR50128 c0_g2  | chr6.CM0055.420.r2.m   | Glyma.15G059500.1      | L-THREONINE 3-DEHYDROGENASE ( <i>TDH</i> )           | 1.62                        | 3.31                     | -1.2317             |
| TR50128 c0_g1  | LjSGA_070619.1         | Glyma.15G059500.1      | L-THREONINE 3-DEHYDROGENASE ( <i>TDH</i> )           | 15.98                       | 4.57                     | 1.6040              |
| TR34200 c0_g1  | chr6.CM0055.420.r2.m   | Glyma.15G059500.1      | L-THREONINE 3-DEHYDROGENASE ( <i>TDH</i> )           | 5.23                        | 3.64                     | 0.3340              |
| TR63600 c0_g2  | chr6.CM0139.260.r2.m   | Glyma.15G106000.1      | GLYCOSYL HYDROLASE                                   | 2.21                        | 2.27                     | -0.2523             |
| TR63600 c0_g1  | chr6.CM0139.260.r2.m   | Glyma.15G106000.1      | GLYCOSYL HYDROLASE                                   | 1.45                        | 0.87                     | 0.5441              |
| TR53088 c0_g1  | chr1.CM0104.480.r2.a   | Glyma.16G050200.1      | V-type H <sup>+</sup> -transporting ATPase subunit C | 108.06                      | 64.97                    | 0.5319              |
| TR20842 c1_g1  | LjSGA_014979.1         | Glyma.18G104100.1      | Transferase family                                   | 1731.14                     | 1737.77                  | 1.4098              |
| TR40987 c0_g1  | chr6.LjT34E09.150.r2.m | Glyma.13G073200.1      | elongation factor EF-1 beta subunit                  | 114.07                      | 34.18                    | 1.5444              |
| TR12259 c0_g1  | LjSGA_075859.1         | Glyma.20G175500.1      | No apical meristem (NAM) protein                     | 2.65                        | 6.65                     | -1.5147             |

| Trinity contig | <i>L. japonicus</i> ID    | <i>G. max</i> BLAST ID | Gene description       | Counts per gene<br>(Yellow) | Counts per gene<br>(Red) | Log <sub>2</sub> FC |
|----------------|---------------------------|------------------------|------------------------|-----------------------------|--------------------------|---------------------|
| TR21604 c0_g2  | chr2.CM0249.600.r2.d      | Glyma.01G169200.1      | ferulate-5-hydroxylase | 268.84                      | 239.65                   | -0.0414             |
| TR60250 c0_g1  | chr6.LjT35D18.30.r2.m     | NA                     |                        | 5.93                        | 0.89                     | 2.5390              |
| TR40104 c1_g1  | LjSGA_035655.2            | NA                     |                        | 7.01                        | 2.59                     | 1.3203              |
| TR13073 c0_g1  | chr3.CM0792.180.r2.d      | NA                     |                        | 3.01                        | 0                        | 5.6687              |
| TR32183 c1_g1  | chr3.CM0792.180.r2.d      | NA                     |                        | 3.99                        | 1.27                     | 1.5431              |
| TR54702 c0_g1  | chr3.CM0792.180.r2.d      | NA                     |                        | 6.21                        | 0                        | 4.8824              |
| TR42774 c0_g1  | chr2.CM0249.600.r2.d      | NA                     |                        | 5.72                        | 3.81                     | 0.6162              |
| TR13250 c0_g1  | chr6.CM0139.240.r2.m      | NA                     |                        | 2.3                         | 0                        | 5.6687              |
| TR26810 c0_g1  | chr6.CM0139.240.r2.m      | NA                     |                        | 9.59                        | 0                        | 5.1945              |
| TR26810 c1_g1  | chr6.CM0139.240.r2.m      | NA                     |                        | 4.9                         | 0                        | 3.9305              |
| TR35810 c0_g1  | chr6.CM0139.240.r2.m      | NA                     |                        | 3.96                        | 0.52                     | 2.6856              |
| TR8127 c0_g2   | chr6.CM0139.240.r2.m      | NA                     |                        | 10.45                       | 0                        | 5.4510              |
| TR34627 c0_g1  | chr1.CM0023.30.r2.d       | NA                     |                        | 1.62                        | 0.95                     | 0.6162              |
| TR61337 c0_g2  | chr5.CM0200.3250.r2.<br>m | Glyma.20G128600.1      |                        | 11.69                       | 1.97                     | 2.3536              |
| TR61337 c0_g4  | chr5.CM0200.3250.r2.<br>m | Glyma.20G128600.1      |                        | 5.23                        | 4.36                     | 0.0739              |
| TR60726 c0_g1  | chr4.CM0042.2100.r2.<br>m | NA                     |                        | 5.45                        | 0                        | 5.19459             |
| TR34461 c3_g1  | chr1.CM0147.370.r2.m      | NA                     |                        | 6.89                        | 0                        | 4.4836              |

| Trinity contig | <i>L. japonicus</i> ID | <i>G. max</i> BLAST ID | Gene description | Counts per gene<br>(Yellow) | Counts per gene<br>(Red) | Log <sub>2</sub> FC |
|----------------|------------------------|------------------------|------------------|-----------------------------|--------------------------|---------------------|
| TR10653 c0_g1  | chr3.LjT45M09.50.r2.d  | NA                     |                  | 2.36                        | 1.12                     | 0.9283              |
| TR19271 c0_g1  | chr3.LjT45M09.50.r2.d  | NA                     |                  | 5.23                        | 0                        | 4.8824              |
| TR65913 c1_g1  | chr2.CM0191.680.r2.m   | NA                     |                  | 1.2                         | 9.4                      | -2.7781             |
| TR36166 c0_g1  | LjSGA_035751.3         | NA                     |                  | 0                           | 4.07                     | -4.2662             |
| TR5553 c0_g1   | chr3.CM0452.240.r2.d   | NA                     |                  | 2.23                        | 3.14                     | -0.3171             |
| TR37416 c0_g1  | chr6.CM0084.30.r2.m    | NA                     |                  | 0                           | 6.27                     | -5.2282             |
| TR3728 c0_g1   | chr1.CM0476.410.r2.a   | NA                     |                  | 2.45                        | 3.5                      | -0.3171             |
| TR45016 c0_g1  | LjSGA_073761.1         | NA                     |                  | 1.36                        | 3.22                     | -1.3166             |

**Table S19.** Tophat Results: Significantly enriched GO terms corresponding to the red stage of a *L. sessilifolius* flower.

| Enriched GO Term: Description  | Number of associated genes | FDR      |
|--|----------------------------|----------|
| GO:0034654nucleobase, nucleoside, nucleotide and nucleic acid biosynthetic process | 21                         | 5.00E-22 |
| GO:0010035response to inorganic substance  | 36                         | 1.80E-14 |
| GO:0055114oxidation reduction  | 41                         | 1.10E-12 |
| GO:0034641cellular nitrogen compound metabolic process                             | 52                         | 2.20E-12 |
| GO:0046686response to cadmium ion  | 20                         | 4.00E-12 |
| GO:0010038response to metal ion  | 29                         | 4.60E-12 |
| GO:0055085transmembrane transport  | 42                         | 5.40E-12 |
| GO:0018130heterocycle biosynthetic process   | 25                         | 5.30E-11 |
| GO:0019438aromatic compound biosynthetic process                                   | 36                         | 1.90E-09 |
| GO:0006725cellular aromatic compound metabolic process                             | 47                         | 3.30E-09 |
| GO:0009611response to wounding   | 28                         | 4.80E-08 |
| GO:0009718anthocyanin biosynthetic process   | 12                         | 5.60E-08 |
| GO:0044271cellular nitrogen compound biosynthetic process                          | 36                         | 6.50E-08 |
| GO:0031540regulation of anthocyanin biosynthetic process                           | 8                          | 4.50E-07 |
| GO:0046283anthocyanin metabolic process  | 12                         | 7.70E-07 |
| GO:0031537regulation of anthocyanin metabolic process                              | 8                          | 6.70E-06 |
| GO:0034655nucleobase, nucleoside, nucleotide and nucleic acid catabolic process    | 6                          | 9.10E-06 |
| GO:0034656nucleobase, nucleoside and nucleotide catabolic process                  | 6                          | 9.10E-06 |
| GO:0042221response to chemical stimulus  | 104                        | 1.10E-05 |
| GO:0046483heterocycle metabolic process  | 40                         | 1.40E-05 |
| GO:0009962regulation of flavonoid biosynthetic process                             | 9                          | 2.00E-05 |
| GO:0048610reproductive cellular process  | 9                          | 5.90E-05 |
| GO:0048868pollen tube development  | 12                         | 6.50E-05 |
| GO:0009605response to external stimulus  | 42                         | 6.70E-05 |
| GO:0009753response to jasmonic acid stimulus                                       | 23                         | 0.00012  |
| GO:0009651response to salt stress  | 27                         | 0.00015  |
| GO:0006970response to osmotic stress   | 31                         | 0.00019  |
| GO:0046148pigment biosynthetic process   | 16                         | 0.00059  |
| GO:0042440pigment metabolic process  | 17                         | 0.00085  |
| GO:0010039response to iron ion   | 6                          | 0.001    |
| GO:0031668cellular response to extracellular stimulus                              | 13                         | 0.0016   |
| GO:0005984disaccharide metabolic process   | 9                          | 0.0023   |
| GO:0016036cellular response to phosphate starvation                                | 8                          | 0.0025   |
| GO:0006811ion transport  | 35                         | 0.0033   |

| Enriched GO Term: Description                                 | Number of associated genes | FDR    |
|---|----------------------------|--------|
| GO:0006814sodium ion transport                                | 6                          | 0.0033 |
| GO:0035295tube development                                    | 12                         | 0.0035 |
| GO:0016137glycoside metabolic process                         | 11                         | 0.0035 |
| GO:0042398cellular amino acid derivative biosynthetic process | 23                         | 0.0037 |
| GO:0009813flavonoid biosynthetic process                      | 15                         | 0.0041 |
| GO:0043455regulation of secondary metabolic process           | 9                          | 0.0042 |
| GO:0031669cellular response to nutrient levels                | 11                         | 0.0043 |
| GO:0009860pollen tube growth                                  | 9                          | 0.0043 |
| GO:0009719response to endogenous stimulus                     | 57                         | 0.0051 |
| GO:0006820anion transport                                     | 13                         | 0.0058 |
| GO:0060918auxin transport                                     | 10                         | 0.0058 |
| GO:0009812flavonoid metabolic process                         | 15                         | 0.0066 |
| GO:0046700heterocycle catabolic process                       | 8                          | 0.0085 |
| GO:0015698inorganic anion transport                           | 9                          | 0.0087 |
| GO:0010224response to UV-B                                    | 9                          | 0.01   |
| GO:0010033response to organic substance                       | 64                         | 0.011  |
| GO:0006595polyamine metabolic process                         | 5                          | 0.012  |
| GO:0042742defense response to bacterium                       | 17                         | 0.012  |
| GO:0009311oligosaccharide metabolic process                   | 9                          | 0.013  |
| GO:0009310amine catabolic process                             | 9                          | 0.013  |
| GO:0006979response to oxidative stress                        | 25                         | 0.013  |
| GO:0009737response to abscisic acid stimulus                  | 26                         | 0.013  |
| GO:0009914hormone transport                                   | 10                         | 0.013  |
| GO:0009932cell tip growth                                     | 10                         | 0.018  |
| GO:0009267cellular response to starvation                     | 9                          | 0.019  |
| GO:0009926auxin polar transport                               | 9                          | 0.019  |
| GO:0019439aromatic compound catabolic process                 | 5                          | 0.023  |
| GO:0009856pollination   | 12                         | 0.027  |
| GO:0005991trehalose metabolic process                         | 5                          | 0.028  |
| GO:0009699phenylpropanoid biosynthetic process                | 17                         | 0.032  |
| GO:0006575cellular amino acid derivative metabolic process    | 25                         | 0.035  |
| GO:0009991response to extracellular stimulus                  | 14                         | 0.035  |
| GO:0009116nucleoside metabolic process                        | 8                          | 0.035  |
| GO:0009725response to hormone stimulus                        | 50                         | 0.035  |
| GO:0048588developmental cell growth                           | 10                         | 0.038  |
| GO:0009063cellular amino acid catabolic process               | 8                          | 0.039  |

| Enriched GO Term: Description                                   | Number of associated genes | FDR      |
|---|----------------------------|----------|
| GO:0032549ribonucleoside binding                                | 32                         | 2.40E-34 |
| GO:0016210naringenin-chalcone synthase activity                 | 8                          | 6.70E-06 |
| GO:0016791phosphatase activity                                  | 20                         | 0.0073   |
| GO:0022891substrate-specific transmembrane transporter activity | 43                         | 0.021    |
| GO:0042578phosphoric ester hydrolase activity                   | 21                         | 0.021    |
| GO:0015103inorganic anion transmembrane transporter activity    | 9                          | 0.021    |
| GO:0008509anion transmembrane transporter activity              | 11                         | 0.049    |
| GO:0005773vacuole   | 55                         | 5.40E-23 |
| GO:0005911cell-cell junction                                    | 24                         | 6.70E-23 |
| GO:0030054cell junction   | 24                         | 4.50E-22 |
| GO:0005774vacuolar membrane                                     | 28                         | 1.40E-09 |
| GO:0044437vacuolar part   | 28                         | 2.10E-09 |
| GO:0009941chloroplast envelope                                  | 17                         | 3.40E-06 |
| GO:0005886plasma membrane                                       | 68                         | 4.50E-06 |
| GO:0009705plant-type vacuole membrane                           | 12                         | 1.00E-05 |
| GO:0000325plant-type vacuole                                    | 15                         | 1.20E-05 |
| GO:0048046apoplast  | 18                         | 7.40E-05 |
| GO:0009526plastid envelope                                      | 17                         | 9.00E-05 |
| GO:0016020membrane  | 158                        | 0.00033  |
| GO:0044459plasma membrane part                                  | 27                         | 0.0004   |
| GO:0009570chloroplast stroma                                    | 13                         | 0.015    |
| GO:0005777peroxisome  | 10                         | 0.016    |
| GO:0042579microbody   | 10                         | 0.016    |
| GO:0005802trans-Golgi network                                   | 7                          | 0.017    |
| GO:0005618cell wall   | 25                         | 0.048    |

**Table S20.** Tophat Results: Significantly enriched GO terms corresponding to the yellow stage of a *L. sessilifolius* flower.

| Enriched GO Term: Description  | Number of associated genes | FDR      |
|--|----------------------------|----------|
| GO:0055114oxidation reduction  | 43                         | 2.10E-20 |
| GO:0034654nucleobase, nucleoside, nucleotide and nucleic acid biosynthetic process | 15                         | 1.80E-16 |
| GO:0034641cellular nitrogen compound metabolic process                             | 44                         | 4.80E-14 |
| GO:0018130heterocycle biosynthetic process   | 24                         | 8.80E-14 |
| GO:0044271cellular nitrogen compound biosynthetic process                          | 34                         | 3.10E-11 |
| GO:0046686response to cadmium ion  | 15                         | 8.40E-10 |
| GO:0016053organic acid biosynthetic process  | 34                         | 4.00E-09 |
| GO:0046394carboxylic acid biosynthetic process                                     | 34                         | 4.00E-09 |
| GO:0019438aromatic compound biosynthetic process                                   | 28                         | 8.00E-09 |
| GO:0006725cellular aromatic compound metabolic process                             | 35                         | 4.70E-08 |
| GO:0043648dicarboxylic acid metabolic process                                      | 14                         | 2.80E-07 |
| GO:0043436oxoacid metabolic process  | 46                         | 3.70E-07 |
| GO:0006082organic acid metabolic process   | 46                         | 3.70E-07 |
| GO:0019752carboxylic acid metabolic process  | 46                         | 3.70E-07 |
| GO:0010035response to inorganic substance  | 20                         | 4.20E-07 |
| GO:0042180cellular ketone metabolic process  | 46                         | 5.10E-07 |
| GO:0010038response to metal ion  | 17                         | 1.90E-06 |
| GO:0046483heterocycle metabolic process  | 32                         | 3.20E-06 |
| GO:0009073aromatic amino acid family biosynthetic process                          | 10                         | 3.20E-06 |
| GO:0046417chorismate metabolic process   | 10                         | 3.20E-06 |
| GO:0006633fatty acid biosynthetic process  | 17                         | 3.30E-06 |
| GO:0009072aromatic amino acid family metabolic process                             | 11                         | 8.40E-06 |
| GO:0006631fatty acid metabolic process   | 20                         | 1.30E-05 |
| GO:0008610lipid biosynthetic process   | 28                         | 1.60E-05 |
| GO:0032787monocarboxylic acid metabolic process                                    | 28                         | 2.20E-05 |
| GO:0030244cellulose biosynthetic process   | 8                          | 0.00015  |
| GO:0010025wax biosynthetic process   | 7                          | 0.00018  |
| GO:0010102lateral root morphogenesis   | 9                          | 0.00026  |

| Enriched GO Term: Description                          | Number of associated genes | FDR     |
|--|----------------------------|---------|
| GO:0010101post-embryonic root morphogenesis            | 9                          | 0.00026 |
| GO:0009886post-embryonic morphogenesis                 | 11                         | 0.00042 |
| GO:0010311lateral root formation                       | 6                          | 0.00042 |
| GO:0010166wax metabolic process                        | 7                          | 0.00042 |
| GO:0019439aromatic compound catabolic process          | 6                          | 0.00046 |
| GO:0006073cellular glucan metabolic process            | 14                         | 0.00059 |
| GO:0030243cellulose metabolic process                  | 8                          | 0.00062 |
| GO:0006629lipid metabolic process                      | 36                         | 0.00071 |
| GO:0044255cellular lipid metabolic process             | 31                         | 0.00079 |
| GO:0044042glucan metabolic process                     | 14                         | 0.0011  |
| GO:0009309amine biosynthetic process                   | 15                         | 0.0015  |
| GO:0008652cellular amino acid biosynthetic process     | 14                         | 0.0017  |
| GO:0044262cellular carbohydrate metabolic process      | 26                         | 0.0026  |
| GO:0009250glucan biosynthetic process                  | 10                         | 0.0042  |
| GO:0008299isoprenoid biosynthetic process              | 10                         | 0.0042  |
| GO:0000162tryptophan biosynthetic process              | 5                          | 0.0046  |
| GO:0009664plant-type cell wall organization            | 8                          | 0.0046  |
| GO:0046219indolalkylamine biosynthetic process         | 5                          | 0.0046  |
| GO:0009416response to light stimulus                   | 27                         | 0.0046  |
| GO:0044264cellular polysaccharide metabolic process    | 15                         | 0.0057  |
| GO:0009314response to radiation                        | 27                         | 0.007   |
| GO:0016109tetraterpenoid biosynthetic process          | 5                          | 0.0095  |
| GO:0016117carotenoid biosynthetic process              | 5                          | 0.0095  |
| GO:0042401cellular biogenic amine biosynthetic process | 6                          | 0.012   |
| GO:0006586indolalkylamine metabolic process            | 5                          | 0.014   |
| GO:0016114terpenoid biosynthetic process               | 8                          | 0.014   |
| GO:0006568tryptophan metabolic process                 | 5                          | 0.014   |
| GO:0010015root morphogenesis                           | 12                         | 0.014   |
| GO:0009555pollen development                           | 10                         | 0.014   |
| GO:0042221response to chemical stimulus                | 63                         | 0.014   |

| Enriched GO Term: Description   | Number of associated genes | FDR      |
|---|----------------------------|----------|
| GO:0051273beta-glucan metabolic process   | 5                          | 0.014    |
| GO:0051274beta-glucan biosynthetic process  | 5                          | 0.014    |
| GO:0044106cellular amine metabolic process  | 20                         | 0.015    |
| GO:0010218response to far red light   | 6                          | 0.016    |
| GO:0048527lateral root development  | 9                          | 0.019    |
| GO:0009628response to abiotic stimulus  | 48                         | 0.02     |
| GO:0005976polysaccharide metabolic process  | 16                         | 0.02     |
| GO:0006720isoprenoid metabolic process  | 10                         | 0.021    |
| GO:0033692cellular polysaccharide biosynthetic process  | 11                         | 0.023    |
| GO:0048528post-embryonic root development   | 9                          | 0.024    |
| GO:0042435indole derivative biosynthetic process  | 5                          | 0.029    |
| GO:0034285response to disaccharide stimulus   | 5                          | 0.029    |
| GO:0006520cellular amino acid metabolic process   | 19                         | 0.032    |
| GO:0000271polysaccharide biosynthetic process   | 11                         | 0.036    |
| GO:0016108tetraterpenoid metabolic process  | 5                          | 0.037    |
| GO:0016116carotenoid metabolic process  | 5                          | 0.037    |
| GO:0042434indole derivative metabolic process   | 5                          | 0.039    |
| GO:0042430indole and derivative metabolic process   | 5                          | 0.039    |
| GO:0030258lipid modification  | 6                          | 0.039    |
| GO:0019321pentose metabolic process   | 5                          | 0.048    |
| GO:0048229gametophyte development   | 13                         | 0.048    |
| GO:0034440lipid oxidation   | 5                          | 0.049    |
| GO:0032549ribonucleoside binding  | 27                         | 3.90E-32 |
| GO:0051213dioxygenase activity  | 10                         | 5.30E-05 |
| GO:0016835carbon-oxygen lyase activity  | 11                         | 0.0075   |
| GO:0005507copper ion binding  | 11                         | 0.0077   |
| GO:0016705oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen | 13                         | 0.025    |
| GO:0043167ion binding   | 81                         | 0.025    |
| GO:0016760cellulose synthase (UDP-forming) activity   | 5                          | 0.025    |

| Enriched GO Term: Description            | Number of associated genes | FDR      |
|--|----------------------------|----------|
| GO:0044434chloroplast part               | 69                         | 1.80E-35 |
| GO:0009941chloroplast envelope           | 41                         | 5.80E-35 |
| GO:0044435plastid part                   | 70                         | 4.20E-33 |
| GO:0009526plastid envelope               | 43                         | 5.30E-33 |
| GO:0009570chloroplast stroma             | 43                         | 1.10E-32 |
| GO:0005911cell-cell junction             | 27                         | 4.30E-32 |
| GO:0030054cell junction                  | 27                         | 4.90E-31 |
| GO:0009532plastid stroma                 | 43                         | 9.40E-30 |
| GO:0005886plasma membrane                | 70                         | 3.20E-15 |
| GO:0031967organelle envelope             | 44                         | 2.00E-14 |
| GO:0031975envelope                       | 44                         | 8.00E-14 |
| GO:0009507chloroplast                    | 81                         | 2.40E-12 |
| GO:0009536plastid                        | 83                         | 4.50E-11 |
| GO:0009534chloroplast thylakoid          | 26                         | 8.10E-11 |
| GO:0031976plastid thylakoid              | 26                         | 3.80E-10 |
| GO:0031984organelle subcompartment       | 26                         | 5.50E-10 |
| GO:0044459plasma membrane part           | 30                         | 2.00E-09 |
| GO:0048046apoplast                       | 20                         | 3.60E-09 |
| GO:0009579thylakoid                      | 28                         | 5.00E-07 |
| GO:0016020membrane                       | 121                        | 9.10E-07 |
| GO:0009535chloroplast thylakoid membrane | 18                         | 4.00E-06 |
| GO:0055035plastid thylakoid membrane     | 18                         | 1.10E-05 |
| GO:0044446intracellular organelle part   | 95                         | 1.20E-05 |
| GO:0044422organelle part                 | 95                         | 1.20E-05 |
| GO:0005576extracellular region           | 28                         | 2.00E-05 |
| GO:0042651thylakoid membrane             | 18                         | 4.00E-05 |
| GO:0044436thylakoid part                 | 19                         | 4.70E-05 |
| GO:0005618cell wall                      | 23                         | 0.00072  |
| GO:0005802trans-Golgi network            | 7                          | 0.0012   |
| GO:0005773vacuole                        | 17                         | 0.0019   |

| Enriched GO Term: Description              | Number of associated genes | FDR    |
|--|----------------------------|--------|
| GO:0030312external encapsulating structure | 23                         | 0.002  |
| GO:0034357photosynthetic membrane          | 18                         | 0.0025 |
| GO:0044444cytoplasmic part                 | 135                        | 0.0026 |
| GO:0010287plastoglobule                    | 6                          | 0.0081 |
| GO:0022626cytosolic ribosome               | 9                          | 0.02   |

**Table S21.** Red Stage TopHat Results: Genes for enzymes annotated to GO:0009813 (Flavonoid biosynthetic process, FDR = 0.0066). The first column lists differentially expressed (FDR<0.005) assembled RNA-seq reads that have mapped to the *Lotus japonicus* genome. The second column is their soybean (*Glycine max*) BLAST homolog, followed by expression at two stages of flower development.

| <i>L. japonicus</i> ID | <i>G. max</i> BLAST ID | <i>G. max</i> description  | Counts per gene<br>(Yellow) | Counts per gene (Red) | Log2FC   |
|------------------------|------------------------|--|-----------------------------|-----------------------|----------|
| chr2.CM0021.2820.r2.m  | Glyma.11G027700.1      | Ribonuclease T(2) / Ribonuclease T2                                  | 11                          | 303                   | -4.87472 |
| chr2.CM0608.560.r2.m   | Glyma.01G048200.1      | NARINGENIN,2-OXOGLUTARATE 3-DIOXYGENASE                              | 4                           | 53                    | -3.79248 |
| chr2.CM0018.1190.r2.m  | Glyma.01G228700.1      | Naringenin-chalcone synthase / Flavonone synthase                    | 0                           | 16                    | -7.06546 |
| chr2.CM0018.760.r2.m   | Glyma.01G228700.1      | Naringenin-chalcone synthase / Flavonone synthase                    | 1                           | 103                   | -6.61962 |
| chr1.LjT39K18.30.r2.m  | Glyma.04G147700.1      | Naringenin-chalcone synthase / Flavonone synthase                    | 78                          | 686                   | -3.24183 |
| chr2.CM0018.730.r2.m   | Glyma.01G228700.1      | Naringenin-chalcone synthase / Flavonone synthase                    | 0                           | 83                    | -9.43178 |
| chr2.CM0018.1300.r2.m  | Glyma.01G228700.1      | Naringenin-chalcone synthase / Flavonone synthase                    | 0                           | 43                    | -8.48495 |
| chr5.CM0077.110.r2.m   | Glyma.02G158700.1      | Naringenin-chalcone synthase / Flavonone synthase                    | 6                           | 108                   | -4.248   |
| chr1.CM0284.240.r2.m   | Glyma.01G228700.1      | Naringenin-chalcone synthase / Flavonone synthase                    | 7                           | 316                   | -5.57781 |
| chr1.CM0104.1140.r2.m  | Glyma.08G109300.1      | Dihydrokaempferol 4-reductase / NADPH-dihydromyricetin reductase     | 27                          | 108                   | -2.10201 |
| chr2.CM0018.1150.r2.m  | Glyma.01G228700.1      | Dihydrokaempferol 4-reductase / NADPH-dihydromyricetin reductase     | 0                           | 20                    | -7.38524 |
| chr4.CM0119.240.r2.m   | Glyma.01G166200.1      | Thromboxane-A synthase / Thromboxane synthetase                      | 11                          | 82                    | -2.99064 |
| chr1.CM0133.560.r2.m   | Glyma.04G052100.1      | Heme oxygenase (biliverdin-producing) / Heme oxygenase (decyclizing) | 0                           | 31                    | -8.01444 |
| chr5.CM0077.210.r2.m   | Glyma.02G158700.1      | Naringenin-chalcone synthase / Flavonone synthase                    | 1                           | 27                    | -4.69274 |
| chr2.CM0018.1200.r2.m  | Glyma.01G228700.1      | LEUCANTHOCYANIDIN DIOXYGENASE-RELATED                                | 1                           | 83                    | -6.30857 |

\*Flavonoid biosynthetic process was not found to be enriched at the yellow stage of floral colour change in *L. sessilifolius*, given the TopHat results.

\*Lignin biosynthetic process was not found to be expressed at the yellow or red stages of floral colour change in *L. sessilifolius*, given the TopHat results.

**Table S22.** STAR Results: Significantly enriched GO terms corresponding to the yellow stage of a *L. sessilifolius* flower.

| Enriched GO Term: Description                             | Number of associated genes | FDR      |
|---|----------------------------|----------|
| GO:0044281small molecule metabolic process                | 105                        | 4.90E-11 |
| GO:0008610lipid biosynthetic process                      | 40                         | 1.10E-08 |
| GO:0044283small molecule biosynthetic process             | 57                         | 1.30E-08 |
| GO:0006629lipid metabolic process                         | 57                         | 1.30E-08 |
| GO:0046394carboxylic acid biosynthetic process            | 41                         | 4.80E-08 |
| GO:0043436oxoacid metabolic process                       | 58                         | 4.80E-08 |
| GO:0016053organic acid biosynthetic process               | 41                         | 4.80E-08 |
| GO:0006082organic acid metabolic process                  | 58                         | 4.80E-08 |
| GO:0019752carboxylic acid metabolic process               | 58                         | 4.80E-08 |
| GO:0042180cellular ketone metabolic process               | 59                         | 5.60E-08 |
| GO:0044255cellular lipid metabolic process                | 41                         | 1.50E-06 |
| GO:0006633fatty acid biosynthetic process                 | 22                         | 9.80E-06 |
| GO:0032787monocarboxylic acid metabolic process           | 34                         | 1.30E-05 |
| GO:0006631fatty acid metabolic process                    | 25                         | 2.00E-05 |
| GO:0050896response to stimulus                            | 144                        | 3.20E-05 |
| GO:0055114oxidation reduction                             | 63                         | 3.20E-05 |
| GO:0046417chorismate metabolic process                    | 11                         | 3.90E-05 |
| GO:0009073aromatic amino acid family biosynthetic process | 11                         | 3.90E-05 |
| GO:0043648dicarboxylic acid metabolic process             | 15                         | 4.30E-05 |
| GO:0009628response to abiotic stimulus                    | 70                         | 6.20E-05 |
| GO:0042221response to chemical stimulus                   | 93                         | 7.70E-05 |
| GO:0008152metabolic process                               | 288                        | 0.00014  |
| GO:0009072aromatic amino acid family metabolic process    | 12                         | 0.00037  |
| GO:0008299isoprenoid biosynthetic process                 | 14                         | 0.00046  |
| GO:0010025wax biosynthetic process                        | 8                          | 0.00047  |
| GO:0010166wax metabolic process                           | 8                          | 0.00047  |
| GO:0009058biosynthetic process                            | 135                        | 0.00065  |

| Enriched GO Term: Description                                  | Number of associated genes | FDR    |
|--|----------------------------|--------|
| GO:0044262cellular carbohydrate metabolic process              | 38                         | 0.0008 |
| GO:0006720isoprenoid metabolic process                         | 14                         | 0.0021 |
| GO:0006950response to stress                                   | 90                         | 0.0021 |
| GO:0044264cellular polysaccharide metabolic process            | 17                         | 0.0025 |
| GO:0044249cellular biosynthetic process                        | 128                        | 0.003  |
| GO:0005976polysaccharide metabolic process                     | 18                         | 0.0041 |
| GO:0009416response to light stimulus                           | 33                         | 0.006  |
| GO:0030244cellulose biosynthetic process                       | 7                          | 0.006  |
| GO:0016114terpenoid biosynthetic process                       | 10                         | 0.0063 |
| GO:0006519cellular amino acid and derivative metabolic process | 36                         | 0.0063 |
| GO:0016126sterol biosynthetic process                          | 5                          | 0.0063 |
| GO:0033692cellular polysaccharide biosynthetic process         | 12                         | 0.0063 |
| GO:0005975carbohydrate metabolic process                       | 47                         | 0.0063 |
| GO:0006073cellular glucan metabolic process                    | 14                         | 0.0063 |
| GO:0009314response to radiation                                | 33                         | 0.0068 |
| GO:0044042glucan metabolic process                             | 14                         | 0.0071 |
| GO:0000271polysaccharide biosynthetic process                  | 12                         | 0.0071 |
| GO:0030243cellulose metabolic process                          | 7                          | 0.0071 |
| GO:0016109tetraterpenoid biosynthetic process                  | 6                          | 0.012  |
| GO:0009607response to biotic stimulus                          | 40                         | 0.012  |
| GO:0016117carotenoid biosynthetic process                      | 6                          | 0.012  |
| GO:0046148pigment biosynthetic process                         | 13                         | 0.012  |
| GO:0010038response to metal ion                                | 28                         | 0.013  |
| GO:0009657plastid organization                                 | 13                         | 0.013  |
| GO:0006520cellular amino acid metabolic process                | 24                         | 0.013  |
| GO:0010218response to far red light                            | 7                          | 0.013  |
| GO:0010102lateral root morphogenesis                           | 9                          | 0.014  |
| GO:0010101post-embryonic root morphogenesis                    | 9                          | 0.014  |
| GO:0044237cellular metabolic process                           | 220                        | 0.014  |
| GO:0071669plant-type cell wall organization or biogenesis      | 12                         | 0.014  |
| GO:0051707response to other organism                           | 38                         | 0.014  |
| GO:0042214terpene metabolic process                            | 7                          | 0.014  |
| GO:0008652cellular amino acid biosynthetic process             | 15                         | 0.014  |
| GO:0006066alcohol metabolic process                            | 23                         | 0.015  |
| GO:0019748secondary metabolic process                          | 24                         | 0.015  |
| GO:0034641cellular nitrogen compound metabolic process         | 58                         | 0.015  |

| Enriched GO Term: Description                             | Number of associated genes | FDR   |
|---|----------------------------|-------|
| GO:0009309amine biosynthetic process                      | 16                         | 0.015 |
| GO:0007275multicellular organismal development            | 74                         | 0.015 |
| GO:0009250glucan biosynthetic process                     | 9                          | 0.015 |
| GO:0009719response to endogenous stimulus                 | 46                         | 0.015 |
| GO:0044106cellular amine metabolic process                | 25                         | 0.015 |
| GO:0010015root morphogenesis                              | 15                         | 0.015 |
| GO:0016108tetraterpenoid metabolic process                | 6                          | 0.015 |
| GO:0016116carotenoid metabolic process                    | 6                          | 0.015 |
| GO:0032502developmental process                           | 78                         | 0.015 |
| GO:0010035response to inorganic substance                 | 32                         | 0.016 |
| GO:0044238primary metabolic process                       | 227                        | 0.016 |
| GO:0000162tryptophan biosynthetic process                 | 5                          | 0.016 |
| GO:0010214seed coat development                           | 5                          | 0.016 |
| GO:0046219indolalkylamine biosynthetic process            | 5                          | 0.016 |
| GO:0007017microtubule-based process                       | 12                         | 0.016 |
| GO:0032501multicellular organismal process                | 76                         | 0.016 |
| GO:0006721terpenoid metabolic process                     | 10                         | 0.017 |
| GO:0046246terpene biosynthetic process                    | 6                          | 0.017 |
| GO:0042335cuticle development                             | 5                          | 0.019 |
| GO:0051704multi-organism process                          | 44                         | 0.02  |
| GO:0019438aromatic compound biosynthetic process          | 38                         | 0.021 |
| GO:0044271cellular nitrogen compound biosynthetic process | 43                         | 0.022 |
| GO:0009834secondary cell wall biogenesis                  | 5                          | 0.024 |
| GO:0046686response to cadmium ion                         | 22                         | 0.027 |
| GO:0010033response to organic substance                   | 53                         | 0.029 |
| GO:0016125sterol metabolic process                        | 5                          | 0.029 |
| GO:0048528post-embryonic root development                 | 10                         | 0.029 |
| GO:0009110vitamin biosynthetic process                    | 7                          | 0.031 |
| GO:0009308amine metabolic process                         | 26                         | 0.032 |
| GO:0006586indolalkylamine metabolic process               | 5                          | 0.032 |
| GO:0051273beta-glucan metabolic process                   | 5                          | 0.032 |
| GO:0051274beta-glucan biosynthetic process                | 5                          | 0.032 |
| GO:0042440pigment metabolic process                       | 13                         | 0.032 |
| GO:0006568tryptophan metabolic process                    | 5                          | 0.032 |
| GO:0071704organic substance metabolic process             | 67                         | 0.033 |
| GO:0006766vitamin metabolic process                       | 7                          | 0.033 |

| Enriched GO Term: Description                       | Number of associated genes | FDR      |
|---|----------------------------|----------|
| GO:0071554cell wall organization or biogenesis      | 18                         | 0.034    |
| GO:0009886post-embryonic morphogenesis              | 12                         | 0.039    |
| GO:0016051carbohydrate biosynthetic process         | 16                         | 0.041    |
| GO:0009611response to wounding                      | 15                         | 0.047    |
| GO:0003824catalytic activity                        | 281                        | 1.20E-11 |
| GO:0016491oxidoreductase activity                   | 70                         | 6.70E-05 |
| GO:0005506iron ion binding                          | 27                         | 0.019    |
| GO:0004506squalene monooxygenase activity           | 5                          | 0.019    |
| GO:0004497monooxygenase activity                    | 11                         | 0.025    |
| GO:0016740transferase activity                      | 100                        | 0.033    |
| GO:0046527glucosyltransferase activity              | 9                          | 0.042    |
| GO:0016760cellulose synthase (UDP-forming) activity | 5                          | 0.047    |
| GO:0016759cellulose synthase activity               | 5                          | 0.047    |
| GO:0044435plastid part                              | 91                         | 1.20E-19 |
| GO:0044434chloroplast part                          | 90                         | 1.20E-19 |
| GO:0009507chloroplast                               | 113                        | 6.70E-17 |
| GO:0009536plastid                                   | 115                        | 8.30E-16 |
| GO:0009526plastid envelope                          | 56                         | 8.90E-15 |
| GO:0009570chloroplast stroma                        | 57                         | 8.90E-15 |
| GO:0009532plastid stroma                            | 57                         | 2.30E-14 |
| GO:0009941chloroplast envelope                      | 53                         | 1.20E-13 |
| GO:0031967organelle envelope                        | 57                         | 1.90E-10 |
| GO:0031975envelope                                  | 57                         | 2.00E-10 |
| GO:0044464cell part                                 | 311                        | 2.00E-09 |
| GO:0005623cell                                      | 311                        | 2.00E-09 |
| GO:0044444cytoplasmic part                          | 197                        | 3.00E-09 |
| GO:0043229intracellular organelle                   | 223                        | 3.00E-08 |
| GO:0043226organelle                                 | 223                        | 3.20E-08 |
| GO:0005737cytoplasm                                 | 204                        | 3.20E-08 |
| GO:0043231intracellular membrane-bounded organelle  | 212                        | 3.60E-08 |
| GO:0043227membrane-bounded organelle                | 212                        | 4.50E-08 |
| GO:0044446intracellular organelle part              | 126                        | 7.30E-08 |
| GO:0044422organelle part                            | 126                        | 9.90E-08 |
| GO:0009579thylakoid                                 | 36                         | 7.30E-07 |

| Enriched GO Term: Description              | Number of associated genes | FDR      |
|--|----------------------------|----------|
| GO:0044424intracellular part               | 242                        | 8.90E-07 |
| GO:0005622intracellular                    | 243                        | 4.10E-06 |
| GO:0005618cell wall                        | 39                         | 6.30E-06 |
| GO:0030312external encapsulating structure | 39                         | 7.20E-06 |
| GO:0031976plastid thylakoid                | 29                         | 9.80E-06 |
| GO:0009534chloroplast thylakoid            | 29                         | 9.80E-06 |
| GO:0031984organelle subcompartment         | 29                         | 1.00E-05 |
| GO:0005576extracellular region             | 36                         | 6.00E-05 |
| GO:0048046apoplast                         | 26                         | 0.0003   |
| GO:0044436thylakoid part                   | 23                         | 0.00082  |
| GO:0016020membrane                         | 154                        | 0.0015   |
| GO:0005886plasma membrane                  | 83                         | 0.0059   |
| GO:0031977thylakoid lumen                  | 8                          | 0.0061   |
| GO:0009535chloroplast thylakoid membrane   | 19                         | 0.0061   |
| GO:0055035plastid thylakoid membrane       | 19                         | 0.0062   |
| GO:0010287plastoglobule                    | 7                          | 0.01     |
| GO:0042651thylakoid membrane               | 19                         | 0.011    |
| GO:0034357photosynthetic membrane          | 19                         | 0.02     |

**Table S23.** STAR Results: Significantly enriched GO terms corresponding to the red stage of a *L. sessilifoliuss* flower.

| Enriched GO Term: Description                                 | Number of associated genes | FDR      |
|---|----------------------------|----------|
| GO:0009605response to external stimulus                       | 60                         | 1.60E-09 |
| GO:0042221response to chemical stimulus                       | 143                        | 7.20E-08 |
| GO:0009611response to wounding                                | 34                         | 4.90E-07 |
| GO:0050896response to stimulus                                | 209                        | 9.70E-07 |
| GO:0006810transport   | 123                        | 9.70E-07 |
| GO:0051234establishment of localization                       | 123                        | 1.00E-06 |
| GO:0051179localization  | 126                        | 1.60E-06 |
| GO:0009753response to jasmonic acid stimulus                  | 30                         | 1.60E-06 |
| GO:0010033response to organic substance                       | 94                         | 2.20E-06 |
| GO:0009714chalcone metabolic process                          | 11                         | 2.70E-06 |
| GO:0042181ketone biosynthetic process                         | 11                         | 2.70E-06 |
| GO:0009715chalcone biosynthetic process                       | 11                         | 2.70E-06 |
| GO:0009719response to endogenous stimulus                     | 79                         | 3.40E-06 |
| GO:0031540regulation of anthocyanin biosynthetic process      | 11                         | 2.50E-05 |
| GO:0009725response to hormone stimulus                        | 71                         | 2.90E-05 |
| GO:0009718anthocyanin biosynthetic process                    | 14                         | 3.30E-05 |
| GO:0009962regulation of flavonoid biosynthetic process        | 12                         | 4.00E-05 |
| GO:0055085transmembrane transport                             | 55                         | 4.40E-05 |
| GO:0006950response to stress                                  | 133                        | 5.00E-05 |
| GO:0009813flavonoid biosynthetic process                      | 18                         | 5.10E-05 |
| GO:0043455regulation of secondary metabolic process           | 14                         | 7.30E-05 |
| GO:0009991response to extracellular stimulus                  | 23                         | 0.00011  |
| GO:0031537regulation of anthocyanin metabolic process         | 11                         | 0.00025  |
| GO:0046283anthocyanin metabolic process                       | 14                         | 0.00047  |
| GO:0006979response to oxidative stress                        | 35                         | 0.00054  |
| GO:0009699phenylpropanoid biosynthetic process                | 20                         | 0.00059  |
| GO:0031668cellular response to extracellular stimulus         | 21                         | 0.00059  |
| GO:0009812flavonoid metabolic process                         | 18                         | 0.00059  |
| GO:0042398cellular amino acid derivative biosynthetic process | 27                         | 0.00059  |

| Enriched GO Term: Description                       | Number of associated genes | FDR     |
|---|----------------------------|---------|
| GO:0071496cellular response to external stimulus    | 21                         | 0.00059 |
| GO:0016137glycoside metabolic process               | 16                         | 0.00088 |
| GO:0005975carbohydrate metabolic process            | 67                         | 0.0012  |
| GO:0060918auxin transport                           | 13                         | 0.0012  |
| GO:0009914hormone transport                         | 13                         | 0.0014  |
| GO:0009056catabolic process                         | 57                         | 0.0014  |
| GO:0009926auxin polar transport                     | 12                         | 0.0015  |
| GO:0009628response to abiotic stimulus              | 87                         | 0.0018  |
| GO:0009629response to gravity                       | 13                         | 0.0018  |
| GO:0031667response to nutrient levels               | 18                         | 0.0018  |
| GO:0044248cellular catabolic process                | 52                         | 0.0019  |
| GO:0046148pigment biosynthetic process              | 18                         | 0.0028  |
| GO:0016139glycoside catabolic process               | 7                          | 0.0033  |
| GO:0016036cellular response to phosphate starvation | 12                         | 0.0033  |
| GO:0010224response to UV-B                          | 14                         | 0.0034  |
| GO:0006811ion transport                             | 41                         | 0.0037  |
| GO:0044283small molecule biosynthetic process       | 54                         | 0.0038  |
| GO:0019748secondary metabolic process               | 34                         | 0.0038  |
| GO:0006527arginine catabolic process                | 5                          | 0.0051  |
| GO:0051704multi-organism process                    | 63                         | 0.0074  |
| GO:0042440pigment metabolic process                 | 19                         | 0.0082  |
| GO:0042594response to starvation                    | 15                         | 0.0083  |
| GO:0031669cellular response to nutrient levels      | 16                         | 0.0093  |
| GO:0006814sodium ion transport                      | 7                          | 0.0098  |
| GO:0009737response to abscisic acid stimulus        | 33                         | 0.01    |
| GO:0009311oligosaccharide metabolic process         | 11                         | 0.01    |
| GO:0009733response to auxin stimulus                | 25                         | 0.012   |
| GO:0005984disaccharide metabolic process            | 10                         | 0.012   |
| GO:0009698phenylpropanoid metabolic process         | 21                         | 0.013   |
| GO:0009607response to biotic stimulus               | 53                         | 0.016   |

| Enriched GO Term: Description                                   | Number of associated genes | FDR      |
|---|----------------------------|----------|
| GO:0006575cellular amino acid derivative metabolic process      | 29                         | 0.016    |
| GO:0046395carboxylic acid catabolic process                     | 13                         | 0.016    |
| GO:0016054organic acid catabolic process                        | 13                         | 0.016    |
| GO:0044282small molecule catabolic process                      | 28                         | 0.017    |
| GO:0009267cellular response to starvation                       | 14                         | 0.018    |
| GO:0009411response to UV  | 15                         | 0.018    |
| GO:0016052carbohydrate catabolic process                        | 17                         | 0.019    |
| GO:0008216spermidine metabolic process                          | 5                          | 0.024    |
| GO:0008295spermidine biosynthetic process                       | 5                          | 0.024    |
| GO:0006595polyamine metabolic process                           | 6                          | 0.024    |
| GO:0051707response to other organism                            | 50                         | 0.024    |
| GO:0030001metal ion transport                                   | 23                         | 0.024    |
| GO:0005991trehalose metabolic process                           | 6                          | 0.024    |
| GO:0006970response to osmotic stress                            | 35                         | 0.026    |
| GO:0010817regulation of hormone levels                          | 19                         | 0.026    |
| GO:0009065glutamine family amino acid catabolic process         | 5                          | 0.031    |
| GO:0009310amine catabolic process                               | 10                         | 0.032    |
| GO:0044275cellular carbohydrate catabolic process               | 15                         | 0.039    |
| GO:0006820anion transport                                       | 15                         | 0.039    |
| GO:0015698inorganic anion transport                             | 10                         | 0.041    |
| GO:0009414response to water deprivation                         | 22                         | 0.045    |
| GO:0010035response to inorganic substance                       | 41                         | 0.047    |
| GO:0030036actin cytoskeleton organization                       | 11                         | 0.047    |
| GO:0005215transporter activity                                  | 79                         | 2.20E-07 |
| GO:0022857transmembrane transporter activity                    | 69                         | 2.20E-07 |
| GO:0022891substrate-specific transmembrane transporter activity | 59                         | 1.40E-06 |
| GO:0022892substrate-specific transporter activity               | 62                         | 1.40E-06 |
| GO:0016210naringenin-chalcone synthase activity                 | 11                         | 1.90E-06 |
| GO:0016798hydrolase activity, acting on glycosyl bonds          | 40                         | 2.10E-05 |
| GO:0004553hydrolase activity, hydrolyzing O-glycosyl compounds  | 38                         | 2.70E-05 |

| Enriched GO Term: Description                                 | Number of associated genes | FDR      |
|---|----------------------------|----------|
| GO:0015291secondary active transmembrane transporter activity | 26                         | 3.20E-05 |
| GO:0016791phosphatase activity                                | 24                         | 0.00012  |
| GO:0042578phosphoric ester hydrolase activity                 | 27                         | 0.00015  |
| GO:0016787hydrolase activity                                  | 152                        | 0.00017  |
| GO:0022804active transmembrane transporter activity           | 36                         | 0.00021  |
| GO:0003779actin binding                                       | 12                         | 0.00066  |
| GO:0015144carbohydrate transmembrane transporter activity     | 11                         | 0.00073  |
| GO:0003824catalytic activity                                  | 346                        | 0.00073  |
| GO:0015075ion transmembrane transporter activity              | 37                         | 0.0041   |
| GO:0015103inorganic anion transmembrane transporter activity  | 11                         | 0.018    |
| GO:0004650polygalacturonase activity                          | 10                         | 0.024    |
| GO:0016746transferase activity, transferring acyl groups      | 26                         | 0.024    |
| GO:0008509anion transmembrane transporter activity            | 14                         | 0.029    |
| GO:0015294solute:cation symporter activity                    | 7                          | 0.031    |
| GO:0015295solute:hydrogen symporter activity                  | 6                          | 0.031    |
| GO:0008092cytoskeletal protein binding                        | 15                         | 0.031    |
| GO:0008234cysteine-type peptidase activity                    | 12                         | 0.031    |
| GO:0008324cation transmembrane transporter activity           | 26                         | 0.031    |
| GO:0051119sugar transmembrane transporter activity            | 7                          | 0.031    |
| GO:0046943carboxylic acid transmembrane transporter activity  | 10                         | 0.031    |
| GO:0015297antiporter activity                                 | 13                         | 0.035    |
| GO:0005342organic acid transmembrane transporter activity     | 10                         | 0.041    |
| GO:0015293symporter activity                                  | 7                          | 0.041    |
| GO:0005773vacuole   | 77                         | 6.50E-07 |
| GO:0000325plant-type vacuole                                  | 20                         | 0.00014  |
| GO:0016020membrane  | 229                        | 0.00017  |
| GO:0009705plant-type vacuole membrane                         | 17                         | 0.00075  |
| GO:0044437vacuolar part                                       | 41                         | 0.022    |
| GO:0005774vacuolar membrane                                   | 41                         | 0.022    |
| GO:0044464cell part   | 397                        | 0.023    |

**Table S24.** Yellow Stage STAR Results: Genes for enzymes annotated to GO:0009813 (Flavonoid biosynthetic process, FDR=1). The first column lists differentially expressed (FDR<0.005) assembled RNA-seq reads that have mapped to the *Lotus japonicus* genome. The second column is their soybean (*Glycine max*) BLAST homolog, followed by expression at two stages of flower development.

| <i>L. japonicus</i> ID | <i>G. max</i> BLAST ID | <i>G. max</i> description   | Counts per gene<br>(Yellow) | Counts per gene (Red) | Log <sub>2</sub> FC |
|------------------------|------------------------|---|-----------------------------|-----------------------|---------------------|
| chr5.CM1077.590.r2.m   | Glyma.03G187000.1      | Trans-zeatin O-beta-D-glucosyltransferase / Zeatin O-glucosyltransferase  | 11.82                       | 0                     | 6.526304            |
| chr3.LjT10E18.60.r2.m  | Glyma.10G210600.1      | B3 DNA binding domain (B3) //<br>Auxin response factor<br>(Auxin_resp)  | 43                          | 6                     | 2.664685            |
| chr1.CM0410.460.r2.m   | Glyma.18G268100.1      | Isoflavone-7-O-beta-glucoside<br>6''-O-malonyltransferase /<br>Flavone/flavonol 7-O-beta-D-glucoside malonyltransferase | 24.83                       | 0                     | 7.577035            |
| chr3.CM0106.330.r2.m   | Glyma.13G344700.1      | B-BOX TYPE ZINC FINGER-CONTAINING PROTEIN-RELATED   | 157                         | 18                    | 2.963609            |
| chr1.CM0476.410.r2.a   | Glyma.04G227700.1      | FLAVONE 3'-O-METHYLTRANSFERASE 1  | 495                         | 98                    | 2.182379            |

**Table S25.** Red Stage STAR Results: Genes for enzymes annotated to GO:0009813 (Flavonoid biosynthetic process, FDR = 0.00059). The first column lists differentially expressed (FDR<0.005) assembled RNA-seq reads that have mapped to the *Lotus japonicus* genome. The second column is their soybean (*Glycine max*) BLAST homolog, followed by expression at two stages of flower development.

| <i>L. japonicus</i> ID | <i>G. max</i> BLAST ID | <i>G. max</i> description  | Counts per gene<br>(Yellow) | Counts per gene (Red) | Log <sub>2</sub> FC |
|------------------------|------------------------|--|-----------------------------|-----------------------|---------------------|
| chr2.CM0608.560.r2.m   | Glyma.01G048200.1      | Ribonuclease T(2) / Ribonuclease T2                                  | 11                          | 128                   | -3.67758            |
| chr4.CM0119.240.r2.m   | Glyma.01G166200.1      | NARINGENIN,2-OXOGLUTARATE 3-DIOXYGENASE                              | 155.05                      | 921.11                | -2.72273            |
| chr3.LjB14006.120.r2.a | Glyma.01G166200.1      | NARINGENIN,2-OXOGLUTARATE 3-DIOXYGENASE                              | 2.95                        | 51.89                 | -4.20965            |
| chr3.CM0590.770.r2.d   | Glyma.01G228700.1      | Naringenin-chalcone synthase / Flavonone synthase                    | 0                           | 39.5                  | -8.40463            |
| chr1.CM0284.250.r2.m   | Glyma.01G228700.1      | Naringenin-chalcone synthase / Flavonone synthase                    | 0                           | 48.1                  | -8.66696            |
| chr3.CM0590.840.r2.m   | Glyma.01G228700.1      | Naringenin-chalcone synthase / Flavonone synthase                    | 0                           | 39.5                  | -8.40463            |
| chr2.CM0018.1190.r2.m  | Glyma.01G228700.1      | Naringenin-chalcone synthase / Flavonone synthase                    | 8.81                        | 182.32                | -4.4707             |
| chr2.CM0018.760.r2.m   | Glyma.01G228700.1      | Naringenin-chalcone synthase / Flavonone synthase                    | 2.85                        | 307.57                | -6.77327            |
| chr2.CM0018.730.r2.m   | Glyma.01G228700.1      | Naringenin-chalcone synthase / Flavonone synthase                    | 8.15                        | 367.98                | -5.65332            |
| chr2.CM0018.1300.r2.m  | Glyma.01G228700.1      | Naringenin-chalcone synthase / Flavonone synthase                    | 0                           | 72.45                 | -9.25074            |
| chr1.CM0284.240.r2.m   | Glyma.01G228700.1      | Naringenin-chalcone synthase / Flavonone synthase                    | 11.42                       | 767.23                | -6.25955            |
| chr2.CM0018.1150.r2.m  | Glyma.01G228700.1      | Naringenin-chalcone synthase / Flavonone synthase                    | 5.77                        | 256.13                | -5.53724            |
| chr2.CM0018.1200.r2.m  | Glyma.01G228700.1      | Naringenin-chalcone synthase / Flavonone synthase                    | 9.38                        | 468.71                | -5.83578            |
| chr5.CM0077.110.r2.m   | Glyma.02G158700.1      | Dihydrokaempferol 4-reductase / NADPH-dihydromyricetin reductase     | 20                          | 321                   | -4.14841            |
| chr1.CM0133.560.r2.m   | Glyma.04G052100.1      | Thromboxane-A synthase / Thromboxane synthetase                      | 0                           | 41                    | -8.44015            |
| chr1.LjT39K18.30.r2.m  | Glyma.04G147700.1      | Heme oxygenase (biliverdin-producing) / Heme oxygenase (decyclizing) | 115                         | 1030                  | -3.31429            |

| <i>L. japonicus</i> ID | <i>G. max</i> BLAST ID | <i>G. max</i> description                            | Counts per<br>gene | Counts per<br>gene (Red) | Log <sub>2</sub> FC<br>(Yellow) |
|------------------------|------------------------|--|--------------------|--------------------------|---------------------------------|
| chr1.CM0104.1140.r2.m  | Glyma.08G109300.1      | Naringenin-chalcone synthase /<br>Flavonone synthase | 70                 | 354                      | -2.48893                        |
| chr2.CM0021.2820.r2.m  | Glyma.11G027700.1      | LEUCOANTHOCYANIDIN<br>DIOXYGENASE-RELATED            | 14                 | 833                      | -6.03436                        |

**Table S26.** Yellow Stage STAR Results: Genes for enzymes annotated to GO:0009808 (Lignin metabolic process, FDR=0.92). The first column lists differentially expressed (FDR<0.005) assembled RNA-seq reads that have mapped to the *Lotus japonicus* genome. The second column is their soybean (*Glycine max*) BLAST homolog, followed by expression at two stages of flower development.

| <i>L. japonicus</i> ID | <i>G. max</i> BLAST ID | <i>G. max</i> description        | Counts per gene | Counts per gene (Red) | Log <sub>2</sub> FC (Yellow) |
|------------------------|------------------------|----------------------------------|-----------------|-----------------------|------------------------------|
| chr6.CM0385.240.r2.d   | Glyma.09G038500.1      | CHITINASE-LIKE PROTEIN 1         | 159             | 16                    | 3.15060                      |
| chr6.CM0084.30.r2.m    | Glyma.02G224800.1      | LACCASE-16                       | 51              | 3                     | 3.88250                      |
| chr4.CM0387.960.r2.m   | Glyma.07G258700.1      | BETA-GLUCOSIDASE 45-RELATED      | 16              | 0                     | 6.93742                      |
| chr2.CM0249.1520.r2.m  | Glyma.01G173500.1      | LACCASE-11                       | 46              | 1                     | 5.21342                      |
| chr1.CM0476.410.r2.a   | Glyma.04G227700.1      | FLAVONE 3'-O-METHYLTRANSFERASE 1 | 495             | 98                    | 2.18237                      |

\*Lignin biosynthetic process was not found to be expressed at the red stage of floral colour change in *L. sessilifolius*, given the STAR results.

**Table S27.** Trinity Results: Significantly enriched GO terms corresponding to the yellow stage of a *L. sessilifolius* flower.

| Enriched GO category   | Number of associated genes | FDR      |
|--|----------------------------|----------|
| GO:0034641cellular nitrogen compound metabolic process       | 80                         | 4.90E-07 |
| GO:0009791post-embryonic development                         | 138                        | 4.90E-07 |
| GO:0009628response to abiotic stimulus                       | 183                        | 4.90E-07 |
| GO:0043436oxoacid metabolic process                          | 119                        | 2.30E-06 |
| GO:0006082organic acid metabolic process                     | 119                        | 2.30E-06 |
| GO:0019752carboxylic acid metabolic process                  | 119                        | 2.30E-06 |
| GO:0042180cellular ketone metabolic process                  | 120                        | 2.40E-06 |
| GO:0006629lipid metabolic process                            | 111                        | 1.70E-05 |
| GO:0009416response to light stimulus                         | 87                         | 2.80E-05 |
| GO:0048610reproductive cellular process                      | 15                         | 2.80E-05 |
| GO:0032787monocarboxylic acid metabolic process              | 71                         | 3.50E-05 |
| GO:0009606tropism  | 29                         | 4.00E-05 |
| GO:0009314response to radiation                              | 88                         | 4.10E-05 |
| GO:0018130heterocycle biosynthetic process                   | 28                         | 0.0002   |
| GO:0009734auxin mediated signaling pathway                   | 28                         | 0.0002   |
| GO:0044262cellular carbohydrate metabolic process            | 77                         | 0.0002   |
| GO:0046394carboxylic acid biosynthetic process               | 64                         | 0.0002   |
| GO:0016053organic acid biosynthetic process                  | 64                         | 0.0002   |
| GO:0046417chorismate metabolic process                       | 16                         | 0.0002   |
| GO:0009073aromatic amino acid family biosynthetic process    | 16                         | 0.0002   |
| GO:0016114terpenoid biosynthetic process                     | 23                         | 0.00024  |
| GO:0009072aromatic amino acid family metabolic process       | 19                         | 0.00029  |
| GO:0016108tetraterpenoid metabolic process                   | 15                         | 0.00039  |
| GO:0016116carotenoid metabolic process                       | 15                         | 0.00039  |
| GO:0009886post-embryonic morphogenesis                       | 24                         | 0.00043  |
| GO:0046148pigment biosynthetic process                       | 29                         | 0.0006   |
| GO:0016109tetraterpenoid biosynthetic process                | 12                         | 0.00071  |
| GO:0016117carotenoid biosynthetic process                    | 12                         | 0.00071  |
| GO:0008299isoprenoid biosynthetic process                    | 25                         | 0.00073  |
| GO:0010382cellular cell wall macromolecule metabolic process | 8                          | 0.00077  |
| GO:0006631fatty acid metabolic process                       | 41                         | 0.0009   |
| GO:0042440pigment metabolic process                          | 31                         | 0.00098  |
| GO:0009629response to gravity                                | 22                         | 0.001    |
| GO:0043648dicarboxylic acid metabolic process                | 20                         | 0.0012   |
| GO:0010876lipid localization                                 | 8                          | 0.002    |
| GO:0006721terpenoid metabolic process                        | 25                         | 0.0021   |
| GO:0006720isoprenoid metabolic process                       | 28                         | 0.0021   |

| Enriched GO category  | Number of associated genes | FDR    |
|---|----------------------------|--------|
| GO:0046246terpene biosynthetic process                              | 11                         | 0.0031 |
| GO:0044264cellular polysaccharide metabolic process                 | 39                         | 0.0037 |
| GO:0008610lipid biosynthetic process                                | 61                         | 0.0038 |
| GO:0019748secondary metabolic process                               | 71                         | 0.004  |
| GO:0044271cellular nitrogen compound biosynthetic process           | 50                         | 0.0046 |
| GO:0006073cellular glucan metabolic process                         | 30                         | 0.0046 |
| GO:0009605response to external stimulus                             | 75                         | 0.0063 |
| GO:0019438aromatic compound biosynthetic process                    | 44                         | 0.0078 |
| GO:0016051carbohydrate biosynthetic process                         | 48                         | 0.0079 |
| GO:0009630gravitropism  | 19                         | 0.0082 |
| GO:0010166wax metabolic process                                     | 11                         | 0.011  |
| GO:0044042glucan metabolic process                                  | 30                         | 0.012  |
| GO:0010025wax biosynthetic process                                  | 10                         | 0.013  |
| GO:0009733response to auxin stimulus                                | 50                         | 0.014  |
| GO:0009637response to blue light                                    | 15                         | 0.014  |
| GO:0050896response to stimulus                                      | 362                        | 0.018  |
| GO:0044255cellular lipid metabolic process                          | 78                         | 0.023  |
| GO:0005976polysaccharide metabolic process                          | 43                         | 0.028  |
| GO:0042214terpene metabolic process                                 | 12                         | 0.028  |
| GO:0006633fatty acid biosynthetic process                           | 26                         | 0.034  |
| GO:0009266response to temperature stimulus                          | 66                         | 0.035  |
| GO:0030244cellulose biosynthetic process                            | 11                         | 0.035  |
| GO:0009638phototropism  | 11                         | 0.038  |
| GO:0005975carbohydrate metabolic process                            | 106                        | 0.038  |
| GO:0009834secondary cell wall biogenesis                            | 12                         | 0.04   |
| GO:0042546cell wall biogenesis                                      | 24                         | 0.045  |
| GO:0033692cellular polysaccharide biosynthetic process              | 27                         | 0.05   |
| GO:0016829lyase activity  | 55                         | 0.017  |
| GO:0016491oxidoreductase activity                                   | 152                        | 0.017  |
| GO:0035251UDP-glucosyltransferase activity                          | 26                         | 0.017  |
| GO:0009055electron carrier activity                                 | 69                         | 0.02   |
| GO:0016860intramolecular oxidoreductase activity                    | 12                         | 0.02   |
| GO:0008194UDP-glycosyltransferase activity                          | 35                         | 0.02   |
| GO:0046527glucosyltransferase activity                              | 28                         | 0.02   |
| GO:0016835carbon-oxygen lyase activity                              | 23                         | 0.028  |
| GO:0016760cellulose synthase (UDP-forming) activity                 | 10                         | 0.029  |
| GO:0008757S-adenosylmethionine-dependent methyltransferase activity | 21                         | 0.029  |

| Enriched GO category  | Number of associated genes | FDR      |
|---|----------------------------|----------|
| GO:0004497monooxygenase activity                            | 37                         | 0.038    |
| GO:0016758transferase activity, transferring hexosyl groups | 46                         | 0.038    |
| GO:0009507chloroplast                                       | 222                        | 2.30E-12 |
| GO:0009536plastid   | 234                        | 1.50E-11 |
| GO:0044434chloroplast part                                  | 80                         | 2.50E-08 |
| GO:0044435plastid part                                      | 85                         | 2.30E-07 |
| GO:0005618cell wall   | 74                         | 5.00E-07 |
| GO:0009505plant-type cell wall                              | 65                         | 1.00E-06 |
| GO:0030312external encapsulating structure                  | 76                         | 1.70E-06 |
| GO:0009535chloroplast thylakoid membrane                    | 42                         | 1.90E-06 |
| GO:0055035plastid thylakoid membrane                        | 42                         | 1.10E-05 |
| GO:0044444cytoplasmic part                                  | 485                        | 1.50E-05 |
| GO:0009534chloroplast thylakoid                             | 42                         | 3.40E-05 |
| GO:0005737cytoplasm   | 542                        | 9.40E-05 |
| GO:0010287plastoglobule                                     | 17                         | 9.40E-05 |
| GO:0042651thylakoid membrane                                | 42                         | 9.80E-05 |
| GO:0031976plastid thylakoid                                 | 42                         | 0.00016  |
| GO:0031984organelle subcompartment                          | 42                         | 0.00023  |
| GO:0044436thylakoid part                                    | 42                         | 0.001    |
| GO:0044464cell part   | 901                        | 0.001    |
| GO:0005623cell  | 901                        | 0.001    |
| GO:0009941chloroplast envelope                              | 21                         | 0.0057   |
| GO:0031225anchored to membrane                              | 27                         | 0.0064   |
| GO:0009514glyoxysome  | 5                          | 0.033    |
| GO:0034357photosynthetic membrane                           | 43                         | 0.04     |
| GO:0043227membrane-bounded organelle                        | 561                        | 0.049    |

**Table S28.** Trinity Results: Significantly enriched GO terms corresponding to the red stage of a *L. sessilifolius* flower.

| Enriched GO category: Description                               | Number of associated genes | FDR      |
|---|----------------------------|----------|
| GO:0050896 response to stimulus                                 | 503                        | 3.40E-08 |
| GO:0010035 response to inorganic substance                      | 54                         | 5.50E-08 |
| GO:0048610 reproductive cellular process                        | 20                         | 4.00E-07 |
| GO:0042221 response to chemical stimulus                        | 284                        | 6.20E-07 |
| GO:0009414 response to water deprivation                        | 57                         | 4.40E-06 |
| GO:0009415 response to water                                    | 58                         | 6.00E-06 |
| GO:0010033 response to organic substance                        | 197                        | 6.00E-06 |
| GO:0006950 response to stress                                   | 325                        | 1.40E-05 |
| GO:0009719 response to endogenous stimulus                      | 168                        | 1.50E-05 |
| GO:0034641 cellular nitrogen compound metabolic process         | 85                         | 6.20E-05 |
| GO:0009737 response to abscisic acid stimulus                   | 75                         | 1.10E-04 |
| GO:0010876 lipid localization                                   | 11                         | 1.10E-04 |
| GO:0009624 response to nematode                                 | 48                         | 1.10E-04 |
| GO:0006970 response to osmotic stress                           | 73                         | 0.00015  |
| GO:0009628 response to abiotic stimulus                         | 202                        | 0.00019  |
| GO:0009651 response to salt stress                              | 61                         | 0.00019  |
| GO:0009725 response to hormone stimulus                         | 151                        | 0.00039  |
| GO:0009607 response to biotic stimulus                          | 137                        | 0.0013   |
| GO:0005975 carbohydrate metabolic process                       | 140                        | 0.0016   |
| GO:0030001 metal ion transport                                  | 52                         | 0.0022   |
| GO:0044262 cellular carbohydrate metabolic process              | 87                         | 0.0024   |
| GO:0051707 response to other organism                           | 126                        | 0.0024   |
| GO:0042398 cellular amino acid derivative biosynthetic process  | 54                         | 0.0056   |
| GO:0010038 response to metal ion                                | 32                         | 0.01     |
| GO:0043436 oxoacid metabolic process                            | 121                        | 0.01     |
| GO:0046174 polyol catabolic process                             | 8                          | 0.01     |
| GO:0006082 organic acid metabolic process                       | 121                        | 0.01     |
| GO:0009611 response to wounding                                 | 41                         | 0.01     |
| GO:0019752 carboxylic acid metabolic process                    | 121                        | 0.01     |
| GO:0051704 multi-organism process                               | 152                        | 0.012    |
| GO:0006813 potassium ion transport                              | 19                         | 0.017    |
| GO:0042180 cellular ketone metabolic process                    | 121                        | 0.017    |
| GO:0006811 ion transport  | 83                         | 0.02     |
| GO:0009605 response to external stimulus                        | 88                         | 0.021    |
| GO:0006575 cellular amino acid derivative metabolic process     | 65                         | 0.026    |
| GO:0006519 cellular amino acid and derivative metabolic process | 107                        | 0.026    |
| GO:0009751 response to salicylic acid stimulus                  | 39                         | 0.026    |

| Enriched GO category  | Number of associated genes | FDR      |
|---|----------------------------|----------|
| GO:0010107potassium ion import                                  | 6                          | 0.034    |
| GO:0044106cellular amine metabolic process                      | 66                         | 0.034    |
| GO:0009991response to extracellular stimulus                    | 33                         | 0.049    |
| GO:0016791phosphatase activity                                  | 50                         | 0.00063  |
| GO:0042578phosphoric ester hydrolase activity                   | 56                         | 0.0011   |
| GO:0022891substrate-specific transmembrane transporter activity | 116                        | 0.0063   |
| GO:0022857transmembrane transporter activity                    | 140                        | 0.008    |
| GO:0015291secondary active transmembrane transporter activity   | 54                         | 0.012    |
| GO:0004553hydrolase activity, hydrolyzing O-glycosyl compounds  | 62                         | 0.012    |
| GO:0022892substrate-specific transporter activity               | 132                        | 0.013    |
| GO:0009055electron carrier activity                             | 83                         | 0.013    |
| GO:0016798hydrolase activity, acting on glycosyl bonds          | 65                         | 0.015    |
| GO:0005506iron ion binding                                      | 85                         | 0.017    |
| GO:0022804active transmembrane transporter activity             | 98                         | 0.017    |
| GO:0005215transporter activity                                  | 173                        | 0.023    |
| GO:0015075ion transmembrane transporter activity                | 86                         | 0.026    |
| GO:0008374O-acyltransferase activity                            | 18                         | 0.031    |
| GO:0020037heme binding  | 51                         | 0.043    |
| GO:0016021integral to membrane                                  | 199                        | 0.0099   |
| GO:0031224intrinsic to membrane                                 | 224                        | 0.03     |
| GO:0050896response to stimulus                                  | 503                        | 3.40E-08 |
| GO:0010035response to inorganic substance                       | 54                         | 5.50E-08 |
| GO:0048610reproductive cellular process                         | 20                         | 4.00E-07 |
| GO:0042221response to chemical stimulus                         | 284                        | 6.20E-07 |
| GO:0009414response to water deprivation                         | 57                         | 4.40E-06 |
| GO:0009415response to water                                     | 58                         | 6.00E-06 |
| GO:0010033response to organic substance                         | 197                        | 6.00E-06 |
| GO:0006950response to stress                                    | 325                        | 1.40E-05 |
| GO:0009719response to endogenous stimulus                       | 168                        | 1.50E-05 |
| GO:0034641cellular nitrogen compound metabolic process          | 85                         | 6.20E-05 |
| GO:0009737response to abscisic acid stimulus                    | 75                         | 1.10E-04 |
| GO:0010876lipid localization                                    | 11                         | 1.10E-04 |
| GO:0009624response to nematode                                  | 48                         | 1.10E-04 |
| GO:0006970response to osmotic stress                            | 73                         | 0.00015  |
| GO:0009628response to abiotic stimulus                          | 202                        | 0.00019  |
| GO:0009651response to salt stress                               | 61                         | 0.00019  |
| GO:0009725response to hormone stimulus                          | 151                        | 0.00039  |
| GO:0009607response to biotic stimulus                           | 137                        | 0.0013   |

| Enriched GO category  | Number of associated genes | FDR     |
|---|----------------------------|---------|
| GO:0005975carbohydrate metabolic process                        | 140                        | 0.0016  |
| GO:0030001metal ion transport                                   | 52                         | 0.0022  |
| GO:0044262cellular carbohydrate metabolic process               | 87                         | 0.0024  |
| GO:0051707response to other organism                            | 126                        | 0.0024  |
| GO:0042398cellular amino acid derivative biosynthetic process   | 54                         | 0.0056  |
| GO:0010038response to metal ion                                 | 32                         | 0.01    |
| GO:0043436oxoacid metabolic process                             | 121                        | 0.01    |
| GO:0046174polyol catabolic process                              | 8                          | 0.01    |
| GO:0006082organic acid metabolic process                        | 121                        | 0.01    |
| GO:0009611response to wounding                                  | 41                         | 0.01    |
| GO:0019752carboxylic acid metabolic process                     | 121                        | 0.01    |
| GO:0051704multi-organism process                                | 152                        | 0.012   |
| GO:0006813potassium ion transport                               | 19                         | 0.017   |
| GO:0042180cellular ketone metabolic process                     | 121                        | 0.017   |
| GO:0006811ion transport   | 83                         | 0.02    |
| GO:0009605response to external stimulus                         | 88                         | 0.021   |
| GO:0006575cellular amino acid derivative metabolic process      | 65                         | 0.026   |
| GO:0006519cellular amino acid and derivative metabolic process  | 107                        | 0.026   |
| GO:0009751response to salicylic acid stimulus                   | 39                         | 0.026   |
| GO:0010107potassium ion import                                  | 6                          | 0.034   |
| GO:0044106cellular amine metabolic process                      | 66                         | 0.034   |
| GO:0009991response to extracellular stimulus                    | 33                         | 0.049   |
| GO:0016791phosphatase activity                                  | 50                         | 0.00063 |
| GO:0042578phosphoric ester hydrolase activity                   | 56                         | 0.0011  |
| GO:0022891substrate-specific transmembrane transporter activity | 116                        | 0.0063  |
| GO:0022857transmembrane transporter activity                    | 140                        | 0.008   |
| GO:0015291secondary active transmembrane transporter activity   | 54                         | 0.012   |
| GO:0004553hydrolase activity, hydrolyzing O-glycosyl compounds  | 62                         | 0.012   |
| GO:0022892substrate-specific transporter activity               | 132                        | 0.013   |
| GO:0009055electron carrier activity                             | 83                         | 0.013   |
| GO:0016798hydrolase activity, acting on glycosyl bonds          | 65                         | 0.015   |
| GO:0005506iron ion binding                                      | 85                         | 0.017   |
| GO:0022804active transmembrane transporter activity             | 98                         | 0.017   |
| GO:0005215transporter activity                                  | 173                        | 0.023   |
| GO:0015075ion transmembrane transporter activity                | 86                         | 0.026   |
| GO:00083740-acyltransferase activity                            | 18                         | 0.031   |
| GO:0020037heme binding  | 51                         | 0.043   |
| GO:0016021integral to membrane                                  | 199                        | 0.0099  |

| Enriched GO category   | Number of associated genes | FDR    |
|--|----------------------------|--------|
| GO:0031224intrinsic to membrane                                | 224                        | 0.03   |
| GO:0022857transmembrane transporter activity                   | 140                        | 0.008  |
| GO:0015291secondary active transmembrane transporter activity  | 54                         | 0.012  |
| GO:0004553hydrolase activity, hydrolyzing O-glycosyl compounds | 62                         | 0.012  |
| GO:0022892substrate-specific transporter activity              | 132                        | 0.013  |
| GO:0009055electron carrier activity                            | 83                         | 0.013  |
| GO:0016798hydrolase activity, acting on glycosyl bonds         | 65                         | 0.015  |
| GO:0005506iron ion binding                                     | 85                         | 0.017  |
| GO:0022804active transmembrane transporter activity            | 98                         | 0.017  |
| GO:0005215transporter activity                                 | 173                        | 0.023  |
| GO:0015075ion transmembrane transporter activity               | 86                         | 0.026  |
| GO:0008374O-acyltransferase activity                           | 18                         | 0.031  |
| GO:0020037heme binding   | 51                         | 0.043  |
| GO:0016021integral to membrane                                 | 199                        | 0.0099 |
| GO:0031224intrinsic to membrane                                | 224                        | 0.03   |

**Table S29.** Red Stage Trinity Results: Genes for enzymes annotated to GO:0009813 (Flavonoid biosynthetic process, FDR=0.11). The first two columns list differentially expressed (FDR<0.05) Trinity contigs and gene names as annotated by BLASTing the *Lotus japonicus* genome. The third column is their soybean BLAST homolog, followed by expression at two stages of flower development.

| Trinity contig                              | L. japonicus ID | G. max BLAST ID   | Gene description   | Counts per gene | Counts per gene (Red) | Log <sub>2</sub> FC (Yellow) |
|---|-----------------|-------------------|--|-----------------|-----------------------|------------------------------|
| TRINITY_DN51676_chr2.CM0641.560.nc<br>c1_g2 |                 | Glyma.01G048200.1 | Ribonuclease T(2) /<br>Ribonuclease T2   | 11              | 353                   | -5.0898                      |
| TRINITY_DN51676_chr2.CM0641.550.nc<br>c1_g1 |                 | Glyma.01G048400.1 | Ribonuclease T(2) /<br>Ribonuclease T2   | 19              | 1992                  | -6.8045                      |
| TRINITY_DN50283_chr2.CM0124.40.nc<br>c0_g1  |                 | Glyma.01G067100.1 | F11M15.8 PROTEIN-RELATED   | 17              | 300                   | -4.2331                      |
| TRINITY_DN52390_chr4.CM0119.240.nc<br>c0_g1 |                 | Glyma.02G048400.1 | NARINGENIN,2-OXOGLUTARATE 3-DIOXYGENASE (F3H)  | 276             | 1893                  | -2.8794                      |
| TRINITY_DN52643_chr2.CM0124.280.nd<br>c1_g1 |                 | Glyma.02G124700.1 | Codeine 3-O-demethylase / Codeine O-demethylase  | 180             | 884                   | -2.3973                      |
| TRINITY_DN44592_chr1.CM0133.170.nc<br>c1_g1 |                 | Glyma.04G052100.1 | Thromboxane-A synthase /<br>Thromboxane synthetase   | 0               | 38                    | -8.3044                      |
| TRINITY_DN52292_LjT09A12.100.nd<br>c0_g1    |                 | Glyma.06G103200.1 | CRYPTOCHROME-1   | 365             | 4597                  | -3.7563                      |
| TRINITY_DN52780_chr4.CM0429.360.nc<br>c0_g1 |                 | Glyma.06G202300.1 | FLAVONOID 3'-MONOOXYGENASE   | 7               | 2073                  | -8.2859                      |
| TRINITY_DN36546_chr3.CM0208.350.nc<br>c0_g1 |                 | Glyma.07G048600.1 | F3H7.17 PROTEIN  | 93              | 520                   | -2.5836                      |
| TRINITY_DN45435_chr4.CM0387.330.nc<br>c0_g1 |                 | Glyma.08G092800.1 | 2-OXOGLUTARATE (2OG) AND FE(II)-DEPENDENT OXYGENASE SUPERFAMILY PROTEIN-RELATED            | 0               | 2374                  | -14.265                      |
| TRINITY_DN46871_LjSGA_066111.1<br>c0_g2     |                 | Glyma.08G110000.1 | Quinate O-hydroxycinnamoyltransferase /<br>Hydroxycinnamoyl coenzyme A-quinate transferase | 5               | 35                    | -2.8775                      |

| Trinity contig                              | <i>L. japonicus</i> ID | <i>G. max</i> BLAST ID | Gene description  | Counts per gene | Counts per gene (Red) (Yellow) | Log <sub>2</sub> FC |
|---|------------------------|------------------------|---|-----------------|--------------------------------|---------------------|
| TRINITY_DN45808_LjSGA_081858.1<br>c0_g1     |                        | Glyma.10G138800.1      | TRANSCRIPTION FACTOR PIF3   | 0               | 47                             | -8.6102             |
| TRINITY_DN54168_chr1.CM0284.240.nc<br>c0_g1 |                        | Glyma.11G011500.1      | Naringenin-chalcone synthase / Flavonone synthase   | 17              | 1134                           | -6.1510             |
| TRINITY_DN54168_chr1.CM0591.360.nd<br>c0_g2 |                        | Glyma.11G011500.1      | Naringenin-chalcone synthase / Flavonone synthase   | 65              | 4382                           | -6.1743             |
| TRINITY_DN53094_chr2.CM0304.350.nc<br>c0_g1 |                        | Glyma.11G027700.1      | LEUCOANTHOCYANIDI N DIOXYGENASE- RELATED (ANS)  | 30              | 3761                           | -7.0659             |
| TRINITY_DN47758_LjT35E03.200.nd<br>c0_g1    |                        | Glyma.12G194000.1      | MULTIDRUG RESISTANCE PROTEIN  | 50              | 664                            | -3.8298             |
| TRINITY_DN53202_chr3.CM0711.140.nd<br>c0_g1 |                        | Glyma.12G199800.1      | Transferase family (Transferase)  | 12              | 456                            | -5.3349             |
| TRINITY_DN53800_chr6.CM0055.290.nc<br>c0_g1 |                        | Glyma.13G255800.1      | Anthocyanidin 3-O-glucosyltransferase / Uridine diphosphoglucose-anthocyanidin 3-O-glucosyltransferase // Flavonol 3-O-glucosyltransferase / UDP-glucose flavonol 3-O-glucosyltransferase | 262             | 3390                           | -3.7951             |
| TRINITY_DN50775_chr3.CM0711.100.nd<br>c0_g1 |                        | Glyma.13G302300.1      | COUMAROYL-COA:ANTHOCYANIDIN 3-O-GLUCOSIDE-6"-O-COUMAROYLTRANSFERASE 1-RELATED   | 0               | 6587.81                        | -15.737             |
| TRINITY_DN49460_LjSGA_019127.1<br>c0_g1     |                        | Glyma.17G061000.1      | SPERMIDINE HYDROXYCINNAMOYL TRANSFERASE   | 1               | 786                            | -9.5451             |
| TRINITY_DN52789_chr5.CM0077.790.nc<br>c0_g1 |                        | Glyma.17G252200.1      | Dihydrokaempferol 4-reductase / NADPH-dihydromyricetin reductase  | 19              | 434                            | -4.6063             |

| Trinity contig                              | <i>L. japonicus</i> ID | <i>G. max</i> BLAST ID | Gene description                                  | Counts per gene | Counts per gene (Red) | Log <sub>2</sub> FC (Yellow) |
|---|------------------------|------------------------|---|-----------------|-----------------------|------------------------------|
| TRINITY_DN52652_chr1.CM0593.380.nc<br>c1_g1 |                        | Glyma.19G105100.1      | Naringenin-chalcone synthase / Flavonone synthase | 145             | 660                   | -2.2875                      |
| TRINITY_DN49414_chr1.CM0012.680.nd<br>c0_g1 |                        | Glyma.19G187000.1      | UDP-GLYCOSYLTRANSFERASE SE 73C7                   | 136             | 1143                  | -3.1720                      |

**Table S30.** Yellow Stage Trinity Results: Genes for enzymes annotated to GO:0009813 (Flavonoid biosynthetic process, FDR=1). The first two columns list differentially expressed (FDR<0.05) Trinity contigs and gene names as annotated by BLASTing the *Lotus japonicus* genome. The third column is their soybean BLAST equivalent, followed by expression at two stages of flower development.

| Trinity contig                                | L. japonicus ID | G. max BLAST ID   | Gene description  | Counts per gene<br>(Yellow) | Counts per gene<br>(Red) | Log2FC |
|---|-----------------|-------------------|---|-----------------------------|--------------------------|--------|
| TRINITY_DN45233_chr4.CM0227.650.nc<br>c0_g1   |                 | Glyma.02G104600.1 | GLUCOSYL/GLUCURON<br>OSYL TRANSFERASES  | 65                          | 9                        | 2.7339 |
| TRINITY_DN45432_chr2.CM0124.350.nd<br>c0_g1   |                 | Glyma.02G125100.1 | 2-OXOGLUTARATE<br>(2OG) AND FE(II)-<br>DEPENDENT<br>OXYGENASE<br>SUPERFAMILY<br>PROTEIN-RELATED | 319                         | 45                       | 2.7201 |
| TRINITY_DN51740_LjSGA_017175.2<br>c2_g2       |                 | Glyma.04G227700.1 | FLAVONE 3'-O-<br>METHYLTRANSFERASE<br>1   | 1247                        | 281                      | 2.0472 |
| TRINITY_DN45545_chr3.LjT10E18.60.nc<br>c0_g1  |                 | Glyma.12G174100.1 | AUXIN RESPONSE<br>FACTOR 10-RELATED   | 23                          | 2.4                      | 3.3450 |
| TRINITY_DN52633_LjSGA_025000.1<br>c4_g1       |                 | Glyma.12G235100.1 | OXIDOREDUCTASE,<br>2OG-FE II OXYGENASE<br>FAMILY PROTEIN  | 962                         | 155                      | 2.5307 |
| TRINITY_DN39800_chr1.LjB17A22.120.nc<br>c0_g1 |                 | Glyma.13G082300.1 | FLAVONOL SYNTHASE<br>3-RELATED  | 1601                        | 112                      | 3.7338 |
| TRINITY_DN51578_LjSGA_065630.1<br>c0_g1       |                 | Glyma.18G208600.1 | GLUCOSYL/GLUCURON<br>OSYL TRANSFERASES  | 1413                        | 162                      | 3.0216 |

**Table S31.** Similarly Expressed Trinity Results: Genes for enzymes annotated to GO:0009813 (Flavonoid biosynthetic process, FDR=). The first two columns list Trinity contigs expresand gene names as annotated by BLASTing the *Lotus japonicus* genome. The third column is their soybean BLAST homolog, followed by expression at two stages of flower development.

| Trinity contig                               | L. japonicus ID | G. max BLAST ID   | Gene description   | Counts per gene<br>(Yellow) | Counts per gene<br>(Red) | Log2FC  |
|--|-----------------|-------------------|--|-----------------------------|--------------------------|---------|
| TRINITY_DN41192_chr5.CM0077.690.nc<br>c0_g1  |                 | Glyma.02G158700.1 | Dihydrokaempferol 4-reductase / NADPH-dihydromyricetin reductase | 2                           | 0                        | 4.0402  |
| TRINITY_DN25538_chr1.CM0122.460.nc<br>c0_g1  |                 | Glyma.03G222100.2 | NITROGEN REGULATORY PROTEIN P-II                                 | 38                          | 14                       | 1.3309  |
| TRINITY_DN48324_chr1.CM0476.340.nd<br>c0_g1  |                 | Glyma.04G228000.1 | PROTEIN TRANSPARENT TESTA GLABRA 1                               | 493                         | 570                      | -0.3114 |
| TRINITY_DN49922_chr4.CM0007.970.nc<br>c0_g1  |                 | Glyma.05G127300.2 | 26S PROTEASOME NON-ATPASE REGULATORY SUBUNIT 8                   | 214                         | 247                      | -0.3088 |
| TRINITY_DN41679_chr4.CM0042.2280.nc<br>c0_g1 |                 | Glyma.05G245800.1 | PATHOGENESIS-RELATED THAUMATIN-LIKE PROTEIN-RELATED              | 3                           | 0                        | 4.5957  |
| TRINITY_DN36305_chr1.CM0033.750.nc<br>c0_g1  |                 | Glyma.10G053500.1 | B3 DNA binding domain (B3) // Auxin response factor (Auxin_resp) | 6                           | 0                        | 5.5655  |
| TRINITY_DN38832_LjSGA_024944.1<br>c0_g1      |                 | Glyma.10G210600.1 | B3 DNA binding domain (B3) // Auxin response factor (Auxin_resp) | 28                          | 4                        | 2.6690  |
| TRINITY_DN31599_chr3.LjT10E18.60.nc<br>c0_g1 |                 | Glyma.11G145500.1 | AUXIN RESPONSE FACTOR 10-RELATED                                 | 8                           | 0                        | 5.9729  |
| TRINITY_DN18931_LjSGA_078422.1<br>c0_g1      |                 | Glyma.11G145500.1 | AUXIN RESPONSE FACTOR 10-RELATED                                 | 1                           | 2                        | -1.0110 |
| TRINITY_DN45545_chr3.LjT10E18.60.nc<br>c0_g1 |                 | Glyma.12G174100.1 | AUXIN RESPONSE FACTOR 10-RELATED                                 | 23                          | 2.4                      | 3.3450  |
| TRINITY_DN56369_chr3.CM0590.460.nd<br>c0_g1  |                 | Glyma.13G279600.1 | COP1-INTERACTING PROTEIN 7 (CIP7)                                | 4                           | 0                        | 4.9957  |

| Trinity contig                               | <i>L. japonicus</i> ID | <i>G. max</i> BLAST ID | Gene description   | Counts per gene | Counts per gene (Red) (Yellow) | Log <sub>2</sub> FC |
|--|------------------------|------------------------|--|-----------------|--------------------------------|---------------------|
| TRINITY_DN50775_chr3.CM0711.100.nd<br>c0_g1  |                        | Glyma.13G302300.1      | COUMAROYL-<br>COA:ANTHOCYANIDIN<br>3-O-GLUCOSIDE-6"-O-<br>COUMAROYLTRANSFE<br>RASE 1-RELATED                                       | 0               | 6587.81                        | -15.7376            |
| TRINITY_DN45545_chr3.LjT10E18.60.nc<br>c2_g1 |                        | Glyma.13G325200.1      | AUXIN RESPONSE<br>FACTOR 10-RELATED  | 4               | 0                              | 4.9957              |
| TRINITY_DN52789_chr5.CM0077.690.nc<br>c0_g2  |                        | Glyma.14G072700.1      | Dihydrokaempferol 4-<br>reductase / NADPH-<br>dihydromyricetin<br>reductase  | 18              | 3                              | 2.4363              |
| TRINITY_DN55140_chr5.CM0077.690.nc<br>c0_g1  |                        | Glyma.14G072700.1      | Dihydrokaempferol 4-<br>reductase / NADPH-<br>dihydromyricetin<br>reductase  | 0               | 2                              | -4.1364             |
| TRINITY_DN53766_chr1.CM0410.100.nc<br>c0_g2  |                        | Glyma.18G268100.1      | Isoflavone-7-O-beta-<br>glucoside 6"-O-<br>malonyltransferase /<br>Flavone/flavonol 7-O-<br>beta-D-glucoside<br>malonyltransferase | 187             | 206                            | -0.2415             |
| TRINITY_DN33124_chr1.CM0410.130.nc<br>c0_g1  |                        | Glyma.18G268100.1      | Isoflavone-7-O-beta-<br>glucoside 6"-O-<br>malonyltransferase /<br>Flavone/flavonol 7-O-<br>beta-D-glucoside<br>malonyltransferase | 10              | 6                              | 0.6246              |
| TRINITY_DN57182_chr1.CM0410.130.nc<br>c0_g1  |                        | Glyma.18G268100.1      | Isoflavone-7-O-beta-<br>glucoside 6"-O-<br>malonyltransferase /<br>Flavone/flavonol 7-O-<br>beta-D-glucoside<br>malonyltransferase | 12              | 7                              | 0.6663              |

| Trinity contig                              | <i>L. japonicus</i> ID | <i>G. max</i> BLAST ID | Gene description  | Counts per gene | Counts per gene (Red) (Yellow) | Log <sub>2</sub> FC |
|---|------------------------|------------------------|---|-----------------|--------------------------------|---------------------|
| TRINITY_DN6325_c chr1.CM0410.130.nc<br>0_g1 |                        | Glyma.18G268100.1      | Isoflavone-7-O-beta-glucoside 6''-O-malonyltransferase / Flavone/flavonol 7-O-beta-D-glucoside malonyltransferase | 5               | 1                              | 2.0924              |
| TRINITY_DN53766_chr1.CM0410.80.nc<br>c0_g2  |                        | Glyma.18G268100.1      | Isoflavone-7-O-beta-glucoside 6''-O-malonyltransferase / Flavone/flavonol 7-O-beta-D-glucoside malonyltransferase | 187             | 206                            | -0.2415             |
| TRINITY_DN54421_chr1.CM0410.40.nc<br>c2_g4  |                        | Glyma.18G268600.1      | Isoflavone-7-O-beta-glucoside 6''-O-malonyltransferase / Flavone/flavonol 7-O-beta-D-glucoside malonyltransferase | 407             | 211.72                         | 0.8384              |
| TRINITY_DN53649_chr1.CM0222.40.nc<br>c3_g1  |                        | Glyma.19G106900.1      | WD REPEAT CONTAINING PROTEIN  | 211             | 148                            | 0.4092              |
| TRINITY_DN41800_LjSGA_045968.2<br>c0_g1     |                        | Glyma.20G180000.1      | Glyma.20G180000.1.p - (M=20)<br>PF02362//PF06507 -  | 4               | 0                              | 4.9957              |
| TRINITY_DN45569_LjSGA_045968.2<br>c1_g1     |                        | Glyma.20G180000.1      | Glyma.20G180000.1.p - (M=20)<br>PF02362//PF06507 -  | 1               | 00                             |                     |

**Table S32.** Yellow Stage Trinity Results: Genes for enzymes annotated to the over-represented GO:0009809 (Lignin biosynthetic process, FDR=1). The first two columns list differentially expressed (FDR<0.05) Trinity contigs and gene names as annotated by BLASTing the *Lotus japonicus* genome. The third column is their soybean BLAST homolog, followed by expression at two stages of flower development.

| Trinity contig                       | L. japonicus ID   | G. max BLAST ID                               | Gene description | Counts per gene<br>(Yellow) | Counts per gene (Red) | Log2FC |
|--------------------------------------|-------------------|---|------------------|-----------------------------|-----------------------|--------|
| TRINITY_DN45532_chr2.CM0249.1210.nc  | Glyma.01G173600.1 | LACCASE-11                                    |                  | 23                          | 0                     | 7.4814 |
| c2_g1                                |                   |   |                  |                             |                       |        |
| TRINITY_DN45532_chr2.CM0249.1210.nc  | Glyma.01G173600.1 | LACCASE-11                                    |                  | 17                          | 0                     | 7.0482 |
| c4_g1                                |                   |   |                  |                             |                       |        |
| TRINITY_DN46194_chr4.CM0227.500.nc   | Glyma.02G103500.1 | O-METHYLTRANSFERASE-RELATED                   |                  | 8146                        | 826                   | 3.1995 |
| c0_g1                                |                   |   |                  |                             |                       |        |
| TRINITY_DN51740_LjSGA_017175.2       | Glyma.04G227700.1 | FLAVONE 3'-O-METHYLTRANSFERASE 1              |                  | 1247                        | 281                   | 2.0472 |
| c2_g2                                |                   |   |                  |                             |                       |        |
| TRINITY_DN44781_LjSGA_035751.3       | Glyma.07G089700.1 | Abieta-7,13-dien-18-ol hydroxylase / CYP720B1 |                  | 8110                        | 225                   | 5.0688 |
| c0_g1                                |                   |   |                  |                             |                       |        |
| TRINITY_DN43710_LjSGA_035751.3       | Glyma.07G089700.1 | Abieta-7,13-dien-18-ol hydroxylase / CYP720B1 |                  | 91.32                       | 12                    | 2.8083 |
| c0_g1                                |                   |   |                  |                             |                       |        |
| TRINITY_DN44818_chr4.CM0042.560.nd   | Glyma.07G089800.1 | Cytochrome P450 (p450)                        |                  | 40                          | 2                     | 4.1399 |
| c0_g1                                |                   |   |                  |                             |                       |        |
| TRINITY_DN45532_chr2.CM0249.1210.nc  | Glyma.11G069500.1 | LACCASE-11                                    |                  | 25                          | 0                     | 7.6011 |
| c0_g1                                |                   |   |                  |                             |                       |        |
| TRINITY_DN52386_LjSGA_011490.1       | Glyma.12G019700.1 | ALCOHOL DEHYDROGENASE-RELATED                 |                  | 76                          | 11                    | 2.6730 |
| c0_g1                                |                   |   |                  |                             |                       |        |
| TRINITY_DN34978_chr1.LjB20I01.110.nd | Glyma.14G062300.1 | MULTI-COPPER OXIDASE                          |                  | 11                          | 0                     | 6.4261 |
| c0_g2                                |                   |   |                  |                             |                       |        |
| TRINITY_DN45845_CM0385.150.nd        | Glyma.15G143600.1 | CHITINASE-LIKE PROTEIN                        |                  | 225                         | 15                    | 3.7940 |
| c0_g1                                |                   |   |                  | 1                           |                       |        |

**Table S33.** Red Stage Trinity Results: Genes for enzymes annotated to the over-represented GO:0009809 (Lignin biosynthetic process, FDR=). The first two columns list differentially expressed (FDR<0.05) Trinity contigs and gene names as annotated by BLASTing the *Lotus japonicus* genome. The third column is their soybean BLAST homolog, followed by expression at two stages of flower development.

| Trinity contig                 | <i>L. japonicus</i> ID | <i>G. max</i> BLAST ID | Gene description                              | Counts per gene<br>(Yellow) | Counts per gene<br>(Red) | Log <sub>2</sub> FC |
|--------------------------------|------------------------|------------------------|---|-----------------------------|--------------------------|---------------------|
| TRINITY_DN49460_LjSGA_019127.1 | c0_g1                  | Glyma.17G061000.1      | SPERMIDINE<br>HYDROXYCINNAMOYL<br>TRANSFERASE | 1                           | 786                      | -9.5451             |
| TRINITY_DN45071_LjSGA_021886.2 | c0_g1                  | Glyma.17G171100.1      | O-METHYLTRANSFERASE-<br>RELATED               | 2                           | 72                       | -5.1840             |
| TRINITY_DN49973_LjSGA_030253.1 | c0_g1                  | Glyma.13G141600.1      | S-ADENOSYLMETHIONINE<br>SYNTHASE 3            | 206                         | 707                      | -1.8805             |

**Table S34.** Similarly Expressed Trinity Results: Genes for enzymes annotated to GO:0009813 (Lignin process, FDR=1). The first two columns list Trinity contigs expresand gene names as annotated by BLASTing the *Lotus japonicus* genome. The third column is their soybean BLAST homolog, followed by expression at two stages of flower development.

| Trinity contig                      | L. japonicus ID | G. max BLAST ID   | Gene description                                  | Counts per gene<br>(Yellow) | Counts per gene<br>(Red) | Log2FC  |
|-------------------------------------|-----------------|-------------------|---|-----------------------------|--------------------------|---------|
| TRINITY_DN53597_chr2.CM0249.290.nd  |                 | Glyma.01G169200.1 | Cytochrome P450 CYP2 subfamily                    | 170                         | 92                       | 0.7828  |
| c0_g1                               |                 |                   |   |                             |                          |         |
| TRINITY_DN41837_LjSGA_021886.2      |                 | Glyma.01G187700.1 | O-METHYLTRANSFERASE- RELATED                      | 1831                        | 863                      | 0.9829  |
| c0_g1                               |                 |                   |   |                             |                          |         |
| TRINITY_DN52969_LjSGA_096258.1      |                 | Glyma.03G122000.1 | CYTOCHROME P450 98A3- RELATED                     | 1429                        | 1471                     | -0.1439 |
| c0_g1                               |                 |                   |   |                             |                          |         |
| TRINITY_DN49973_LjSGA_030253.1      |                 | Glyma.03G184000.1 | S-ADENOSYLMETHIONINE SYNTHASE 3                   | 4222                        | 10944                    | -1.4762 |
| c2_g1                               |                 |                   |   |                             |                          |         |
| TRINITY_DN16999_chr1.CM0063.200.nc  |                 | Glyma.05G203000.1 | OMEGA-HYDROXYPALMITATE O-FERULOYL TRANSFERASE     | 0                           | 10                       | -6.3911 |
| c0_g1                               |                 |                   |   |                             |                          |         |
| TRINITY_DN49267_LjT16L08.100.nd     |                 | Glyma.05G233200.1 | Cytochrome P450 CYP2 subfamily                    | 144                         | 441                      | -1.7159 |
| c1_g2                               |                 |                   |   |                             |                          |         |
| TRINITY_DN17837_chr3.LjT40P18.60.nc |                 | Glyma.07G021600.1 | SHIKIMATE O-HYDROXYCINNAMOYLTRANSFERASE           | 5                           | 6                        | -0.3570 |
| c0_g1                               |                 |                   |   |                             |                          |         |
| TRINITY_DN53428_LjSGA_040039.1      |                 | Glyma.07G026300.1 | CINNAMOYL-COA:NADP OXIDOREDUCTASE-LIKE 1- RELATED | 156                         | 46                       | 1.6571  |
| c1_g1                               |                 |                   |   |                             |                          |         |
| TRINITY_DN33065_LjSGA_027787.1      |                 | Glyma.07G050600.1 | NAC DOMAIN-CONTAINING PROTEIN 43- RELATED         | 3                           | 0                        | 4.59573 |
| c0_g1                               |                 |                   |   |                             |                          |         |
| TRINITY_DN33065_LjSGA_027787.1      |                 | Glyma.07G050600.1 | NAC DOMAIN-CONTAINING PROTEIN 43- RELATED         | 0                           | 00                       |         |
| c0_g2                               |                 |                   |   |                             |                          |         |
| TRINITY_DN66560_LjSGA_027787.1      |                 | Glyma.07G050600.1 | NAC DOMAIN-CONTAINING PROTEIN 43- RELATED         | 1                           | 00                       |         |
| c0_g1                               |                 |                   |   |                             |                          |         |
| TRINITY_DN17072_LjSGA_035751.3      |                 | Glyma.07G089700.1 | Abieta-7,13-dien-18-ol hydroxylase / CYP720B1     | 102                         | 31                       | 1.61231 |
| c0_g1                               |                 |                   |   |                             |                          |         |
| TRINITY_DN49973_LjSGA_140570.1      |                 | Glyma.07G233800.1 | S-ADENOSYLMETHIONINE SYNTHASE 4                   | 13                          | 15                       | -0.3058 |
| c3_g1                               |                 |                   |   |                             |                          |         |
| TRINITY_DN51745_chr3.CM0452.240.nd  |                 | Glyma.08G220200.1 | SHIKIMATE O-HYDROXYCINNAMOYLTRANSFERASE           | 568                         | 908                      | -0.7788 |
| c0_g1                               |                 |                   |   |                             |                          |         |

| Trinity contig                                | <i>L. japonicus</i> ID | <i>G. max</i> BLAST ID | Gene description  | Counts per gene | Counts per gene (Red) (Yellow) | Log <sub>2</sub> FC |
|---|------------------------|------------------------|---|-----------------|--------------------------------|---------------------|
| TRINITY_DN42496_chr1.CM0410.190.nc<br>c1_g1   |                        | Glyma.08G246100.1      | LRR RECEPTOR-LIKE SERINE/THREONINE- PROTEIN KINASE RPK2 | 10              | 7                              | 0.406346            |
| TRINITY_DN47225_chr1.CM0410.190.nc<br>c0_g1   |                        | Glyma.08G246100.1      | LRR RECEPTOR-LIKE SERINE/THREONINE- PROTEIN KINASE RPK2 | 24              | 5                              | 2.13427             |
| TRINITY_DN37023_LjSGA_030670.1<br>c0_g2       |                        | Glyma.08G311800.1      | SPERMIDINE HYDROXYCINNAMOYL TRANSFERASE                 | 4               | 1                              | 1.77955             |
| TRINITY_DN37010_chr1.CM0147.770.nc<br>c0_g1   |                        | Glyma.08G359100.1      | LACCASE-17  | 1               | 1                              | -0.0908             |
| TRINITY_DN32484_LjSGA_011490.1<br>c0_g1       |                        | Glyma.12G019700.1      | ALCOHOL DEHYDROGENASE- RELATED                          | 5               | 4                              | 0.21381             |
| TRINITY_DN41325_LjSGA_011490.1<br>c0_g1       |                        | Glyma.12G019700.1      | ALCOHOL DEHYDROGENASE- RELATED                          | 5               | 1                              | 2.09241             |
| TRINITY_DN41325_LjSGA_011490.1<br>c0_g2       |                        | Glyma.12G019700.1      | ALCOHOL DEHYDROGENASE- RELATED                          | 4               | 6                              | -0.6698             |
| TRINITY_DN49071_LjSGA_015763.2<br>c2_g3       |                        | Glyma.12G019700.1      | ALCOHOL DEHYDROGENASE- RELATED                          | 2               | 2                              | -0.0961             |
| TRINITY_DN54121_LjSGA_013806.1<br>c0_g1       |                        | Glyma.12G109800.1      | O-METHYLTRANSFERASE FAMILY PROTEIN                      | 124             | 228                            | -0.9800             |
| TRINITY_DN49611_LjT45M09.100.nd<br>c0_g1      |                        | Glyma.13G369800.1      | NAD DEPENDENT EPIMERASE/DEHYDRATA SE                    | 1434            | 375                            | 1.83260             |
| TRINITY_DN35253_chr1.LjB20I01.110.nd<br>c1_g1 |                        | Glyma.14G062300.1      | MULTI-COPPER OXIDASE                                    | 3               | 0                              | 4.59573             |
| TRINITY_DN50309_LjSGA_070619.1<br>c0_g1       |                        | Glyma.15G059500.1      | ALCOHOL DEHYDROGENASE RELATED                           | 48              | 24                             | 0.89451             |
| TRINITY_DN51988_LjT35D18.30.nc<br>c1_g1       |                        | Glyma.15G190500.1      | S-ADENOSYLMETHIONINE SYNTHASE 4                         | 8441            | 2869                           | 1.45468             |

| Trinity contig                               | <i>L. japonicus</i> ID | <i>G. max</i> BLAST ID | Gene description  | Counts per gene | Counts per gene (Red) | Log <sub>2</sub> FC (Yellow) |
|--|------------------------|------------------------|---|-----------------|-----------------------|------------------------------|
| TRINITY_DN2300_c chr1.CM0593.100.nd<br>0_g1  |                        | Glyma.16G047300.1      | Protein kinase domain<br>(Pkinase) // Leucine rich<br>repeat N-terminal domain<br>(LRRNT_2) // Leucine rich<br>repeat (LRR_8) | 6               | 4                     | 0.47076                      |
| TRINITY_DN38374_chr1.CM0593.100.nd<br>c0_g1  |                        | Glyma.16G047300.1      | Protein kinase domain<br>(Pkinase) // Leucine rich<br>repeat N-terminal domain<br>(LRRNT_2) // Leucine rich<br>repeat (LRR_8) | 0               | 10                    | -6.3911                      |
| TRINITY_DN38272_chr1.CM0593.40.nd<br>c0_g1   |                        | Glyma.16G047300.1      | Protein kinase domain<br>(Pkinase) // Leucine rich<br>repeat N-terminal domain<br>(LRRNT_2) // Leucine rich<br>repeat (LRR_8) | 10              | 0                     | 6.29031                      |
| TRINITY_DN49071_LjT48C16.160.nd<br>c1_g1     |                        | Glyma.18G091500.1      | ALCOHOL<br>DEHYDROGENASE-<br>RELATED  | 93              | 70                    | 0.30726                      |
| TRINITY_DN37010_chr1.CM0147.770.nc<br>c1_g1  |                        | Glyma.18G177200.1      | MULTI-COPPER OXIDASE  | 4               | 0                     | 4.99577                      |
| TRINITY_DN47225_chr1.CM0410.190.nc<br>c1_g1  |                        | Glyma.18G267000.1      | LRR RECEPTOR-LIKE<br>SERINE/THREONINE-<br>PROTEIN KINASE RPK2   | 61.37           | 47                    | 0.27337                      |
| TRINITY_DN47225_chr1.CM0410.190.nc<br>c1_g2  |                        | Glyma.18G267000.1      | LRR RECEPTOR-LIKE<br>SERINE/THREONINE-<br>PROTEIN KINASE RPK2   | 1.63            | 0                     | 4.04029                      |
| TRINITY_DN50519_chr5.CM0200.1180.nc<br>c0_g1 |                        | Glyma.20G128600.1      | CINNAMYL ALCOHOL<br>DEHYDROGENASE 4-<br>RELATED   | 423             | 183                   | 1.1061                       |
| TRINITY_DN58801_LjSGA_075859.1<br>c0_g1      |                        | Glyma.20G175500.1      | No apical meristem (NAM)<br>protein (NAM)   | 4               | 1                     | 1.7795                       |

**Table S35.** *L. filicaulis* Yellow Stage Trinity Results: Genes for enzymes annotated to GO:00016117 (Carotenoid biosynthetic process, FDR=0.11). The first two columns list differentially expressed (FDR<0.05) Trinity contigs and gene names as annotated by BLASTing the *Lotus japonicus* genome. The third column is their soybean BLAST homolog, followed by expression at two stages of flower development.

| Trinity contig | <i>L. japonicus</i> ID    | <i>G. max</i> BLAST ID | Gene description   | Counts per gene<br>(Yellow) | Counts per gene (Red) | Log2FC |
|----------------|---------------------------|------------------------|--|-----------------------------|-----------------------|--------|
| TR50146 c0_g1  | chr3.CM0996.270.r2.a      | Glyma.01G028900.1      | ATP-CITRATE<br>SYNTHASE  | 34.96                       | 2                     | 3.7015 |
| TR45451 c0_g1  | chr4.CM0570.280.r2.a      | Glyma.01G064200.1      | AGAMOUS-LIKE MADS-<br>BOX PROTEIN AGL8-<br>RELATED                         | 1414                        | 151                   | 2.8697 |
| TR18321 c0_g1  | LjSGA_059500.1            | Glyma.01G186200.1      | ZEAXANTHIN<br>EPOXIDASE,<br>CHLOROPLASTIC                                  | 310                         | 63                    | 1.9404 |
| TR32448 c0_g2  | chr5.CM1125.160.r2.m      | Glyma.02G149400.1      | Not Available  | 10.54                       | 0.64                  | 3.1901 |
| TR52234 c0_g2  | LjSGA_013562.2            | Glyma.02G188200.1      | Prolycopene isomerase<br>/ CRTISO  | 6.01                        | 18.45                 | 2.2958 |
| TR33198 c0_g1  | chr2.LjT08I01.60.r2.a     | Glyma.02G240200.1      | PHYTOENE SYNTHASE,<br>CHLOROPLASTIC  | 4765                        | 1049                  | 1.8268 |
| TR64960 c0_g1  | chr3.CM0282.920.r2.m      | Glyma.06G238100.1      | SQUAMOSA<br>PROMOTER-BINDING-<br>LIKE PROTEIN 4-<br>RELATED                | 117                         | 1                     | 6.3654 |
| TR22838 c0_g1  | chr5.CM0456.560.r2.m      | Glyma.09G132200.1      | Beta-carotene 3-<br>hydroxylase / Beta-<br>carotene 3,3'-<br>monooxygenase | 4                           | 00                    |        |
| TR60004 c0_g2  | chr5.CM1077.670.r2.m      | Glyma.10G062900.1      | Carotene epsilon-<br>monooxygenase / LUT1                                  | 33.2                        | 8                     | 7.9261 |
| TR3908 c0_g1   | chr5.CM1323.150.r2.m      | Glyma.10G160500.1      | 9,9'-di-cis-zeta-carotene<br>desaturase / Zeta-<br>carotene desaturase     | 21.7                        | 5.36                  | 1.9696 |
| TR53492 c0_g1  | chr4.CM0044.50.r2.m       | Glyma.11G252500.1      | Zeta-carotene<br>isomerase / 15-cis-zeta-<br>carotene isomerase            | 446                         | 18                    | 4.2661 |
| TR15422 c0_g1  | chr1.CM0104.3370.r2.<br>m | Glyma.16G036100.1      | POLYRIBONUCLEOTIDE<br>NUCLEOTIDYLTRANSF<br>ERASE 1,<br>CHLOROPLASTIC       | 27.03                       | 0                     | 7.5950 |
| TR18321 c1_g1  | LjSGA_078276.1            | NA                     | zeaxanthin epoxidase   | 4274.46                     | 305.41                | 3.4517 |
| TR18321 c2_g1  | LjSGA_024159.1            | NA                     | zeaxanthin epoxidase   | 366                         | 77                    | 1.8908 |

**Table S36.** *L. filicaulis* Similarly Expressed Trinity Results: Genes for enzymes annotated to GO:00016117 (Carotenoid biosynthetic process, FDR=1). The first two columns list Trinity contigs expresand gene names as annotated by BLASTing the *Lotus japonicus* genome. The third column is their soybean BLAST homolog, followed by expression at two stages of flower development.

| Trinity contig | <i>L. japonicus</i> ID    | <i>G. max</i> BLAST ID | Gene description   | Counts per gene | Counts per gene (Red) | Log2FC (Yellow) |
|----------------|---------------------------|------------------------|--|-----------------|-----------------------|-----------------|
| TR52234 c0_g1  | LjSGA_013562.2            | Glyma.02G188200.1      | ATP-CITRATE<br>SYNTHASE  | 6.01            | 18.45                 | -1.9168         |
| TR15191 c0_g1  | chr6.CM0013.1810.r2.d     | Glyma.09G066200.1      | Not Available  | 143             | 46                    | 1.27775         |
| TR7103 c0_g1   | LjSGA_075584.1            | Glyma.07G273600.1      | Prolycopene isomerase<br>/ CRTISO  | 129.46          | 16                    | 3.93057         |
| TR32448 c0_g1  | chr5.CM1125.160.r2.m      | Glyma.02G149400.1      | Glutathione transferase<br>/ S-<br>(hydroxyalkyl)glutathio<br>ne lyase     | 10.54           | 0.64                  | 2.97110         |
| TR15422 c0_g1  | chr1.CM0104.3370.r2.<br>m | Glyma.16G036100.1      | LYCOPENE BETA<br>CYCLASE,<br>CHLOROPLASTIC                                 | 27.03           | 0                     | 1.93647         |
| TR51506 c0_g1  | chr6.CM0367.660.r2.d      | Glyma.09G008500.1      | Prolycopene isomerase<br>/ CRTISO  | 2279            | 594                   | 1.58315         |
| TR42882 c0_g1  | LjSGA_050034.1            | Glyma.09G000600.1      | MONOOXYGENASE  | 4               | 0                     | 4.88247         |
| TR64376 c0_g1  | LjSGA_046377.1            | Glyma.02G283400.1      | UBIQUINOL OXIDASE 4,<br>CHLOROPLASTIC/CHR<br>OMOPLASTIC                    | 222             | 571                   | -1.7188         |
| TR61986 c0_g1  | LjSGA_045520.1            | Glyma.03G128900.1      | LYCOPENE EPSILON<br>CYCLASE,<br>CHLOROPLASTIC                              | 635             | 164                   | 1.59588         |
| TR60004 c0_g1  | chr5.CM1077.670.r2.m      | Glyma.10G062900.1      | Beta-carotene 3-<br>hydroxylase / Beta-<br>carotene 3,3'-<br>monooxygenase | 33.2            | 8                     | 1.67434         |
| TR13886 c0_g1  | chr5.CM1323.150.r2.m      | Glyma.10G160500.1      | PROTEIN LUTEIN<br>DEFICIENT 5,<br>CHLOROPLASTIC                            | 21.7            | 5.36                  | 1.75885         |
| TR33253 c0_g1  | chr1.CM1413.250.r2.a      | Glyma.09G252800.1      | Carotene epsilon-<br>monooxygenase / LUT1                                  | 963             | 463                   | 0.69985         |
| TR61205 c0_g1  | chr6.LjT13N05.10.r2.m     | Glyma.11G253000.1      | 9,9'-di-cis-zeta-carotene<br>desaturase / Zeta-<br>carotene desaturase     | 1488            | 691                   | 0.7499          |

| Trinity contig | <i>L. japonicus</i> ID | <i>G. max</i> BLAST ID | Gene description  | Counts per gene | Counts per gene (Red) | Log <sub>2</sub> FC (Yellow) |
|----------------|------------------------|------------------------|---|-----------------|-----------------------|------------------------------|
| TR6924 c0_g1   | chr2.CM0545.170.r2.m   | Glyma.14G046600.1      | 15-cis-phytoene desaturase / Plant-type phytoene desaturase | 81              | 23                    | 1.45539                      |
| TR24925 c0_g1  | LjSGA_031176.1         | Glyma.17G174500.1      | LYCOPENE BETA/EPSILON CYCLASE PROTEIN                       | 241             | 58                    | 1.6964                       |
| TR22838 c0_g1  | chr5.CM0456.560.r2.m   | Glyma.09G132200.1      | POLYRIBONUCLEOTIDE NUCLEOTIDYLTRANSF ERASE 1, CHLOROPLASTIC | 4               | 0                     | 4.88247                      |
| TR50146 c2_g1  | chr2.CM0008.330.r2.m   | Glyma.01G028900.1      | ZEAXANTHIN EPOXIDASE, CHLOROPLASTIC                         | 840             | 625                   | 0.06998                      |

**Table S37.** *L. sessilifolius* Yellow Stage Trinity Results: Genes for enzymes annotated to GO:00016117 (Carotenoid biosynthetic process, FDR=0.00027). The first two columns list differentially expressed (FDR<0.05) Trinity contigs and gene names as annotated by BLASTing the *Lotus japonicus* genome. The third column is their soybean BLAST homolog, followed by expression at two stages of flower development.

| Trinity contig                                | <i>L. japonicus</i> ID | <i>G. max</i> BLAST ID | Gene description  | Counts per gene | Counts per gene (Red) | Log <sub>2</sub> FC (Yellow) |
|---|------------------------|------------------------|---|-----------------|-----------------------|------------------------------|
| TRINITY_DN53748_LjSGA_045520.1<br>c0_g1       |                        | Glyma.03G128900.1      | LYCOPENE BETA CYCLASE,<br>CHLOROPLASTIC   | 38.81           | 0                     | 8.240016                     |
| TRINITY_DN50287_LjSGA_046377.1<br>c0_g1       |                        | Glyma.08G306200.1      | Glutathione transferase / S-<br>(hydroxyalkyl)glutathione lyase // 15-cis-phytoene synthase / PSase | 102             | 10                    | 3.232895                     |
| TRINITY_DN49175_LjT17B03.90.nd<br>c0_g1       |                        | Glyma.09G008500.1      | UBIQUINOL OXIDASE 4,<br>CHLOROPLASTIC/CHROMOPLASTIC   | 1268            | 232                   | 2.347616                     |
| TRINITY_DN50928_LjSGA_055879.1<br>c1_g1       |                        | Glyma.09G233300.1      | Protein disulfide-isomerase / S-S rearrangase   | 187             | 42                    | 2.0493                       |
| TRINITY_DN48832_LjSGA_013562.2<br>c0_g1       |                        | Glyma.10G086700.1      | Prolycopene isomerase / CRTISO  | 249             | 43                    | 2.428307                     |
| TRINITY_DN50878_chr2.CM0018.70.nc<br>c0_g1    |                        | Glyma.11G016200.1      | CYTOCHROME P450 97B3, CHLOROPLASTIC   | 170             | 13                    | 3.59459                      |
| TRINITY_DN50044_chr6.LjT13N05.100.nc<br>c0_g1 |                        | Glyma06g11820.1        | 15-cis-phytoene desaturase / Plant-type phytoene desaturase   | 2187            | 425                   | 2.260955                     |
| TRINITY_DN53304_LjT01G11.200.nd<br>c1_g1      |                        | Glyma.17G174500.1      | ZEAXANTHIN EPOXIDASE,<br>CHLOROPLASTIC  | 73              | 14                    | 2.270513                     |
| TRINITY_DN38311_LjT40E15.150.nc<br>c0_g2      |                        | Glyma.20G227900.1      | 9,9'-di-cis-zeta-carotene desaturase / Zeta-carotene desaturase                                     | 8.33            | 7.97                  | -0.10057                     |
| TRINITY_DN53720_LjSGA_021331.1<br>c2_g1       |                        | Glyma10g07210.1        | carotene epsilon-monooxygenase  | 204             | 21                    | 3.170623                     |
| TRINITY_DN53304_LjSGA_078276.1<br>c3_g1       |                        | Glyma11g05960.1        | zeaxanthin epoxidase  | 185             | 12                    | 3.830864                     |
| TRINITY_DN53304_LjSGA_024159.1<br>c5_g1       |                        | Glyma17g20020.1        | zeaxanthin epoxidase  | 2027            | 321                   | 2.556112                     |

Table S38 Red stage *L. filicaulis*: 19 contigs with greatest magnitude of log fold-change recovered from entire transcriptome.

| Trinity_ID    | LogFC    | Lotus1.0_ID         | Lotus2.5_ID           | Annotation (UniProt and AnnoMine)  |
|---------------|----------|---------------------|-----------------------|--|
| TR7064 c2_g1  | -16.5797 | NA                  | LjT31N05.100.r2.d     | SJCHGC01014 protein<br>(Fragment)<br>OS=Schistosoma<br>japonicum         |
| TR34298 c0_g1 | -13.9764 | NA                  | chr4.CM0026.290.r2.m  | Putative senescence-associated protein<br>(Fragment) OS=Pisum<br>sativum |
| TR50987 c0_g1 | -13.3631 | LjSGA_061080.1.1    | chr4.CM0026.290.r2.m  | Putative senescence-associated protein<br>(Fragment) OS=Pisum<br>sativum |
| TR3216 c0_g3  | -13.3391 | LjSGA_089366.0.1    | LjSGA_089366.0.1      | NA   |
| TR29498 c0_g1 | -13.3057 | chr4.CM0042.1410.nc | chr4.CM0042.1350.r2.m | Dynamin OS=Cucumis<br>melo subsp. melo                                   |
| TR48381 c3_g1 | -13.1261 | chr5.CM1439.180.nd  | chr5.CM1439.390.r2.m  | RING/U-box<br>superfamily protein  |
| TR42412 c0_g1 | -13.1231 | LjT03L03.160.nd     | chr5.CM0696.1060.r2.a | NA   |
| TR53102 c0_g1 | -13.1113 | chr4.CM0229.70.nc   | chr4.CM0229.70.r2.m   | Probable<br>polygalacturonase<br>At2g43860                               |
| TR40587 c0_g1 | -13.0933 | chr3.CM0129.380.nd  | chr3.CM0129.50.r2.d   | NA   |
| TR2085 c0_g1  | -13.0644 | LjSGA_078330.2      | chr4.CM0026.290.r2.m  | NA   |
| TR1538 c0_g2  | -12.7012 | LjSGA_125503.1      | LjT31N05.100.r2.d     | SJCHGC01014 protein<br>(Fragment)<br>OS=Schistosoma<br>japonicum         |
| TR3124 c0_g3  | -12.6853 | chr5.CM0040.150.nd  | chr5.CM0040.150.r2.d  | Protein of unknown<br>function DUF1677,<br>plant                         |
| TR4260 c0_g1  | -12.5775 | chr4.CM0288.1020.nd | chr4.CM0288.1020.r2.d | 1-aminocyclopropane-<br>1-carboxylate oxidase<br>OS=Trifolium repens     |
| TR14717 c0_g3 | -12.558  | LjSGA_070603.1      | chr3.CM0213.540.r2.m  | ATP-binding cassette<br>transporter, putative<br>OS=Ricinus communis     |
| TR20264 c0_g1 | -12.4303 | LjSGA_025923.1      | LjSGA_025923.1        | NA   |

| <b>Trinity_ID</b> | <b>LogFC</b> | <b>Lotus1.0_ID</b>   | <b>Lotus2.5_ID</b>     | <b>Annotation (UniProt and AnnoMine)</b>                |
|-------------------|--------------|----------------------|------------------------|---|
| TR48491 c0_g1     | -12.3818     | chr1.LjT16003.100.nd | chr1.LjT16003.100.r2.d | Legumain-like protease (Precursor)<br>OS=Ixodes ricinus |
| TR46724 c0_g3     | -12.3162     | chr5.CM0200.550.nc   | chr5.CM0200.2620.r2.m  | Zinc finger protein<br>OS=Cicer arietinum               |
| TR49591 c0_g1     | -12.3111     | LjT07J01.20.nd       | chr5.CM0052.370.r2.a   | GmCK2p OS=Glycine max                                   |
| TR23809 c0_g6     | -12.1554     | LjSGA_022872.2       | LjSGA_022872.2         | Elongation factor 1-alpha OS=Ricinus communis           |

Table S39 Yellow stage *L. filicaulis*: 19 contigs with greatest magnitude of log fold-change recovered from entire transcriptome.

| <b>Trinity_ID</b> | <b>LogFC</b> | <b>Lotus1.0_ID</b>     | <b>Lotus2.5_ID</b> | <b>Annotation<br/>(Annomine and<br/>UniProt)</b>                  |
|-------------------|--------------|------------------------|--------------------|---|
| TR24089 c0_g1     | 14.89616     | chr1.CM0088.370.r2.d   | NA                 | NA  |
| TR15262 c0_g2     | 14.61414     | chr5.CM0072.330.r2.d   | NA                 | Flowering-promoting factor 1-like protein 1                       |
| TR43132 c0_g1     | 14.19693     | LjSGA_013861.2         | LjSGA_013861.2     | Putative auxin efflux carrier protein 6<br>OS=Medicago truncatula |
| TR55706 c0_g1     | 14.16986     | chr6.CM0679.380.r2.m   | NA                 | member of Alpha-Expansin Gene Family                              |
| TR47113 c0_g4     | 13.92623     | LjSGA_045866.1         | LjSGA_045866.1     | Early-responsive to dehydration<br>OS=Medicago truncatula         |
| TR33662 c0_g2     | 13.54451     | LjSGA_020750.1         | LjSGA_020750.1     | ribonuclease T2   |
| TR16126 c0_g1     | 13.45947     | LjSGA_032186.1         | LjSGA_032186.1     | NA  |
| TR33992 c0_g1     | 13.24861     | chr1.LjT44L17.240.r2.d | NA                 | NA  |
| TR50837 c0_g1     | 13.23161     | LjSGA_071404.1         | LjSGA_071404.1     | Lachrymatory-factor synthase, putative<br>OS=Ricinus communis     |
| TR56525 c0_g2     | 13.20029     | chr3.CM0152.240.r2.m   | NA                 | Beta-amylase  |
| TR17308 c2_g2     | 13.02148     | chr6.CM0037.300.r2.m   | NA                 | Cellulose synthase 3<br>OS=Eucalyptus grandis                     |
| TR63082 c0_g1     | 12.93094     | LjSGA_009749.1         | LjSGA_009749.1     | Putative ripening related protein<br>OS=Cicer arietinum           |
| TR50996 c0_g1     | 12.92962     | chr3.CM0129.210.r2.d   | NA                 | TPR domain protein<br>OS=Arthrosphaera platensis NIES-39          |
| TR48869 c0_g3     | 12.91239     | LjSGA_012731.1         | LjSGA_012731.1     | DEAD (Asp-Glu-Ala-Asp) box polypeptide 5<br>OS=Mus musculus       |

| <b>Trinity_ID</b> | <b>LogFC</b> | <b>Lotus1.0_ID</b>   | <b>Lotus2.5_ID</b> | <b>Annotation (UniProt and AnnoMine)</b>                                      |
|-------------------|--------------|----------------------|--------------------|---|
| TR3230 c0_g2      | 12.8773      | chr5.CM0052.240.r2.d | NA                 | RANBP2-like and<br>GRIP domain-containing protein<br>5/6                      |
| TR16751 c0_g1     | 12.83714     | chr6.CM0066.630.r2.m | NA                 | NA  |
| TR53791 c0_g1     | 12.81876     | LjSGA_020451.1       | LjSGA_020451.1     | WD repeat-containing protein<br>OS=Medicago truncatula                        |
| TR8063 c0_g1      | 12.76956     | chr4.CM0126.800.r2.d | NA                 | Glucose-methanol-choline oxidoreductase<br>OS=Halococcus salifodinae DSM 8989 |
| TR49365 c1_g1     | 12.73229     | chr3.CM0136.330.r2.m | NA                 | Fasciclin-like AGP 11<br>OS=Populus canescens                                 |

Table S40 Red stage *L. sessilifolius* 19 contigs with greatest magnitude of log fold-change recovered from entire transcriptome.

| Trinity_ID            | LogFC    | Lotus1.0_ID          | Lotus2.5_ID           | Annotation<br>(Annomine and<br>Uniprot)                             |
|-----------------------|----------|----------------------|-----------------------|---|
| TRINITY_DN50775_c0_g1 | -15.7376 | chr3.CM0711.100.nd   | chr3.CM0711.100.r2.d  | Malonyl-coenzyme:anthocyanin 5-O-glucoside-6''-O-malonyltransferase |
| TRINITY_DN45435_c0_g1 | -14.2652 | chr4.CM0387.330.nc   | chr4.CM0387.1100.r2.m | Thebaine 6-O-demethylase  |
| TRINITY_DN11784_c0_g2 | -13.6968 | LjT33L17.60.nc       | chr2.CM0660.220.r2.m  | Bark storage protein A  |
| TRINITY_DN54303_c0_g1 | -12.8579 | LjSGA_138785.1       | LjSGA_138785.1        | Raffionse synthase 2 OS=Glycine max                                 |
| TRINITY_DN49759_c0_g1 | -12.802  | LjSGA_010954.1       | LjSGA_010954.1        | Catalytic, putative OS=Ricinus communis                             |
| TRINITY_DN50868_c0_g1 | -12.5777 | chr2.CM1150.550.nd   | chr2.CM0028.320.r2.d  | Metalloendoproteinase 1   |
| TRINITY_DN52783_c0_g1 | -12.3214 | chr4.CM0307.20.nc    | chr4.CM0307.20.r2.m   | DNA polymerase III PolC-type  |
| TRINITY_DN40888_c0_g2 | -11.9258 | chr1.CM0141.350.nd   | chr1.CM0141.360.r2.a  | R2R3 MYB related transcription factor OS=Ipomoea batatas            |
| TRINITY_DN16795_c0_g1 | -11.8435 | LjT03L03.160.nd      | chr5.CM0696.1060.r2.a | Peroxidase OS=Populus trichocarpa                                   |
| TRINITY_DN47218_c0_g1 | -11.5483 | chr1.LjT43005.150.nd | chr1.LjT43005.70.r2.a | pathogenesis-related family protein                                 |
| TRINITY_DN42182_c0_g1 | -11.5201 | LjSGA_034431.0.1     | LjSGA_034431.0.1      | TIFY Transcription factor   |
| TRINITY_DN50876_c1_g1 | -11.4787 | LjT03E06.40.nd       | chr1.CM0012.70.r2.d   | Protein of unknown function DUF3741                                 |
| TRINITY_DN48639_c0_g2 | -11.4787 | chr3.CM0164.80.nd    | chr3.CM0164.80.r2.d   | dichloro-2,5-cyclohexadiene-1,4-diol dehydrogenase                  |
| TRINITY_DN49642_c0_g1 | -11.4448 | LjSGA_111612.1.2     | chr4.CM0007.970.r2.a  | Putative senescence-associated protein (Fragment) OS=Ipomoea nil    |

| <b>Trinity_ID</b>     | <b>LogFC</b> | <b>Lotus1.0_ID</b> | <b>Lotus2.5_ID</b>     | <b>Annotation (UniProt and AnnoMine)</b>                 |
|-----------------------|--------------|--------------------|------------------------|--|
| TRINITY_DN34011_c0_g1 | -11.4362     | LjSGA_053993.1     | LjSGA_053993.1         | Beta-fructofuranosidase<br>OS=Citrus sinensis            |
| TRINITY_DN48126_c0_g1 | -11.3968     | LjT45L18.200.nd    | LjT45L18.200.r2.d      | Proteinase inhibitor I3,<br>Kunitz legume                |
| TRINITY_DN50442_c0_g1 | -11.3789     | LjSGA_147445.1.1   | chr4.LjT13G24.170.r2.a | SJCHGC01393 protein<br>OS=Schistosoma<br>japonicum       |
| TRINITY_DN49028_c1_g1 | -11.3333     | chr1.CM0295.60.nd  | chr1.CM0295.140.r2.m   | Phosphate transporter<br>OS=Lotus japonicus              |
| TRINITY_DN53079_c2_g1 | -11.3287     | LjSGA_008068.1     | LjSGA_008068.1         | D-galactose<br>transporter GalP<br>OS=Edwardsiella tarda |

Table S41 Yellow stage *L. sessilifolius*: 19 contigs with greatest magnitude of log fold-change recovered from entire transcriptome.

| <b>Trinity_ID</b>     | <b>LogFC</b> | <b>Lotus1.0_ID</b>  | <b>Lotus2.5_ID</b>    | <b>Annotation<br/>(Annomine and<br/>UniProt)</b>   |
|-----------------------|--------------|---------------------|-----------------------|--|
| TRINITY_DN11722_c0_g1 | 11.10024     | chr1.CM0141.260.nc  | chr1.CM0141.260.r2.m  | Expansin-A15<br>Ribose-phosphate<br>pyrophosphokinase<br>3 OS=Capsaspora<br>owczarzaki (strain |
| TRINITY_DN51824_c2_g1 | 10.13103     | LjSGA_043454.1      | LjSGA_043454.1        | ATCC 30864)<br>Homeodomain-<br>related (Fragment)<br>OS=Medicago                               |
| TRINITY_DN48232_c0_g1 | 10.02803     | chr1.CM0088.1030.nc | chr1.CM0088.1030.r2.a | truncatula<br>Calmodulin-binding<br>protein<br>OS=Medicago                                     |
| TRINITY_DN43262_c0_g2 | 9.784361     | chr6.LjT10I04.70.nd | chr6.CM0778.70.r2.d   | truncatula<br>Gibberellin induced<br>protein<br>OS=Medicago                                    |
| TRINITY_DN51657_c0_g1 | 9.652068     | chr1.CM0398.130.nd  | chr1.CM0398.250.r2.d  | truncatula<br>Glucan 1,3-beta-<br>glucosidase<br>OS=Medicago                                   |
| TRINITY_DN43721_c0_g2 | 9.624089     | LjSGA_011684.1      | LjSGA_011684.1        | truncatula<br>Disease resistance<br>response protein 1   |
| TRINITY_DN47886_c0_g1 | 9.595556     | LjSGA_025651.1.1    | chr3.CM2163.270.r2.m  | OS=Glycine max   |
| TRINITY_DN52257_c0_g4 | 8.907039     | chr6.CM1829.200.nc  | chr6.CM0139.1580.r2.m | NA<br>energized vacuolar<br>membrane proton  |
| TRINITY_DN46161_c0_g1 | 8.907039     | chr4.CM0219.190.nc  | chr4.CM0219.190.r2.m  | pump   |
| TRINITY_DN40465_c0_g3 | 8.811031     | chr1.CM0009.350.nc  | chr1.CM0009.350.r2.m  | CER1-like 2  |
| TRINITY_DN34483_c0_g2 | 8.760519     | LjT26P12.80.nd      | LjT26P12.100.r2.a     | NA<br>Heparanase-like<br>protein<br>OS=Medicago  |
| TRINITY_DN47039_c0_g2 | 8.708175     | chr4.CM0219.380.nd  | chr4.CM0219.380.r2.d  | truncatula   |
| TRINITY_DN47671_c0_g1 | 8.708175     | LjSGA_043568.1      | LjSGA_043568.1        | NA   |

| <b>Trinity_ID</b>     | <b>LogFC</b> | <b>Lotus1.0_ID</b> | <b>Lotus2.5_ID</b>    | <b>Annotation (UniProt and AnnoMine)</b>                                |
|-----------------------|--------------|--------------------|-----------------------|---|
| TRINITY_DN40925_c0_g3 | 8.681273     | LjSGA_031888.1.1   | chr1.CM0104.3590.r2.m | alpha/beta-Hydrolases<br>superfamily protein<br>PMR5 N-terminal         |
| TRINITY_DN42547_c0_g1 | 8.681273     | LjSGA_027078.2     | LjSGA_027078.2        | domain<br>Major facilitator<br>superfamily domain,<br>general substrate |
| TRINITY_DN36333_c0_g1 | 8.65386      | LjSGA_092888.1     | LjSGA_092888.1        | transporter<br>xylosidase/alpha-L-arabinofuranosidase                   |
| TRINITY_DN25191_c0_g1 | 8.625916     | chr5.CM0239.470.nd | chr5.CM0239.440.r2.d  | 2   |
| TRINITY_DN44884_c1_g1 | 8.625916     | CM0466.10.nd       | chr5.CM0466.460.r2.d  | NA  |
| TRINITY_DN46831_c0_g2 | 8.568349     | LjSGA_031037.1     | chr4.CM0387.700.r2.a  | NA  |

**Table S42** Results of hand pollination experiment. Hand pollinated treatments are denoted by P, controls are denoted by N.

| Treatment | Plant number | Days to PACC from anthesis (mean of 3 reps) |
|-----------|--------------|---|
| P         | 1            | 3.33333                                     |
| P         | 2            | 3.33333                                     |
| P         | 3            | 3   |
| P         | 4            | 3   |
| P         | 5            | 2.66667                                     |
| P         | 6            | 3.33333                                     |
| P         | 7            | 2.33333                                     |
| P         | 8            | 2.66667                                     |
| P         | 9            | 2.33333                                     |
| P         | 10           | 2.33333                                     |
| P         | 11           | 2.33333                                     |
| P         | 12           | 2.66667                                     |
| P         | 13           | 2   |
| P         | 14           | 2   |
| P         | 15           | 2   |
| P         | 16           | 2.66667                                     |
| P         | 17           | 2.33333                                     |
| P         | 18           | 2   |
| P         | 19           | 2   |
| P         | 20           | 2   |
| P         | 21           | 2   |
| N         | 1            | 5   |
| N         | 2            | 5.33333                                     |
| N         | 3            | 4.6667                                      |
| N         | 4            | 4.66667                                     |
| N         | 5            | 4.66667                                     |
| N         | 6            | 5   |

| Treatment | Plant number | Days to PACC from anthesis (mean of 3 reps) |
|-----------|--------------|---|
| N         | 7            | 4.66667                                     |
| N         | 8            | 4.33333                                     |
| N         | 9            | 4.33333                                     |
| N         | 10           | 4   |
| N         | 11           | 4.33333                                     |
| N         | 12           | 4.66667                                     |
| N         | 13           | 4   |
| N         | 14           | 4   |
| N         | 15           | 4.66667                                     |
| N         | 16           | 4   |
| N         | 17           | 5   |
| N         | 18           | 5   |
| N         | 19           | 4.33333                                     |
| N         | 20           | 5   |
| N         | 21           | 4.66667                                     |

Mean number of days to observe colour change in hand pollinated treatment =  **$2.5 \pm 0.2$  days**

Mean number of days to observe colour change in control =  **$4.6 \pm 0.2$  days**

$p = 2.2 \times 10^{-16}$

$n = 21$

## Appendix 4

Supplementary Figures S1 – S10 Amino acid alignments for non-differentially expressed carotenoid genes in *Lotus filicaulis*

Figure S1. Amino acid alignment for β-CHY

|      |     |  |
|------|-----|--|
| Lfil | 1   | -----KPYPLTLTVKSSINKNSTKPSSWVSPDWLTSRSLTAGKNDDSGIPI  |
| Ljap | 1   | SLSLSPLSLFPPKPYPPLTLSVKSSINKNSTKPSSWVSPDWLTSRSLTAGKNDDSGIPI  |
| Gmax | 1   | SLSLTPLSVFPPPTPSFKRLSVKSSINKPTTKPSSWISPDWLTSRSLTAGNDVSGIPV   |
| Mtru | 1   | SIPLSHLSISSLFCKRYPLRILTKSSTNKNTTKSISWVSPDWLTSLSHSLTTSKNDDSNIP  |
| Atha | 1   | SSSYSSSLFTAKPSPPKTFSirSSIEKPKSSQSWSVSPDWLTTLTRILSSGKNDDESGIPI  |
| <br> |     |  |
| Lfil | 49  | ASAKLDDVSDLLGGALFLPLFKWMKEYGPIYRLAAGPRNFVVVSDPAIAKHVLKNYGKG  |
| Ljap | 61  | ASAKLDDVSDLLGGALFLPLFKWMKEYGPIYRLAAGPRNFVVVSDPAIAKHVLKNYGKG  |
| Gmax | 60  | ASAKLDDVSDLLGGALFLPLFKWMQDGPIYRLAAGPRNFVVVSDPAIAKHVLKNYGKA   |
| Mtru | 61  | ASAKLDDVSDLLGGALFLPLFKWMNEYGPIYRLAAGPRNFVVVSDPAIAKHVLKNYGKG  |
| Atha | 61  | ANAKLDDVADLLGGALFLPLYKWMNEYGPIYRLAAGPRNFVIVSDPAIAKHVLKNYPKYA   |
| <br> |     |  |
| Lfil | 109 | KGLVAEVSEFLFGSGFAIAAEGLWTARRRAVVPSPSLHKRYLSIVDRVFCRCAERLVEKLQ  |
| Ljap | 121 | KGLVAEVSEFLFGSGFAIAAEGLWTARRRAVVPSPSLHKRYLSIVDRVFCRCAERLVEKLQ  |
| Gmax | 120 | KGLVAEVSEFLFGSGFAIAAEGLWTARRRAVVPSPSLHKRYLSIVDRVFCRCAERLVEKLQ  |
| Mtru | 121 | KGLVAEVSEFLFGDGFAIAAEGLWTARRRAVVPSPSLHKRYLSIMVDRVFCCKCAERLVEKLQ  |
| Atha | 121 | KGLVAEVSEFLFGSGFAIAAEGLWTARRRAVVPSPSLHRRYLSIVVERVFCCKCAERLVEKLQ  |
| <br> |     |  |
| Lfil | 169 | PDALNGTAVNMEDKFSQLTLDVIGLSVFNYDFDSLNAADSPVIGAVYTALKEEARSTDLL   |
| Ljap | 181 | PDALNGTAVNMEDKFSQLTLDVIGLSVFNYDFDSLNAADSPVIGAVYTALKEEARSTDLL   |
| Gmax | 180 | PDALNGTAVNMEAKFSQLTLDVIGLSVFNYNFDLNTDSPVIEAVYTALKEEARSTDLL   |
| Mtru | 181 | ADA <sup>1</sup> NGTAVNMEDKFSQLTLDVIGLSVFNYNFD <sup>2</sup> ALNSDSPVIEAVYTALKEEARSTDLL   |
| Atha | 181 | PYAEDGSAVNMEAKFSQLTLDVIGLSFNYNFD <sup>2</sup> LDSPVIEAVYTALKEEARSTDLL  |
| <br> |     |  |
| Lfil | 229 | PYWQ <sup>1</sup> VEFLRKIIIPRQIKAEKAVSIIRKTVENLIECKKEIVESEGERIDG <sup>2</sup> DEYVNDSDPSI  |
| Ljap | 241 | PYWQ <sup>1</sup> VEFLRKIIIPRQIKAENAVTIIRKTVEDLIECKKEIVESEGERIDG <sup>2</sup> DEYVNDSDPSI  |
| Gmax | 240 | PYWK <sup>1</sup> FKFLCKIIIPRQIKAE <sup>2</sup> EAVSVIRKTVEDLIECKREIVESEGERID <sup>3</sup> EYVNDSDPSI                            |
| Mtru | 241 | PYWK <sup>1</sup> IDFLCKIIIPRQIKAENAVTVIRKTVEDLIE <sup>2</sup> QCKEIVESEGERID <sup>3</sup> DEYVNADPSI                            |
| Atha | 241 | PYWK <sup>1</sup> DALCKIVPRQVKAEKAVT <sup>2</sup> LIRET <sup>3</sup> VEDLIAKCKEIVE <sup>4</sup> REGERINDE <sup>5</sup> EYVNADPSI |
| <br> |     |  |
| Lfil | 289 | LRFLLASREEVSSDQLRDDLLSMLVAGHETTGSVLWTLYLLSKDSSSLAKVQEEVDRVL  |
| Ljap | 301 | LRFLLASREEVSSDQLRDDLLSMLVAGHETTGSVLWTLYLLSKDSSSLAKVQEEVDRVL  |
| Gmax | 300 | LRFLLASREEVSSVQLRDDLLS <sup>1</sup> LVAGHETTGSVLWTLYLLSKDSSSLAKAQEEVDRVL   |
| Mtru | 301 | LRFLLASREEVSSVQLRDDLLSMLVAGHETTGSVLWTLYLLSKDSSSLAKAQEEVDRVL  |
| Atha | 301 | LRFLLASREEVSSVQLRDDLLSMLVAGHETTGSVLWTLYLLSKN <sup>1</sup> SSA <sup>2</sup> LRKAQEEVDRVL  |
| <br> |     |  |
| Lfil | 349 | QGRRPTFEDMKS <sup>1</sup> LKF <sup>2</sup> LCIT <sup>3</sup> ESLR <sup>4</sup> LYPHPPVLIRRAQVPDELPGAYKVNAGQDIMISVY               |
| Ljap | 361 | QGRRPTFEDMKN <sup>1</sup> LKF <sup>2</sup> LCIT <sup>3</sup> ESLR <sup>4</sup> LYPHPPVLIRRAQVPDELPGAYKVNAGQDIMISVY               |

|      |     |   |
|------|-----|---|
| Gmax | 360 | QGRRPTYEDIKDLKFLTRCIIESLRLYPHPVLIRRAQVPDELPGCYKLDAGQDIMISVY   |
| Mtru | 361 | QGRRPTYEDMKDLKFLNRCIIESLRLYPHPVLIRRQIPDELPGDYKIDAGQDIMISVY    |
| Atha | 361 | EGRNPAFEDIKEALKYITRCINESMRLYPHPPVLIRRAQVPDTLPGNYKVNTGQDIMISVY |

|      |     |  |
|------|-----|--|
| Lfil | 409 | NIHHSSEVWDRAEEFM <del>F</del> PERFDLDGPMPNETNTDFRFIPFSGGPRKCVGDQFALLEATVSL             |
| Ljap | 421 | NIHHSSEVWDRAEEFLPERFDLDGPMPNETNTDFRFIPFSGGPRKCVGDQFALLEATVSL                           |
| Gmax | 420 | NIHRSSEVWDRAEEFV <del>V</del> PERFDLDGPVPNETNTDFRFIPFSGGPRKCVGDQFALMEAI <del>VAL</del> |
| Mtru | 421 | NIHHSSKVWDRAEEFLPERFDLDGPVPNETNTDFRFIPFSGGPRKCVGDQFALLEATVAL                           |
| Atha | 421 | NIHRSSEVWEKAEEFLPERFDIDGAIPNETNTDFKFIPFSGGPRKCVGDQFALMEAI <del>VAL</del>               |

|      |     |   |
|------|-----|---|
| Lfil | 469 | AIFLQHMNFELVPDQNISM <b>TGATIHTTNGLYM</b> KLSQRVK  |
| Ljap | 481 | AIFLQHMNFELVPDQNISM <b>TGATIHTTNGLYM</b> KLSQRVK  |
| Gmax | 480 | AIFLQHMNFELVPDQNISM <b>TGATIHTTNGLYM</b> KLSRRIK  |
| Mtru | 481 | AVFLQHMNFELVPDQNICMTTGATIHTTNGLYM <b>KLSQR</b> IK |
| Atha | 481 | AVFLQRLNVELVPDQTISMTTGATIHTTNGLYM <b>KVSQR</b> -- |

Figure S2. Amino acid alignment for CDS

|      |     |  |
|------|-----|--|
| Lfil | 1   | MAGCGIILSAVNLYHG[GARNTIKLC[SSSS[TDSSISLSFGGSES[LRLG--PSTRARLRNH    |
| Ljap | 1   | MAGCGIILSAVNLYHAGARNTIKLG[SSS[TDSSISLSFGGSES[LRLG--PSTRARLRNH      |
| Gmax | 1   | MAACGYIISAANFNYLVGARNISKFAS---SDATISESFGGSDSMGLTLRA---PKRNH        |
| Mtru | 1   | MALYGSISSPNLNWQIGPKTISKSTSSSMNSYTISLSFSSSVSMGLNLRPSTRARLRNH        |
| Atha | 1   | MVVFGNVSAANLPYQONGFLE-----ALSSGGCELMGHFSRPTSQARRST                 |
| <br> |     |  |
| Lfil | 59  | GSPLRVVCIDYPRPQLENTVNLFLEAAYLSSTFRASPRPTKPLKVVIAGAGLAGLSTAKYL      |
| Ljap | 59  | GSPFDRVVCIDYPRPQLENTVNLFLEAAYLSSTFRASPRPAKPLKVVIAGAGLAGLSTAKYL     |
| Gmax | 53  | E[SPLRVVCMDYPRPELENTVNFEAA[YLSSSTFRASPR[EAKPLKPLNIVIAGAGLAGLSTAKYL |
| Mtru | 61  | GSPLKVCIDYPRPELD[DTVNFI[EASYLSSTFRASPRPTKPLKVVIAGAGLAGLSTAKYL      |
| Atha | 46  | AGPLQVVCVDIPRPELENTVNLFLEAASLSASFRSAPRPAKPLKVVIAGAGLAGLSTAKYL      |
| <br> |     |  |
| Lfil | 119 | ADAGHKPILLEARDVLGGKVAAWKDEDGDWYETGLHIFFGAYPNVQNLFGELGINDRQLQW      |
| Ljap | 119 | ADAGHKPILLEARDVLGGKVAAWKDEDGDWYETGLHIFFGAYPNVQNLFGELGINDRQLQW      |
| Gmax | 113 | ADAGHKPILLEARDVLGGKVAAWKD[KDGDWYETGLHIFFGAYPNVQNLFGELGINDRQLQW     |
| Mtru | 121 | ADAGHKPILLEARDVLGGKVAAWKDEDGDWYETGLHIFFGAYPNVQNLFGELGINDRQLQW      |
| Atha | 106 | ADAGHKPILLEARDVLGGKIAAWKDEDGDWYETGLHIFFGAYPNVQNLFGELGINDRQLQW      |
| <br> |     |  |
| Lfil | 179 | KEHSMIFAMPSKPGEFSRFDFAEVLPAP                                       |
| Ljap | 179 | KEHSMIFAMPSKPGEFSRFDFAEVLPAP                                       |
| Gmax | 173 | KEHSMIFAMP[KPGEFSRFDFPEVLPS[P                                      |
| Mtru | 181 | KEHSMIFAMPSKPGEFSRFDFPEVLPS[P                                      |
| Atha | 166 | KEHSMIFAMPSKPGEFSRFDPVLPAP   |

Figure S3. Amino acid alignment for CRTISO-2

|      |       |   |
|------|-------|---|
| Lfil | 1     | EADVVVIGSGI <span style="background-color: black; color: white;">GGLCCAALLARYEONVLVLESHDLPGGAAHSFDIKGYKFDGPSLFSGL</span>  |
| Ljap | 1     | EADVVVIGSGI <span style="background-color: black; color: white;">GGLCCAALLARYEONVLVLESHDLPG</span> -----  |
| Gmax | 1     | EADVVVIGSGIGGLCCG <span style="background-color: black; color: white;">CALLARYEEEDVLVLESHDQPGGAAHSFDIKGYNFDGPSLFSGL</span>  |
| Mtru | 1     | ETDV <span style="background-color: black; color: white;">TIVIGSGIGGLSCAALLARYEQDVVVFESHDHAGGAAHSFDVKGYKFDGPSLFSGL</span>   |
| Atha | 1     | EADVVVIGSGIGGLCCG <span style="background-color: black; color: white;">CALLARYEQDVIVLESHDHPGGAAHSFEIKGYKFDGPSLFSGL</span>   |
| <br> |       |   |
| Lfil | 61    | QSRGPQANPLAQVLDALGES <span style="background-color: black; color: white;">LPCATYDSWKVYIPEGEFLSRIGPTEFSKDLEKYGGPN</span> AV  |
| Ljap | 38    | ----- <span style="background-color: black; color: white;">SWMRWVSHSHALLM</span> -----  |
| Gmax | 61    | QSRGPQANPLAQVLDALGES <span style="background-color: black; color: white;">LPCATYDSWMVHIPEGQFLSRIGPTEFFKDLQQYAGPN</span> AV  |
| Mtru | 61    | QSRGPQANPLAQVLDALGES <span style="background-color: black; color: white;">VPCATYDSWMVHVPEADFLSRIGPTEFLKDLHN</span> YAGPEAV  |
| Atha | 61    | QSRGPQANPLAQVLDALGES <span style="background-color: black; color: white;">FPCKKYDSWMVYIPEGDFLSRIGPTDFFKDLEKYAGPS</span> AV  |
| <br> |       |   |
| Lfil | 121   | QEWRKLLDAVLPLSTAAMALPPLSIRGDLGV <span style="background-color: black; color: white;">VYTA</span> AAARYAPS <span style="background-color: black; color: white;">LLKSFIQMGPQCALGATK</span>  |
| Ljap | 52    | -----   |
| Gmax | 121   | LEWRKLLDAVLPLSTAAMALPPLSIRGDLGV <span style="background-color: black; color: white;">VYTA</span> AAARYAPS <span style="background-color: black; color: white;">LFKSFIQMGPRA</span> SLRATK   |
| Mtru | 121   | QEWRKLLDAVLPLSTAAMALPPLS <span style="background-color: black; color: white;">VRGD</span> FGV <span style="background-color: black; color: white;">YTA</span> AAARYAPS <span style="background-color: black; color: white;">LFNTFFQMGPQ</span> ALARST <span style="background-color: black; color: white;">Q</span>   |
| Atha | 121   | QEWEKLLGA <span style="background-color: black; color: white;">I</span> LP <span style="background-color: black; color: white;">LSSAAMALPPLSIRGDLGV</span> V <span style="background-color: black; color: white;">YTA</span> AAARYAPS <span style="background-color: black; color: white;">LLKSFIKMGPKCALGATK</span>  |
| <br> |       |   |
| Lfil | 181   | LLRP <span style="background-color: black; color: white;">FKEILDG</span> EL <span style="background-color: black; color: white;">LKDPF</span> IRN <span style="background-color: black; color: white;">WIDLLSFLLAG</span> -----   |
| Ljap | 52    | -----   |
| Gmax | 181   | LLRP <span style="background-color: black; color: white;">FSEILDD</span> QLNDPFIRN <span style="background-color: black; color: white;">WIDLLSFLLAGV</span> KANAV <span style="background-color: black; color: white;">LSAEMIYMFAEWYKPG</span> CCLEYP   |
| Mtru | 181   | LLSP <span style="background-color: black; color: white;">FSQILD</span> SLQLKNPFIRN <span style="background-color: black; color: white;">WIDLLSFLLAGV</span> KSDS <span style="background-color: black; color: white;">ILSAEMVYMFAEWYKPG</span> CCLEYP  |
| Atha | 181   | LLRP <span style="background-color: black; color: white;">FSEIV</span> D <span style="background-color: black; color: white;">SELKDPF</span> IRN <span style="background-color: black; color: white;">WIDLLAF</span> LLAGV <span style="background-color: black; color: white;">KSDGILSAEMIYMFAEWYKPG</span> C <span style="background-color: black; color: white;">CTLEYP</span> |
| <br> |       |   |
| Lfil | ----- | I <span style="background-color: black; color: white;">HGRFTIYLKVNV</span> V  |
| Ljap | 52    | ----- <span style="background-color: black; color: white;">IHGRFTIYLKVNV</span> V   |
| Gmax | 241   | L <span style="background-color: black; color: white;">HGSAAIIDALVRGLEKF</span> GGRI <span style="background-color: black; color: white;">SLQSHVENIVVENDRAI</span> GV <span style="background-color: black; color: white;">VKGSGQFIRAKKAVV</span>   |
| Mtru | 241   | LDGAAGIV <span style="background-color: black; color: white;">DALIRGLEKF</span> GGRI <span style="background-color: black; color: white;">SLQSHVEKIVVENDRAI</span> GV <span style="background-color: black; color: white;">VKLRSQFIRAKKAVV</span>   |
| Atha | 241   | IDGT <span style="background-color: black; color: white;">GAVVEALVRGLEKF</span> GGRL <span style="background-color: black; color: white;">SLKSHVENIVIENGKAV</span> VG <span style="background-color: black; color: white;">VKGSGQFIRAKKAVV</span>   |

Figure S4. Amino acid alignment for CRTISO-3

|      |     |  |
|------|-----|--|
| Lfil | 1   | SSSIFSSLNKFNPLSLRSPLRIRATVVKDTEGKVVEKTFPSKIPLQGTGAEEEDSDSSPPT  |
| Ljap | 1   | SSSIFSSLNKFSPLSLRSPLRVRATVVKDTEGKVVEKTFPSKIPLQGTGAEEEDSDSSPPT  |
| Gmax | 1   | SSSLFSPPLPK---HTAPQLRARSSLQDNEKKVIVHDSFPSKTPHTADK-GGN SINTS    |
| Mtru | 1   | CDSSSSLFTPLNKFRPLSFRPPLRIRSSLQDKEDKVITQNTFPSKTPLDSVTENDDTS     |
| Atha | 1   | SGGTLRPLVTHLPLSSRRLRVQATIILQDDEEKVVVEESFKAETSTGTEPLENMSSSSTS   |
| <br> |     |  |
| Lfil | 61  | AFELGVVIKVEQSINIFLTDSEVIKILDALYRDRNYARFFVLETIARVPYFAFMSVLHMYES |
| Ljap | 61  | AFELGVVIKVEQSINIFLTDSEVIKILDALYRDRNYARFFVLETIARVPYFAFMSVLHMYES |
| Gmax | 56  | AFEKRIIKVEQSINIFLTDSEVIKILDLYHADRHYARFFVLETIARVPYFAFMSVLHMYES  |
| Mtru | 61  | STSAWEKGVIKVEQSINIFLTDSEVIKILDALYRDRNYARFFVLETIARVPYFAFMSILHM  |
| Atha | 61  | AFETWIIKLEQGVNVFLTDSEVIKILDLYRDRTYARFFVLETIARVPYFAFMSVLHMYET   |
| <br> |     |  |
| Lfil | 121 | FGWWRRADYLKVHFAESWNEMHLLIMEELGGNAWWFDRFLAQHIAIFYYFMTALMYTIS    |
| Ljap | 121 | FGWWRRADYLKVHFAESWNEMHLLIMEELGGNAWWFDRFLAQHIAIFYYFMTALMYTIS    |
| Gmax | 116 | FGWWRRADYLKVHFAESWNEMHLLIMEELGGNAWWFDRFLAQHIAIFYYIMTVLMYAVS    |
| Mtru | 121 | YESFGWWRRADYLKVHFAESWNEMHLLIMEELGGNAWWFDRFLAQHIAIFYYFMTALMY    |
| Atha | 121 | FGWWRRADYLKVHFAESWNEMHLLIMEELGGNSWWFDRFLAQHIAIFYYFMTVFLYIIS    |
| <br> |     |  |
| Lfil | 181 | PRMAYHFSECVENHAFETYDKFIKDQ---                                  |
| Ljap | 181 | PRMAYHFSECVENHAFETYDKFIKDQ---                                  |
| Gmax | 176 | PRMAYHFSECVESHAFETYDKFIKVQ---                                  |
| Mtru | 181 | LISPRMAYHFSECVESHA <del>FETYDKFIKEQ</del>                      |
| Atha | 181 | PRMAYHFSECVESHAY <del>EETYDKFLKAS</del> --                     |

Figure S5. Amino acid alignment for CYB-1

Lfil 1 -----

Ljap 1 ILVETDHIGRQASGSVTVDGETIVYTTVCLDDVPSEPSDFPFLSVNYQERFSAAGRTSG

Gmax 1 ILVETAHIGRQASGSVTVDGETIVYTTVCLDDVPCEPSDFPFLSVIYQERFSAAGRTSG

Mtru 1 ILVETGHMGRQASGSVTVDGETIVYTTVCLDDTPSEPSDFPFLSVNYQERFSAAGRTSG

Atha 1 ILVETGLMGRQASSAVTVDGETIVYT<sub>S</sub>VCLADVPSEPSDFLPLYVHYQERFSAVGRTSG

Lfil 1 -----

Ljap 61 GFFKREGKTKDHEVLICRLIDRPLRPSMPKGFYHETQILSWVLSYDGLHSPDSLAVTAAG

Gmax 61 GFFKREGKIKDHEVLICRLIDRPLRPTMPKGFYHETQILSWVLSYDGLHSPDSLAVTAAG

Mtru 61 GFFKREGKTKDHEVLICRLIDRPLRPTMPKGFYHETQILSWVLSYDGLHIPDSLAVTAAG

Atha 61 GFFKREGRTKDHEVLICRLIDRPLRPTMPKGFYNETQILSWVLSYDGLHAPDALAVTSAG

Lfil 1 -----

Ljap 121 IAVALSEVPMSKTVAGVR<sub>I</sub>GLVGDKFLVNPTTEEMENSELDDL<sub>L</sub>LAGTD<sub>S</sub>AILMIEGYSNF

Gmax 121 IAVALSEVPMSKAVAGVRVGLVGDKYIVNPTTEEMEHSELDDL<sub>L</sub>LAGTD<sub>S</sub>AILMIEGYSNF

Mtru 121 IALALSEVP<sub>T</sub>SKAVAGVRVGLVGDKYIVNPTSEEMENSELDLMLAGTD<sub>S</sub>AILMIEGYGNF

Atha 121 IAVALSEVPNAKAIAGVRVGLIGGE<sub>F</sub>IVNPTVKEMEESQLDLFLAGTD<sub>T</sub>AILTIEGYSNF

Lfil 1 -----

Ljap 181 LPEEKLLAVEVGQDAVRAICKEVAALVKKCGKPKMFDAIKLPPPELYKHVEEIAGDELV

Gmax 181 LPEEKLLKAVEVGQNAVRAICKNEVEALVKKCGKPKMLDAIKLPPPELYEHVEAIAASDELV

Mtru 181 LPEEKLLKAVEVGQDAVRAICKNEVEALVKKCGKPKMVDAIKLPPPELYKHVEEIAGDELV

Atha 181 LPEEMLLQAVKVGQDAVQATCIAEV<sub>L</sub>AKKY<sub>G</sub>KPKMLDAIRLPPPELYKHVKELAGEELT

Lfil 1 -----

Ljap 241 KVLQIRNKIPRRKALSSLEEKV<sub>I</sub>KILTENGV<sub>V</sub>TNDATQRSNTETIAEILEDEDED<sub>E</sub>EVIV

Gmax 241 KVLQIRNKIPRRKALSSLEEKV<sub>L</sub>KILTENG<sub>F</sub>V<sub>N</sub>DSL<sub>T</sub>RSNSSETIAEILEVEDEDED<sub>E</sub>EVIV

Mtru 241 KVLQIRNKIPRRKALSSLEEKV<sub>L</sub>KIL<sub>S</sub>ENG<sub>F</sub>V<sub>T</sub>NDTAPRNNAETIAEILEDEDED<sub>E</sub>EVIV

Atha 241 KALQIKSKISRRKA<sub>T</sub>SSLEEKV<sub>L</sub>T<sub>I</sub>L<sub>T</sub>EKG<sub>V</sub>IDEVA-FGTIEAQPD<sub>D</sub>ILEDEDED<sub>E</sub>EVVP

Lfil 1 ----- VTSKFLRKRIVEGGKRS<sub>D</sub>GRKPNEIRPIN

Ljap 301 DGEVDEGDVH<sub>I</sub>KPT<sub>R</sub>KPLFSEVDV<sub>K</sub>LVFKEVTSKFLRKRIVEGGKRS<sub>D</sub>GRKPNEIRPIN

Gmax 301 DGEVDEGDVH<sub>I</sub>KPT<sub>P</sub>RKPLFSEVDV<sub>K</sub>LVFKEVTSK<sub>Y</sub>WRKRMVEGGKRS<sub>D</sub>GRTPD<sub>G</sub>IRPIY

Mtru 301 DGEVDEGDVH<sub>I</sub>KPT<sub>P</sub>RKPLFSEVDV<sub>K</sub>LVFKEVTSKFLRKRIVEGGKRS<sub>D</sub>GRTPNEIRPIN

Atha 300 EGEVDQGDVHIRP<sub>I</sub>PRKPLFSEVDV<sub>K</sub>LVFKEVSSKLLR<sub>R</sub>IVEGGKRS<sub>D</sub>GRTLDEIRPIN

Lfil 30 SRCG<sub>L</sub>LPRAHGSTLFTRGETQSLAVVTLGDKQMAQRIDNLMGVDEFKKFYLQYSFP<sub>P</sub>SCV

Ljap 361 SRCG<sub>L</sub>LPRAHGSTLFTRGETQSLAVVTLGDKQMAQRIDNLMGVDEFKKFYLQYSFP<sub>P</sub>SCV

Gmax 361 SRCG<sub>L</sub>LPRAHGSTLFTRGETQ<sub>A</sub>LAVVTLGDKQMAQRID<sub>S</sub>LDGVDEFKRFYLQYSFP<sub>P</sub>SCV

Mtru 361 SSCG<sub>L</sub>LPRAHGSA<sub>L</sub>FTRGETQSLAVATLGDNKQMAQRIDNLM<sub>D</sub>VDDYKRFYLQYSFP<sub>P</sub>SCV

Atha 360 SRCG<sub>L</sub>LPRAHGSTLFTRGETQ<sub>A</sub>LAVVTLGDKQMAQRIDNLEGSDEYKRFYLQYTFP<sub>S</sub>SCV

Lfil 90 GEVGRIGAPS<sub>R</sub>REIGHGMLAERSLE<sub>P</sub>ILPSDKDFPYTIRVESTITESNGSSMASVC<sub>GG</sub>C

Ljap 421 GEVGRIGAPS<sub>R</sub>REIGHGMLAERSLE<sub>P</sub>ILPSDKDFPYTIRVESTITESNGSSMASVC<sub>GG</sub>C

Gmax 421 GEVGRIGAPS<sub>R</sub>REIGHGMLAERSLE<sub>P</sub>ILPS<sub>E</sub>DFPYTIRVESTITESNGSSMASVC<sub>GG</sub>C

Mtru 421 GEVGRIGAPS<sub>R</sub>REIGHGMLAERSLET<sub>I</sub>LPSDKDFPYTIRVESTITESNGSSMASVC<sub>GG</sub>C

Atha 420 GEVGRIGAPS<sub>R</sub>REIGHG<sub>T</sub>LAERA<sub>L</sub>ET<sub>I</sub>LPS<sub>D</sub>DFPYTIRVESTVIESNGSSMASVC<sub>GG</sub>C

|      |     |   |
|------|-----|---|
| Lfil | 150 | LALQDAGVPIKSAIAGIAMGMVLDTKEFGGDGTPLILSDITGSEDASGDMDFKVAGNEHG    |
| Ljap | 481 | LALQDAGVPIKSAIAGIAMGMVLDTKEFGGDGTPLILSDITGSEDASGDMDFKVAGNEHG    |
| Gmax | 481 | LALQDAGVPIKGSIAAGIAMGMVLDTKEFGGDGTPLILSDITGSEDASGDMDFKVAGNEHG   |
| Mtru | 481 | LALQDAGVPIKSSIAAGIAMGLVLDTKEFGGDGTPLILSDITGSEDASGDMDFKVAGNEHG   |
| Atha | 480 | LALQDAGVPIVKCSVAGIAMGMVWDTEEFGGDGSPLILSDITGAEDASGDMDFKVAGNEHG   |
| <br> |     |   |
| Lfil | 210 | ITAFQMDIKVGGITLPMREALLQARDGRKHILGEMMNCSPPPAKILSKYAPLIHIMKVR     |
| Ljap | 541 | ITAFQMDIKVGGITLPMREALLQARDGRKHILGEMMNCSPPPAKILSKYAPLIHIMKVR     |
| Gmax | 541 | ITAFQMDIKVGGITLPMRKALLQARDGRKHILGAMMNSSPPPAKRLSKYAPLIHVMKVR     |
| Mtru | 541 | ITAFQMDIKVGGITLAIMREALLQAKDGRKHILGEMMKCLPPPAKRLSKYAPLIHVMKVR    |
| Atha | 540 | ITAFQMDIKVGGITLTIMEKALIQAKAGRHHILAEMAKCSPPTLSSLKYAPLIHIMKVR     |
| Lfil | 270 | PDKVNLIIGSGGKKVKSIIIESGIEAIDTDDNGTVKIFARDLSSLERSKAMISNLTMVPA    |
| Ljap | 601 | PDKVNLIIGSGGKKVKSIIIESGIEAIDTDDNGTVKIFARDLSSLERSKAMISNLTMVPA    |
| Gmax | 601 | PDRINLIIGSGGKKVKSIIIESGVDTIDTEDDGTVKIFADLPSLEMSKAIINSLTMVPT     |
| Mtru | 601 | PDKINLIIGSGGKKVKSIIIESQSGIEAIDTEDNGTVKIFARDLASLEMASKAIISNLTMVPT |
| Atha | 600 | PSKVYSLIGSGGKKVKSIIIESGVVEAIDMQDDGTVKIMAIDVASLERAKAIISGLTMVPS   |
| <br> |     |   |
| Lfil | 330 | IGDIYRNCEIKSIAPYGVFVEIAPGREGLCHISELSSGWLWPKAEDVFVGDRIDVKLIEI    |
| Ljap | 661 | IGDIYRNCEIKSIAPYGVFVEIAPGREGLCHISELSSGWLWPKAEDVFVGDRIDVKLIEI    |
| Gmax | 661 | IGDIYRSCIEIKSIASYGVFVEIAPGREGLCHISELSSGWLWPKAEDVFVGDRIDVKLIEI   |
| Mtru | 661 | IGDIYRNCEIKSIVPYGAFVEIAPGREGLCHISELSSAWLAKAEDAFVGDRIDVKLIEI     |
| Atha | 660 | VGDIYRNCEIKSMAPYGAFVEIAPGREGLCHISELSAEWLAKPEDANVGDRIDVKLIEV     |
| <br> |     |   |
| Lfil | 390 | NDKGQLRLSHRALLPDTDPDGIAEEKIEQPKDKINTPKGDSSKRSSRDDSALPSKKFIRR    |
| Ljap | 721 | NDKGQLRLSHRALLPDTDPDGIAEEKIEQPKDKINTPKGDSSKRSSRDDSALPSKKFIRR    |
| Gmax | 721 | NEKGQLRLSHRALLPDTASGLVEEKTEQSKDKSSTPNVTSSKRSSSEDDSVLPSKKFVRR    |
| Mtru | 721 | NDKGQLRLSHRALLPDAEPDGLVEEKTEQN-AKVNTRKGRSSKRSSSEDSALPSKKFIRR    |
| Atha | 720 | NEKGQLRLSVRALLPESETDDSTKDQSQR-KYVNTSSKDASKVSSGDELVL-KKKDVRR     |
| <br> |     |   |
| Lfil | 450 | MVSPSQDKPVTNKDKIKRSSNKASDKDESSLVGEA                             |
| Ljap | 781 | MVSPSQDKPVTNKDKIKRSSNKASDKDESSLVSEEA                            |
| Gmax | 781 | LVSSSLDKFTNKDKTKKSSNKASGKDESSLVSGEA                             |
| Mtru | 780 | S---SEDKPVTNKDKIKKSQN---KDESSLVSEEA                             |
| Atha | 778 | ATGGSSDKTMNS-----SSTNEESLVNGEA                                  |

Figure S6. Amino acid alignment for CYB-2

|      |     |   |
|------|-----|---|
| Lfil | 1   | LRASSSALLELVPEFKKENLDFELPLYDSTKGAVVNLA<br>VVGGGPAGLAVAQQVSEAGLSV  |
| Ljap | 1   | LRASSSALLELVPEVKKENLDFELPLYDSMKGAVVD<br>AVVGGGPAGLAVAQQVSEAGLSV   |
| Gmax | 1   | LRASSSALLELVPEFKKENLDFELPLYDSSKGAM<br>VLDLVVGGGPAGLAVAQQVSEAGLSV  |
| Mtru | 1   | LRASSSALLELVPEFKKENLDFELPLYDSTKGTV<br>VDLVVGGGPAGLAVAQQVSEAGLSV   |
| Atha | 1   | VVSGSAALLDLVPETKKENLDFELPLYDTSKSQV<br>VVDLAVVGGGPAGLAVAQQVSEAGLSV   |
| <br> |     |   |
| Lfil | 61  | CAIDPNPRLIWPNNYGVWVDEFEA<br>MDLLCDDTTWSGAVVYIDDKTKKYLDRPYGRVNRK   |
| Ljap | 61  | CAIDPNPRLIWPNNYGVWVDEFEA<br>MDLLCDDTTWSGAVVYIDDKTKKYLDRPYGRVNRK   |
| Gmax | 61  | CAIDPNPRLIWPNNYGVWVDEFEA<br>MDLLCDDTITWSGAVVYIDDKTKKLDLDRPYGRVNRK   |
| Mtru | 61  | CAIDPNPRLIWPNNYGVWVDEFEA<br>MDLLCDDKTWSGAVVYIDDKTKKLDLDRPYGRVNRK  |
| Atha | 61  | CSIDPSPKLIWPNNYGVWVDEFEA<br>MDLLCDDTTWSGAVVYDEGVKKDLSRPYGRVNRK  |
| <br> |     |   |
| Lfil | 121 | LLKSMLQKCISNGVKFHQA<br>KVIHEESKSMLICNDGVTIQATVVL<br>DATGFSRSLVQY  |
| Ljap | 121 | LLKSMLQKCISNGVKFHQA<br>KVIHEESKSMLICNDGVTIQATVVL<br>DATGFSRSLVQY  |
| Gmax | 121 | LLKSMLQKCISNGVKFHQA<br>KVIHEEAKSLLICNDGVTIATVVL<br>DATGFSRCLVQY   |
| Mtru | 121 | LLKSMLQKCITANGVKFHQA<br>KVIHEESKSMLICNDGVTQATVVL<br>DATGFSRCLVQY  |
| Atha | 121 | QLKSMLQKCITNGVKFHQS<br>KVTNVHEEANSTVVCSDGVKI<br>QASVVL<br>DATGFSRCLVQY  |
| <br> |     |   |
| Lfil | 181 | DKPYNPGYQVAYGILA<br>EVEEHPFDVKMLFMDWRD<br>SHLDNNMELKEKNSRIP<br>TFLYAMPF   |
| Ljap | 181 | DKPYNPGYQVAYGILA<br>EVEEHPFDVKMLFMDWRD<br>SHLDNNMELKEKNSRIP<br>TFLYAMPF   |
| Gmax | 181 | DKPYNPGYQVAYGILA<br>EVDEHPFDVKMLFMDWRD<br>SHLDNDMELKQRNSRIP<br>TFLYAMPF   |
| Mtru | 181 | DKPYNPGYQVAYGILA<br>EVEEHPFDVKMLFMDWRD<br>SHLDNDLKLKERN<br>NSKIP<br>TFLYAMPF  |
| Atha | 181 | DKPYNPGYQVAYGIVAEV<br>DGH<br>PF<br>DVKM<br>FMDWRD<br>KHLDS<br>YPE<br>LKER<br>NSKIP<br>TFLYAMPF                        |
| <br> |     |   |
| Lfil | 241 | SSTKIFLEETSLVARPGLRM<br>DDIQDRMVARLKHLGI<br>KVKSIEEDEHC<br>VIPMGGPLPVLPQ  |
| Ljap | 241 | SSTKIFLEETSLVARPGLRM<br>DDIQDRMVARLKHLGI<br>KVKSIEEDEHC<br>VIPMGGPLPVLPQ  |
| Gmax | 241 | SSTKIFLEETSLVARPGLRM<br>DDIQDRMVARLKHLGI<br>RVKSIEEDEHC<br>VIPMGGPLPVLPQ  |
| Mtru | 241 | SSTKIFLEETSLVARPGLRM<br>DDIQDRMVARLKHLGI<br>INVKSIEEDEQC<br>VIPMGGPLPVLPQ   |
| Atha | 241 | SSN<br>RIFLEETSLVARPGLRM<br>EDIQDRM<br>AARLKHLGI<br>NVKRI<br>EDEPC<br>VIPMGGPLPVLPQ                                   |
| <br> |     |   |
| Lfil | 301 | RVIGIGGTAGMVHP<br>STGYM<br>VARTLAA<br>APIVANA<br>IVQYLGS<br>DRGI<br>SGDGL<br>LSSIV<br>WKDLW<br>PI                     |
| Ljap | 301 | RVIGIGGTAGMVHP<br>STGYM<br>VARTLAA<br>APIVANA<br>IVQYLGS<br>DRGI<br>SGDGL<br>LSSIV<br>WKDLW<br>PI                     |
| Gmax | 301 | RVVGIGGTAGMVHP<br>STGYM<br>VARTLAA<br>APIVAN<br>SIVQCL<br>GS<br>DRG<br>FSG<br>DE<br>ISAQV<br>WKDLW<br>PI              |
| Mtru | 301 | RVVGIGGTAGMVHP<br>STGYM<br>VARTLAA<br>APIVAN<br>IVQYLGS<br>DRGI<br>LGS<br>DRG<br>L<br>G<br>DE<br>VSARV<br>WKDLW<br>PI |
| Atha | 301 | RVVGIGGTAGMVHP<br>STGYM<br>VARTLAA<br>APIVAN<br>IVRYLG<br>SPNSL<br>RGD<br>Q<br>L<br>SAE<br>VWR<br>DLW<br>PI           |
| <br> |     |   |
| Lfil | 361 | ERRRQREFFCFGMD<br>ILLKLDL<br>PGT<br>RRFFDAFF<br>DLEPHYWHG<br>FLSSRL<br>FPELLV<br>FGLSLF                               |
| Ljap | 361 | ERRRQREFFCFGMD<br>ILLKLDL<br>PGT<br>RRFFDAFF<br>DLEPHYWHG<br>FLSSRL<br>FPELLV<br>FGLSLF                               |
| Gmax | 361 | QRRRQREFFCFGMD<br>ILLKLDL<br>PGT<br>RFFDAFF<br>DLEPHYWHG<br>FLSSRL<br>FHELL<br>FFGLSLF                                |
| Mtru | 361 | ERRRQREFFCFGMD<br>VLLKLDL<br>PGT<br>RRFFDAFF<br>NLEPHYWHG<br>FLSSRL<br>YLP<br>ELFT<br>FGLSLF                          |
| Atha | 361 | ERRRQREFFCFGMD<br>ILLKLDL<br>DA<br>TRRF<br>DAFF<br>D<br>Q<br>PHYWHG<br>FLSSRL<br>FPELLV<br>FGLSLF                     |
| <br> |     |   |
| Lfil | 421 | SYASNTSRIEIMAKGT<br>VTP   |
| Ljap | 421 | SYASNTSRIEIMAKGT<br>VTP   |
| Gmax | 421 | SYASNTSRVEIMAKGT<br>VTP   |
| Mtru | 421 | SYASNTSRLEIMAKGT<br>VTP   |
| Atha | 421 | SHASNTSRLEIMT<br>KGT<br>VTP   |

Figure S7. Amino acid alignment for CYE

Lfil 1 MLLLQLQPFNGSHLTVGTPFPLKKCSRRTLLIRPQAVPSRTQRIMESVSVSGEVGGAGGA  
Ljap 1 MLLLQLQPFNGSHLTVGTPFPQKKCSRRTLLIRPQAVPSRTQRIMESVSVSGEVGGAGGA  
Gmax 1 MLLI**R**LQPI**N**GSQ**I**LSVGTSFPHKKCSRPLLSRPQAVPSRTQRIMESVSVSGEVGGAGGA  
Mtru 1 MSLLQLQPI**N**GSQ**I**LV-----QKKCSRKA**F**RRAQAVPS**T**QRIMESVSVSGEVGGAGGA  
Atha 1 MVV**L**Q**I**H**Q**FNGSSVSRA**Q**TNQGR**R**ASTTVC**V**QT**Q**LAPS**L**T**Q**K**I**M**E**S**I**SVG**G**EAGGAGGA

Lfil 61 YSYEALKRLDQLWSSICSTQEVVQE**P**QQV**V**SSIPSLFRSSDLANKAEGSYDV**V**CGGT**L**  
Ljap 61 YSYEALKRLDQLWSSICSA**Q**EVVQE**P**QQV**V**SSIPSLFRSSDLANKAEGSYDV**V**CGGT**L**  
Gmax 61 YSYEALKRLD**Q**WSSICSS**Q**EVVQE**P**QQV**V**STIPSLFTSSDLAD**K**IEG**S**YDV**V**CGGT**L**  
Mtru 56 YSYEALKRLDQLW**S**NICSP**Q**EVVQE**P**QQIV**V**STIPSLFTSSDLAD**K**IEG**S**YDV**V**CGGT**L**  
Atha 61 YSY**N**ALKRLD**N**IWSNIC**I**QPTGP**Q**ET**Q**QIV**V**SR**V**SGFS**Q**DYS**M**GNNL**V**GT**F**D**I**V**V**CGGT**L**

Lfil 121 I**F**IAT**A**LCARGLKVA**I**VERNV**L**K**G**REQ**D**WN**I**SR**K**EL**L**LE**V**EV**G**V**L**EE**D**IER**A**T**S****I**NF**N**P  
Ljap 121 I**F**IAT**A**LCARGLKVA**I**VERNV**L**K**G**REQ**D**WN**I**SR**K**EL**L**LE**V**EV**G**V**L**EE**D**IER**A**T**S****I**NF**N**P  
Gmax 121 I**F**IAT**A**LCARGLRV**A**VERNV**L**K**G**REQ**E**WN**I**SR**K**EL**L**LE**V**EV**G**V**L**EE**D**IER**A**T**A**K**F**N**P**  
Mtru 116 I**F**IAT**A**LCARGLRV**A**VERNV**L**K**G**REQ**E**WN**I**SR**K**EL**L**LE**V**EV**G****I**LE**E****D**IER**R****V****T****A**K**F**N**P**  
Atha 121 I**F**IAT**A**LC**A**GLRV**A**VER**N**A**I**K**G**R**D**QE**W**N**I**SR**K****E****M**K**E****L**T**E****V**R**V****I**T**E****D****E****I**E**V****I**A**K****F**N**P**

Lfil 181 NRCGFESKGDIWVNNILHLGVSPVKL**I**EIV**K**ERFISLGGVIFEGCSVSCIN**I**YEDAAVL**K**  
Ljap 181 NRCGFESKGDIWVNNILHLGVSPVKL**I**EIV**K**ERFISLGGVIFEGCSVSCIN**I**YEDAAVL**K**  
Gmax 181 NRCGFERKGDIWVN**D**ILNLGVSPAKL**I**EIV**K**TRFISLGGVIFEG**C**GVSCIN**V**YEDAAVL**K**  
Mtru 176 NRCGFESKGDIWVNNILNLGVSPV**R**L**I**EIV**K**KRFISLGGV**F**FE**G**YSV**S****F**INV**V**YEDAAVL**K**  
Atha 181 NRCGFENL**I**GDIW**V****E**DILNLGVSPAKL**V**ETVK**Q**RFISLGGV**I**LEDSS**S****I**S**V**I**Y**ND**L**AV**M****Q**

Lfil 241 LSGDKILSSRLIIDAMGNFSPIV**K**Q**I**RR**G**R**K**PD**G**V**C**LV**V**G**T**CARG**F**E**S****N****T****S****D****V****I****F****S****S****S**  
Ljap 241 LSGDKILSSRLIIDAMGNFSPIV**K**Q**I**RR**G**R**K**PD**G**V**C**LV**V**G**T**CARG**F**E**S****N****T****S****D****V****I****F****S****S****S**  
Gmax 241 LSGDKILSSRLIIDAMGNFSP**V**V**K**Q**I****R****G**R**K**PD**G**V**C**LV**V**G**T**CARG**F**E**T****N****S****T****S****D****V****I****F****S****S****S**  
Mtru 236 LSGG**K**V**L**SSRLIIDAMGNFSP**V**V**K**Q**I**RR**G**R**K**PD**G**V**C**LV**V**G**T**CARG**F**EN**N****S****N****D****V****I****F****S****S****S**  
Atha 241 LSKGDT**L**SSRL**I**VIDAMGNFSP**I**I**K**Q**I****R****G**R**K**PD**G****M**CL**V**V**G****S**AH**G****F**K**E****N****S****S****D****V****I****Y****S****S****S**

Lfil 301 VKKVGD**S****Q**A**Q**YFWEAFPA**A**SGPLDR**T**TYMFTY**V****E****A**Q**P**G**S**P**K**LE**E****L**LE**A****Y**W**D**L**M**P**E****Y****Q****G****V****S**  
Ljap 301 VKKVGD**S****Q**A**Q**YFWEAFPA**A**SGPLDR**T**TYMFTY**V****E****A**Q**P**G**S**P**K**LE**E****L**LE**A****Y**W**D**L**M**P**E****Y****Q****G****V****S**  
Gmax 301 IKKVGD**S****Q**A**Q**YFWEAFPA**A**SGPLDR**T**TY**I****L**F**T**Y**V****E****P****Q****P****A****S****P****K**LE**E****L**LE**A****Y**W**D**L**M**P**E****Y****Q****G****V****S**  
Mtru 296 VK**V**VGD**S****Q**A**Q**YFWEAFPA**A**PG**S**GPLDR**T**TYMFTY**V****E****P****Q****P****G****S**P**K**LE**E****L**LE**E****Y****W****N**L**M**P**E****Y****Q****G****V****P**  
Atha 301 VT**V**V**A**DS**N****V****O****L**FWEAFPA**A**PG**S**GPLDR**T**TYMFTY**T****E****P****Q****S****T****S****P****L****E****D****L****L****E****Y****W****K**L**M****P****K****Y****Q****G****V****S**

Lfil 361 LD**N****L**E**I**LRVI**Y****G****I**FPT**Y****R****E****S****P****L****P****A****F****S****R****V****L****Q****F****G****D****A****S****G****I****Q****S****P****V****S****F****GG****F****G****S****L****T****R****H****L****G****R****I****S****A****G**  
Ljap 361 LD**N****L**E**I**LRVI**Y****G****I**FPT**Y****R****E****S****P****L****P****A****F****C****R****V****L****Q****F****G****D****A****S****G****I****Q****S****P****V****S****F****GG****F****G****S****L****T****R****H****L****G****R****I****S****A****G**  
Gmax 361 LD**N****L**E**I**LRVI**Y****G****I**FPT**Y****R****Q****S****P****L****P****A****F****S****R****V****L****Q****F****G****D****A****S****G****I****Q****S****P****V****S****F****GG****F****G****S****L****T****R****H****L****G****R****L****S****A****G**  
Mtru 356 LD**N****L**E**I**LRVI**Y****G****I**FPT**Y****R****E****S****P****L****P****A****F****S****R****V****L****Q****F****G****D****A****S****G****I****Q****S****P****V****S****F****GG****F****G****S****L****T****R****H****L****G****R****L****S****A****G**  
Atha 361 LD**E****L**E**I**LRV**V****Y****G****I**FPT**Y****R****N****S****P****L****P****A****F****D****R****V****L****Q****F****G****D****A****S****G****I****Q****S****P****V****S****F****GG****F****G****S****L****T****R****H****L****G****R****L****S****N****G**

Lfil 421 IYEAINGDYLD**S****Y****N****L****S****L****N****P****Y****M****P****N****L****S****A****S****W****L****F****Q****R****A****M****S****A****K****K****Q****S****N****V****P****P****D****F****I****N****E****L****L****Y****A****N****F****S****C****M****Q**  
Ljap 421 IYEAINGDYLD**S****Y****N****L****S****L****N****P****Y****M****P****N****L****S****A****S****W****L****F****Q****R****A****M****S****A****K****K****Q****S****N****V****P****P****D****F****I****N****E****L****L****Y****A****N****F****S****C****M****Q**  
Gmax 421 IHEAINGDYLD**S****Y****N****L****S****L****N****P****Y****M****P****N****L****S****A****S****W****L****F****Q****R****A****M****S****A****K****K****Q****S****P****V****S****F****GG****F****G****S****L****T****R****H****L****G****R****L****S****A****G**  
Mtru 416 I**Q****E****A****I****D****G****D****Y****L****D****S****Y****N****L****S****L****N****P****Y****M****P****N****L****S****A****S****W****L****F****Q****R****A****M****S****A****K****Q****S****D****V****P****E****D****F****I****N****E****L****L****Y****A****N****F****S****C****M****Q**  
Atha 421 IY**D****A****I****D****G****D****L****D****S****D****L****S****K****L****N****P****Y****M****P****N****L****S****A****S****W****L****F****Q****R****A****M****S****A****K****Q****S****D****V****R****G****F****T****N****E****L****L****H****V****N****F****S****C****M****Q**

|      |     |  |
|------|-----|--|
| Lfil | 481 | RLGDPVLRPFLQDVVQFGPLSKTLGLVMLTKPQILPSIFK |
| Ljap | 481 | RLGDPVLRPFLQDVVQFGPLSKTLGLVMLTKPQILPSIFK |
| Gmax | 481 | RLGDPVLRPFLQDVVQFGPLSKTLGLVMLTNPKILPSIFK |
| Mtru | 476 | RLGDPVLRPFLQDVVQFGALSCTLGLVMLTRPQILPSIFK |
| Atha | 481 | RLGDPVLRPFLQDIIQFGPLAKTLGLVMLTKPQIIPSIFR |

Figure S8. Amino acid alignment for E-CHY

Lfil 1 MASHAALLHVPPPLSIP[TQRFYHKQICINRLKPSITSSSSW[FPCSSSSVIVCSSSNGKS  
Ljap 1 MASHAALLHVPPPLSIP[TQRFYHKQICINRLKPSITSSSSW[FPCSSSSVIVCSSSNGKS  
Gmax 1 MASHVALLRVPPPLSISTQRFHAKQICINGLKLTNTSSSSCFPCSCSSVITCSSSNGRD  
Mtru 1 MASHLTLLHAPPPLSLQTKTFHSKYITIKPLKPTTTSSSCSLSFPCSCSSFIACCSSSNGRS  
Atha 1 MAMAFPL-SYTPTI-----TVKP-----V-SNFVVFSSSSNGRD

Lfil 61 SDSGDDG-----IKSKQRAELSARIAKGEGFTVNQSGFPSDLKKSLSQVGVPNEILDFL  
Ljap 61 SDSGDDG-----IKSKQRAELSARIAKGEGFTVNQSGFPSDLKKSLSQVGVPNEILDFL  
Gmax 61 PNSVD[EKOVERILEEKRRRAELSAKIASGEFTVKQKSGLISI-MEGLAKVGVPNEVLEFL  
Mtru 61 PDSVDDGKSAQDQLEEKRRRAELSAKIASGEFTVKQESGLPSILKKSLSNLGVSNEILEFL  
Atha 33 PNSVPNGKSL[EKQEEKRRAELSAIASGAFTVRKS-SFPSTVKNGLSKIGIPESNVLDFM

Lfil 115 FGWIIEGGYYYPKVPEAKGSINAVRNEAFFIPLYELYLYTGGIFRLTFGPKSFLIVSDPSI  
Ljap 115 FGWIIEGGYYYPKVPEAKGSINAVRNEAFFIPLYELYLYTGGIFRLTFGPKSFLIVSDPSI  
Gmax 120 FGWFEGGGEHPKIPEAKGSIKAVRSVAFFIPLYELYLYTGGIFRLTFGPKSFLIVSDPSI  
Mtru 121 FG-----IYPKIPPEAKGSISAI[RSEAFFIPLYELYIITYGGIFRLNFGPKSFLIVSDPAI  
Atha 92 FDWTGSDQDYPKVPEAKGSIQAVRNEAFFIPLYELFLTYGGIFRLTFGPKSFLIVSDPSI

Lfil 175 AKHILKDNAKGSKGILAEILDVMGKGLIPADGEIWVRVRRRAIVPALHQKYVAAMIGLF  
Ljap 175 AKHILKDNAKGSKGILAEILDVMGKGLIPADGEIWVRVRRRAIVPALHQKYVAAMIGLF  
Gmax 180 AKHIL[RENSKAYSKGILAEILDVMGKGLIPADGEIWVRVRRRAIVPALHQKYVAAMIGLF  
Mtru 175 AKHILKDNSKAYSKGILAEILDVMGKGLIPADGEIWVRVRRRTIVPALH[K]FVAAMIGLF  
Atha 152 AKHILKDNAKAYSKGILAEILDVMGKGLIPADGEIWRRRRAIVPALHQKYVAAMISLF

Lfil 235 GQATDRLCKLDTAAASDGEDVEMESLFSRLTLDVIGKAVFNYDFDSLSDNTGIIEAVYNV  
Ljap 235 GQATDRLCKLDTAAASDGEDVEMESLFSRLTLDVIGKAVFNYDFDSLSDNTGIIEAVYNV  
Gmax 240 GQAADRLCQKLDAAASDGEDVEMESLFSRLTD[I]IGKAVFNYDFDSLSDNTGIYEAVYTV  
Mtru 235 GQATDRLCQKLDAAASDGEDVEMESLFSRLTLDVIGKAVFNYDFDSLSDNTGIIEAVYTV  
Atha 212 GEASDRLCQKLDAAALKGEFVEMESLFSRLTD[I]IGKAVFNYDFDSLTDNTGVIEAVYTV

Lfil 295 LREAEDRSVSPPIPTWEIPIWKDISPROKKVNAALKLVNDTLDDLIAICKRMVDEEEELQFH  
Ljap 295 LREAEDRSVSPPIPTWEIPIWKDISPROKKVNAALKLVNDTLDDLIAICKRMVDEEEELQFH  
Gmax 300 LREAEDRSVAPIPVWEIPIWKDVSPRLRKVNAALKLINDTLLDLIAICKRMVDEEEELQFH  
Mtru 295 LREAEDRSITSPIPWWDIPIWKDISPRQRKVTAALKLVNDTLNNLIAICKRMVDEEEELQFH  
Atha 272 LREAEDRSVSPPIPVDIPIWKDISPRQRKVATSLKLINDTLLDLIA[CKRMV]EEELQFH

Lfil 355 EEYMNEQDPSILHFLLAAGDDVSSKQLRDDLMTLILIAGHETSAAVLTWTFYLLSKEPSVM  
Ljap 355 EEYMNEQDPSILHFLLAAGDDVSSKQLRDDLMTLILIAGHETSAAVLTWTFYLLSKEPSVM  
Gmax 360 EEYMNEQDPSILHFLLASGDVSSKQLRDDLMTMLIAGHETSAAVLTWTFYLLSKEPRVM  
Mtru 355 EEYMNEQDPSILHFLLASGDVT[SKQLRDDLMTMLIAGHETSAAVLTWTFYLLSKEPSVM  
Atha 332 EEYMNERDPSILHFLLASGDVSSKQLRDDLMTMLIAGHETSAAVLTWTFYLLTEPSVV

Lfil 415 SKLQEEVDSVLGDRFPTIEDMKKLKYTRVINESLRLYPQPPVLIIRRSIDDDVLGEYPIK  
Ljap 415 SKLQEEVDSVLGDRFPTIEDMKKLKYTRVINESLRLYPQPPVLIIRRSIDDDVLGEYPIK  
Gmax 420 SKLQEEVDSVLGDQYPTIEDMKKLKYTRVINESLRLYPQPPVLIIRRSLEDDVLGEYPIK  
Mtru 415 SKLQEEVDSVLGDRFPTIEDMKKLKYTRVINESLRLYPQPPVLIIRRSIEDDVVLGEYPIK  
Atha 392 AKLQEEVDSVIGDRFPTIOMMKKLKYTRVMNESLRLYPQPPVLIIRRSIDNDILGEYPIK

|      |     |  |
|------|-----|--|
| Lfil | 475 | KGEDIFISI <span style="background-color: black; color: black;">WNLHRS</span> PHLWDDADKFEPE <span style="background-color: black; color: black;">RPLDGP</span> NPNETNQNFRYLPF <span style="background-color: black; color: black;">GGGQ</span> RKCIGDM  |
| Ljap | 475 | KGEDIFISI <span style="background-color: black; color: black;">WNLHRS</span> PHLWDDADKFEPE <span style="background-color: black; color: black;">RPLDGP</span> NPNETNQNFRYLPF <span style="background-color: black; color: black;">GGGQ</span> RKCIGDM  |
| Gmax | 480 | R <span style="background-color: black; color: black;">NEDIFISV</span> WNLHRS <span style="background-color: black; color: black;">P</span> HLWDDADKFEPE <span style="background-color: black; color: black;">RWA</span> DGP <span style="background-color: black; color: black;">SP</span> NETNQNFKYLPF <span style="background-color: black; color: black;">GGGPR</span> KC <span style="background-color: black; color: black;">VG</span> DL  |
| Mtru | 475 | R <span style="background-color: black; color: black;">GEDIFISV</span> WNLHRS <span style="background-color: black; color: black;">PTLW</span> NDA <span style="background-color: black; color: black;">DKF</span> EPE <span style="background-color: black; color: black;">RPLDGP</span> NPNETNQN <span style="background-color: black; color: black;">QGF</span> KYLPF <span style="background-color: black; color: black;">GGGPR</span> KC <span style="background-color: black; color: black;">IG</span> DM  |
| Atha | 452 | R <span style="background-color: black; color: black;">GEDIFISV</span> WNLHRS <span style="background-color: black; color: black;">PLHW</span> DDA <span style="background-color: black; color: black;">F</span> K <span style="background-color: black; color: black;">E</span> NPER <span style="background-color: black; color: black;">RPLDGP</span> NPNETNQN <span style="background-color: black; color: black;">FSY</span> LPF <span style="background-color: black; color: black;">GGGPR</span> KC <span style="background-color: black; color: black;">IG</span> DM |
| <br> |     |  |
| Lfil | 535 | FAT <span style="background-color: black; color: black;">FETVV</span> VALAMLVRRFN <span style="background-color: black; color: black;">FQMA</span> I <span style="background-color: black; color: black;">GAPPV</span> VMTTGATI <span style="background-color: black; color: black;">HTTQGLN</span> MTVTRRI <span style="background-color: black; color: black;">QPPIVPSL</span> H <span style="background-color: black; color: black;">HM</span>  |
| Ljap | 535 | FAT <span style="background-color: black; color: black;">FETVV</span> VALAMLVRRFN <span style="background-color: black; color: black;">FQMA</span> I <span style="background-color: black; color: black;">GAPPV</span> VMTTGATI <span style="background-color: black; color: black;">HTTQGLN</span> MTVTRRI <span style="background-color: black; color: black;">QPPIVPSL</span> H <span style="background-color: black; color: black;">HM</span>  |
| Gmax | 540 | FAS <span style="background-color: black; color: black;">YETVV</span> VALAMLVRRFN <span style="background-color: black; color: black;">FQ</span> I <span style="background-color: black; color: black;">AVGAPPV</span> E <span style="background-color: black; color: black;">EMTTGATI</span> HTTQGL <span style="background-color: black; color: black;">KMTVT</span> <span style="background-color: black; color: black;">HRIKPPIVPSL</span> QM  |
| Mtru | 535 | FAS <span style="background-color: black; color: black;">YEVVV</span> VALAMLVRRFN <span style="background-color: black; color: black;">FQMA</span> V <span style="background-color: black; color: black;">GAPPV</span> VMTTGATI <span style="background-color: black; color: black;">HTTQGLN</span> MTVTRRI <span style="background-color: black; color: black;">KPPIVPSL</span> QM  |
| Atha | 512 | FAS <span style="background-color: black; color: black;">FENVV</span> VAT <span style="background-color: black; color: black;">AML</span> I <span style="background-color: black; color: black;">IRRNFQ</span> I <span style="background-color: black; color: black;">APGAPPV</span> K <span style="background-color: black; color: black;">MTTGATI</span> HTTE <span style="background-color: black; color: black;">GLKLTV</span> T <span style="background-color: black; color: black;">KRTKPLD</span> <span style="background-color: black; color: black;">IPSVP</span> I |
| <br> |     |  |
| Lfil | 595 | ST <span style="background-color: black; color: black;">VEVDQ</span> KDQVY <span style="background-color: black; color: black;">QAO</span>   |
| Ljap | 595 | ST <span style="background-color: black; color: black;">VEVDQ</span> KDQVY <span style="background-color: black; color: black;">QAO</span>   |
| Gmax | 600 | ST <span style="background-color: black; color: black;">VEVDQ</span> K <span style="background-color: black; color: black;">GEV</span> VY <span style="background-color: black; color: black;">QAO</span>  |
| Mtru | 595 | ST <span style="background-color: black; color: black;">VEVDQ</span> KDQVY <span style="background-color: black; color: black;">QAO</span>   |
| Atha | 572 | LP <span style="background-color: black; color: black;">MD--SRD</span> E <span style="background-color: black; color: black;">VSSAL</span>   |

Figure S9. Amino acid alignment for ZEP-1

|      |     |   |
|------|-----|---|
| Lfil | 1   | LCYNSLNPSTA<br>AFSRTNFSVPSNKD<br>HSLETSPFSYVHRL<br>GРАTKQGKKVTHVKA<br>AAVAERE                                   |
| Ljap | 1   | LCYNSLNPSTA<br>AFSRTNFSVPSNKD<br>HSLETSPFSYVHRL<br>GРАTKQGKKVTHVKA<br>AAVAERE                                   |
| Gmax | 1   | LCYNSLNPSTA<br>IVFSRTHFSVPLNKE<br>LPLDASPFGYNCG<br>VGRTRKQRKKV<br>MHVKA<br>CAVV--E                              |
| Mtru | 1   | LSHKCLSPSM<br>TFSRTHFSNP<br>-----<br>FSYGN--<br>RTTKQRKLMQVKAT<br>VM-HE   |
| Atha | 1   | FCY-SINPSKLDFT<br>TRTHVFS<br>PVSKQFY<br>LDLSSFGKPGGV<br>-SGFRSRRALL<br>GVKAALVEKE                               |
| <br> |     |   |
| Lfil | 61  | APKKQLRVLVAGGGIGGLVF<br>ALA<br>AKKKGF<br>EVVV<br>FEKDL<br>SA<br>VRGE<br>GQYRG<br>PIQI<br>QSNA<br>LAA            |
| Ljap | 61  | APKKQLRVLVAGGGIGGLVF<br>ALA<br>AKKKGF<br>EVVV<br>FEKDL<br>SA<br>VRGE<br>GQYRG<br>PIQI<br>QSNA<br>LAA            |
| Gmax | 59  | APKKQLR<br>ILVAGGGIGGLVF<br>ALA<br>AKRK<br>GFEV<br>VFEK<br>DL<br>AIRGE<br>GQYRG<br>PIQI<br>QSNA<br>LAA          |
| Mtru | 46  | APKKQL<br>KLVAGGGIGGLVF<br>ALA<br>AKRK<br>GFEV<br>VFEK<br>DL<br>AIRGE<br>GQYRG<br>PIQI<br>QSNA<br>LAA           |
| Atha | 59  | EKKK<br>KS<br>RVLVAGGGIGGLVF<br>ALA<br>AKKKGF<br>DVL<br>VFEK<br>DL<br>AIRGE<br>GK<br>YRG<br>PIQI<br>QSNA<br>LAA |
| <br> |     |   |
| Lfil | 121 | LEAID<br>-----  |
| Ljap | 121 | LEAID<br>PDVA<br>DEVMR<br>LWCITGDR<br>INGL  |
| Gmax | 119 | LEAID<br>SEVA<br>DEVMR<br>VG<br>CITGDR<br>INGL  |
| Mtru | 106 | LEAID<br>DMN<br>VA<br>DEV<br>VMR<br>VG<br>CITGDR<br>INGL  |
| Atha | 119 | LEAID<br>IEVA<br>EQV<br>MEA<br>AGC<br>ITGDR<br>INGL   |

Figure S10. Amino acid alignment for ZEP-2

|      |     |  |
|------|-----|--|
| Lfil | 1   | ----KIREYDSKRLVKEHFKRLSGKELPIKSAQVTGSTDFSELQDKEPWLSSSKLVVKPD     |
| Ljap | 1   | MARKKIREYDSKRLVKEHFKRLSGKELPIKSAQVTGSTDFSELQDKEPWLSSSKLVVKPD     |
| Gmax | 1   | MARKKIREYDSKRLIKEHFKRISGQELPIKSAQVTTESTNFSELAFKEPWLLSSSKLVVKPD   |
| Mtru | 1   | MARKKIREYDSKRLIKEHFKRLSGKELPIKSAQVTEATDFTELQDKEQWLSSSKLVVKPD     |
| Atha | 1   | MARKKIREYDSKRLVKEHFKRLSGQELPIRSVQINQETDLNELVEREPWLSSEKLVVKPD     |
| <br> |     |  |
| Lfil | 57  | MLFGKRGKSGLVALNLDFAQASFVKERLGKEVEMGGCKGPITTFIVEPFIPHNEEFYLN      |
| Ljap | 61  | MLFGKRGKSGLVALNLDFAQASFVKERLGKEVEMGGCKGPITTFIVEPFIPHNEEFYLN      |
| Gmax | 61  | MLFGKRGKSGLVALNLDAEVDASFVKERLGKEVEMGGCKGPITTFIVEPFIPHNEEFYLN     |
| Mtru | 61  | MLFGKRGKSGLVALNLDAQASFVKERLGKEVEMGGCKGPITTFIVEPFIPHNEYLYLN       |
| Atha | 61  | MLFGKRGKSGLVALNLDFADVATFVKERLGKEVEMSGCKGPITTFIVEPFVPHNEEFYLN     |
| <br> |     |  |
| Lfil | 117 | IVSERLGNSISFSEC GGIDIEENWDKVKT VF IPTGVSLTSEIIVAPLVATLPLEIKGEIEE |
| Ljap | 121 | IVSERLGNSISFSEC GGIDIEENWDKVKT VF IPTGVSLTSEIIVAPLVATLPLEIKGEIEE |
| Gmax | 121 | IVSERLGNSISFSEC GGIEFIEENWDKVKT FMPTGVSLTSEISIAPLVATLPLEIKGEIEE  |
| Mtru | 121 | IVSDRLGNSISFSEC GGIDIEENWDKVKT F IPTGVSLTSEIIAPLVATLPLEIKGEIEE   |
| Atha | 121 | IVSDRLGCISFSEC GGIDIEENWDKVKT ITIPTGASLTFEICAPLVATLPLEIKGELED    |
| <br> |     |  |
| Lfil | 177 | FLKVIFTLFQDL DFTFLEMNPFTLVDGKPYPLDMRGELDDTA AFKNFKKWGNIEFPLPFG   |
| Ljap | 181 | FLKVIFTLFQDL DFTFLEMNPFTLVDGKPCPLDMRGELDDTA AFKNFKKWGNIEFPLPFG   |
| Gmax | 181 | FLKVIFTLFQDL DFTFLEMNPFTLNVNGKPYPLDMRGELDDTA AFKNFKKWGNIEFPLPFG  |
| Mtru | 181 | FLKVIFTLFQDL DFTFLEMNPFTLVDGKPYPLDMRGELDDTA AFKNFKKWGNIEFPLPFG   |
| Atha | 181 | FIQVIFTLFEDLDFTFLEMNPFTLVDGKPYPLDMRGELDDTA AFKNFKKWGDIEFPMPFG    |
| <br> |     |  |
| Lfil | 237 | RVMSATESFVHE LDEKTSASLKFTVLNP EGR IWTMVAGGGASVIYADTVGDLGYAAELGN  |
| Ljap | 241 | RVMSATESFVHE LDEKTSASLKFTVLNPKGRIWTMVAGGGASVIYADTVGDLGFASELGN    |
| Gmax | 241 | RVMSTTEAFIHL DEKTSASLKFTVLNPMPGRIWTMVAGGGASVIYADTVGDLGYAPELGN    |
| Mtru | 241 | RVMSATESFIHL DEKTSASLKFTVLNPKGRIWTMVAGGGASVIYADTVGDLGIANELGN     |
| Atha | 241 | RVMSSTESFIHL DEKTSASLKFTVLNPKGRIWTMVAGGGASVIYADTVGDLGYASELGN     |
| <br> |     |  |
| Lfil | 297 | YAEYSGAPKEDEV LQYARVV IDCATANPDGQKRALVIGGGIANFTDVAATFSGIIRALKE   |
| Ljap | 301 | YAEYSGAPKEDEV LQYARVV IDCATANPDGQKRALVIGGGIANFTDVAATFSGIIRALKE   |
| Gmax | 301 | YAEYSGAPKEDEV LQYARVV IDCATSNDGQKRALVVGGGIANFTDVAATFSGIIRALKE    |
| Mtru | 301 | YAEYSGAPNEDEV LQYARVV IDCATANPDGQKRALVIGGGIANFTDVAATFSGIIRALKE   |
| Atha | 301 | YAEYSGAPKEDEV LQYARVV IDCATANPDGKSRALVIGGGIANFTDVAATFNGIIRALKE   |
| <br> |     |  |
| Lfil | 357 | KEQKLKEAKMHIYVR RGGPNYQKGLAKMRALGEEIGIPIEVYGPEATMTGICKEAIQCIT    |
| Ljap | 361 | KESKLKAARMHIYVR RGGPNYQKGLALMRALGEEIGIPIEVYGPEATMTGICKEAIQCIT    |
| Gmax | 361 | KEQKLKEAKMHIYVR RGGPNYQKGLAKMRALGEEIGIPIEVYGPEATMTGICKQAIQYIT    |
| Mtru | 361 | KESKLKAARMHIYVR RGGPNYQKGLEKMRALGEEIGIPIEVYGPEATMTGICKQAIQCIT    |
| Atha | 361 | KEAKLKAARMHIFVR RGGPNYQKGLAKMRSLGDEIGVPIEVYGPEATMTGICKEAIQYIT    |
| <br> |     |  |
| Lfil | 417 | AAA  |
| Ljap | 421 | AAA  |
| Gmax | 421 | AAA  |
| Mtru | 421 | ASA  |
| Atha | 421 | AAA  |

## Appendix 5

Supplementary Figures S11 – S17 Amino acid alignments for yellow-differentially expressed carotenoid genes in *Lotus filicaulis*

Figure S11. Amino acid alignment for CDS-1

|      |     |  |
|------|-----|--|
| Lfil | 1   | M--ASLIHCPAATSLSATR-ADSVCGFLVLPSSSRFIKTHKCRVRCSLDNVSDMSVNAPK   |
| Ljap | 1   | M--ASLIHCPAATSLSATR-GDSVCGFLV-PSSSRFIKTHKCRVRCSLDNVSDMSVNAPK   |
| Gmax | 1   | MAMASLIQGS-ATSLSAV-----PIITTRFTTRTHKSRLRCSDLANVSDMSVNAPK   |
| Mtru | 1   | MM-TSLIHCP-ATSLSTTRPGDSV AFFV-PSRFTKTRSYKPVRVRCSDLNVSDMSTNAPK  |
| Atha | 1   | M--ASSVVFAATGSLSLVP-----P-----LKSRRFYVNSSLSDVSDMSVNAPK   |
| <br> |     |  |
| Lfil | 58  | GLFPPEPEHYRGPKLKVAIIAGLAGMSTAVELLDQGHEVDIYESRPFIGGKVGSFVDKR  |
| Ljap | 57  | GLFPPEPEHYRGPKLKVAIIAGLAGMSTAVELLDQGHEVDIYESRPFIGGKVGSFVDKR  |
| Gmax | 49  | GLFPPEPEHYRGPKLKVAIIAGLAGMSTAVELLDQGHEVDIYESRPFIGGKVGSFVDKG  |
| Mtru | 58  | GLFPPEPEHYRGPKLKVAIIAGLAGMSTAVELLDQGHEVDIYESRTFIGGKVGSFVDKR  |
| Atha | 43  | GLFPPEPVYKGPKLKVAIIAGLAGMSTAVELLDQGHEVDIYDSRTFIGGKVGSFVDRR   |
| <br> |     |  |
| Lfil | 118 | GNHIEMGLHVFFGCYNNLFRLMKKVGAENNLLVKDHHTHTFVN <span style="background-color: black; color: white;">T</span> GGQIGELDFRFPIGAPI  |
| Ljap | 117 | GNHIEMGLHVFFGCYNNLFRLMKKVGAENNLLVKDHHTHTFVN <span style="background-color: black; color: white;">T</span> GGQIGELDFRFPIGAPI  |
| Gmax | 109 | GNHIEMGLHVFFGCYNNLFRLLKKVGAENNLLVKDHHTHTFVN <span style="background-color: black; color: white;">K</span> GGQIGELDFRFPIGAPI  |
| Mtru | 118 | GNHIEMGLHVFFGCYNNLFRLLKKVGAENNLLVKDHHTHTFVN <span style="background-color: black; color: white;">K</span> GGQIGELDFRFPVGAPI  |
| Atha | 103 | GNHIEMGLHVFFGCYNNLFRLMKKVGAENLLVKDHHTHTF <span style="background-color: black; color: white;">T</span> NKDG <span style="background-color: black; color: white;">T</span> IGELDFRFPVGAPI   |
| <br> |     |  |
| Lfil | 178 | HGI <span style="background-color: black; color: white;">T</span> AFLT TNQLKYDKARNALALALSPVVRALVDPDGALRDIRNLDVSFSDWFLSKGG  |
| Ljap | 177 | HGI <span style="background-color: black; color: white;">T</span> AFLT TNQLKYDKARNALALALSPVVRALVDPDGALRDIRNLDVSFSDWFLSKGG  |
| Gmax | 169 | HGI <span style="background-color: black; color: white;">R</span> AFLT TNQLNTYDKARNAVALALALSPVVRALVDPDGALRDIRNLD <span style="background-color: black; color: white;">S</span> FSDWFLSKGG  |
| Mtru | 178 | HGI <span style="background-color: black; color: white;">R</span> AFLT TNQLKYDKARNAVALALALSPVVRALVDPDGALRDIRNLDVSFSDWFMSKGG  |
| Atha | 163 | HGI <span style="background-color: black; color: white;">R</span> AFLV <span style="background-color: black; color: white;">T</span> NQLKPYDK <span style="background-color: black; color: white;">L</span> RNSLALALALSPVVRALVDPDGAMRDIRNLD <span style="background-color: black; color: white;">S</span> FSDWFLSKGG |
| <br> |     |  |
| Lfil | 238 | TRTSIQKMWDVPAYALGFIDCDNISARCMLTIFALFATKTEASLLRMLKGSPDVYLSGPI   |
| Ljap | 237 | TRTSIQKMWDVPAYALGFIDCDNISARCMLTIFALFATKTEASLLRMLKGSPDVYLSGPI   |
| Gmax | 229 | TRMSITKMWDVPAYALGFIDCDNISARCMLTIFALFATKTEASLLRMLKGSPDVYLSGPI   |
| Mtru | 238 | TRTSIKKMWDVPAYALGFIDCDNISARCMLTIFALFATKTEASLLRMLKGSPDVYLSGPI   |
| Atha | 223 | TRASIQRMWDVPAYALGFIDCDNMSARCMLTIFS <span style="background-color: black; color: white;">L</span> FATKTEASLLRMLKGSPDVYLSGPI   |
| <br> |     |  |
| Lfil | 298 | RKYITDRGGRFHLRWGCREILYDE <span style="background-color: black; color: white;">S</span> ADGSTYVKGLSMSKATAKQIV <span style="background-color: black; color: white;">I</span> VKADAYVAACDVPGI   |
| Ljap | 297 | RKYITDRGGRFHLRWGCREILYDE <span style="background-color: black; color: white;">S</span> ADGSTYVKGLSMSKATAKQIV <span style="background-color: black; color: white;">I</span> VKADAYVAACDVPGI   |
| Gmax | 289 | RKYIMDRGGRFHLRWGCREILYDKSADGSI <span style="background-color: black; color: white;">I</span> YVRGLSMSKATAKKIV <span style="background-color: black; color: white;">I</span> VKADAYVAACDVPGI  |
| Mtru | 298 | RKYITDRGGRFHLRWGCREVLYDKSADGSTYVTGLSISKATEKKIV <span style="background-color: black; color: white;">E</span> ADAYVAACDVPGI   |
| Atha | 283 | KQYITDRGGRFHLRWGCREILYDKSADGETYVTGLAISKATNNKKIV <span style="background-color: black; color: white;">K</span> ADVYVAACDVPGI  |
| <br> |     |  |
| Lfil | 358 | KRLIPSEWRNEFFNNIYELVGVPVTVQLRYNGWVTELQNLEKSRQLKKATGLDNLLYT   |
| Ljap | 357 | KRLIPSEWRNEFFNNIYELVGVPVTVQLRYNGWVTELQNLEKSRQLKKATGLDNLLYT   |

|      |     |  |
|------|-----|--|
| Gmax | 349 | KRLLPSEWREQEFFNNIYELVGVPVVTVQLRYNGWVTELQDLEKSRLGKAVGLDNLLYT    |
| Mtru | 358 | KRLLPSEWREKEFFNNIYELVGVPVVTVQLRYNGWVTELQNLLELSRQLKKATGLDNLLYT  |
| Atha | 343 | KRLLPKIEWRESRFFNDIYELEEGVPVVTVQLRYNGWVTELQDIELARQLKRAVGLDNLLYT |
| <br> |     |  |
| Lfil | 418 | PDADFSCFADLALTSPEDYYIEGQGSLLQCVLTPGDPYMPNPEEIIARVAKQ-----      |
| Ljap | 417 | PDADFSCFADLALTSPEDYYIEGQGSLLQCVLTPGDPYMPNPEEIIARVAKQ-----      |
| Gmax | 409 | PDADFSCFADLALSSPEDYYIEGQGSLLQCVLTPGDPYMPNDEIIARVAKQVLALFPS     |
| Mtru | 418 | PDADFSCFADLALASPEDYYIEGQGSLLQCVLTPGDPYMPNPEEII SRVAKQVISLFPS   |
| Atha | 403 | PDADFSCFADLALASPADYYIEGQGTLLQCVLTPGDPYMRMPNDKIIEKVAMQVTELFPS   |
| <br> |     |  |
| Lfil |     | -----  |
| Ljap |     | -----  |
| Gmax | 469 | SQGLEVTWSSVVKIGQSLYREGPGKDPYRPDQKTPVRNFFLAGSYTKQDYIDSMEGATLS   |
| Mtru | 478 | SQGLEVTWSSVVKIGQSLYREGPGKDPFRPDQKTPVKNFFLGSYTKQDYIDSMEGATLS    |
| Atha | 463 | SRGLEVTWSSVVKIAQSLYREAPGKDPFRPDQKTPIKNFFLAGSYTKQDYIDSMEGATLS   |
| <br> |     |  |
| Lfil |     | -----  |
| Ljap |     | -----  |
| Gmax | 529 | GRQASAYICDAGEELVALRKKLDAEFKDDLKI SNTKDELSLVX                   |
| Mtru | 538 | GRQTSAYICDAGEELVALRKELLAQSKDDIKFTNTKDELSLVX                    |
| Atha | 523 | GRQASSYICDAGEELAELNKKLSSS-----AVPDELSLVX                       |

Figure S12. Amino acid alignment for CIS

|      |     |   |
|------|-----|---|
| Lfil | 1   | MASSPLVFSAPFFSHRTSSTKTHLSLPLSSSRSVFPSKPLLSRKLLAPASVAEVGEDSA   |
| Ljap | 1   | MASSPLVFSAPFFSHRTSSTKTHLSLPLSSSRSVFPSKPLLSRKLLAPASVAEVGEDSA   |
| Gmax | 1   | MAASVVF-STSLSVYGY-HRPPSISITKTNLKSNSNSVAPIYCRKFVARTSIGEVGEDSA  |
| Mtru | 1   | MASTPLVFSTSISSSHLL-HHHP--SLPSSSLCQYFSSNPLLLSHKLVTRVSSSEVGEDSA |
| Atha | 1   | MAVYHLLILSSPPSLLPPSPRRPNLRIPAH-PRSLLSSSPV-IRKTLVRSTIRETGEDSA  |
| <br> |     |   |
| Lfil | 61  | AFDLQSQKVSSWVYFTVILGVVLFAALNVVWIDPSTGFGKDFVDAVSGISDSPEVVMLLI  |
| Ljap | 61  | VFDLQSQKISSWVYFTVILGVVLFAALNVVWIDPSTGFGKDFVDAVSGISDSPEVVMLLI  |
| Gmax | 59  | VFEGLGKQKVSSWIYFTAIGGVVLYVLNVAWIDNSTGYGKAFIDAVSTSDSSEVVMLILI  |
| Mtru | 58  | TFDIONQKLSWWFYFTAIGGVVLSVLNVIWIDDSTGFGKAFVDSISGISDSHEVVMLVLV  |
| Atha | 59  | AFFELGKQKLVSWVYFGVVLGVVLFTLNVVWIDNSTGFGKSFIDAVSNISGSPEVAMLMLI |
| <br> |     |   |
| Lfil | 121 | LIFAGVHGLASFRTGEKLIGERAFRVLFAGTSPLALTTIVYFINHRYDGLQLWQLQN     |
| Ljap | 121 | LIFAGVHGLASFRTGEKLIGERAFRVLFAGTSPLALTTIVYFINHRYDGEQLWQLQN     |
| Gmax | 119 | LIFAGVHGLASFRTGEKLIGERPFRTVLFAGISLPLAVSTVYFINHRYDGLQLWQLQD    |
| Mtru | 118 | LIFAGVHGLASFRTGEKLIGERAIRVLFAGTSPLALTMIVYFINHRYDGLQLWQLQD     |
| Atha | 119 | LIFATVHGLASLRDIEKLIGERAFRVLFAGISLPLAMSTIVYFINHRYDGSQLWQLQG    |
| <br> |     |   |
| Lfil | 181 | VPGLHQLLWLSNFISFYFLYPSTFNLLLEVAAVDKPKLHLWETGIMRITRHPQLVGQVIWC |
| Ljap | 181 | VPGLHQLLWLSNFISFYFLYPSTFNLLLEVAAVDKPKLHLWETGIMRITRHPQLVGQVIWC |
| Gmax | 179 | APGLHQLLWLSNFISFYFLYPSTFNLLLEVAAVDKPKLHLWETGIMRITRHPQMVGQVIWC |
| Mtru | 178 | VPGVHQLVWISNFISFYFLYPATFNLLLEVAAVDKPKLHLWETGIMRITRHPQMVGQVMWC |
| Atha | 179 | VPGVHEAIWVANFVSFFFLYPSTFNLLLEVAAVDKPKMHLWETGIMRITRHPQVNISLMC  |
| <br> |     |   |
| Lfil | 241 | LAHTIWIGNSVAVAASVGLIGHHLFGAWNGDRRLAKRYGEDFDLVKERTSIVPFAAILDG  |
| Ljap | 241 | LAHTIWIGNSVAVAASVGLIGHHLFGAWNGDRRLAKRYGEDFDLVKERTSIVPFAAILDG  |
| Gmax | 239 | LAHTIWIGNSVAVAASIGLIAHHLFGWNGDRRLAIRYGEDFELVKSRSTSVPFAAILDG   |
| Mtru | 238 | LAHTIWIGNSVAVAASFALIAHHLFGWNGDRKLAERYGEDFEIVKGRTSVPFAAILDG    |
| Atha | 239 | LNMKT-----I   |
| <br> |     |   |
| Lfil | 301 | RQELPKDFYKEFIRLPYLTITFLTLGAYFAHPLMQSASFNLHW                   |
| Ljap | 301 | RQELPKDFYKEFIRLPYLTITFLTLGAYFAHPLMQSASFNLHW                   |
| Gmax | 299 | RQELPKDFYKEFIRLPYLTVAITLGAYFAHPLMQAASFNLHW                    |
| Mtru | 298 | RQELPKDFYKEFIRLPYFTITALTTLGAYFAHPLMRAASLKLHW                  |
| Atha | 245 | -----SFIFVFKYRKIGFL-----                                      |

Figure S13. Amino acid alignment for CRTISO

Lfil 1 VKGARVLVLEKYVIPGGSSGFYQRDGTYFDVGSSVMFGSDKGNLNLTQALEAVGCKMQ  
 Ljap 1 VKGARVLVLEKYVIPGGSSGFYQRDGTYFDVGSSVMFGSDKGNLNLTQALEAVGCKMQ  
 Gmax 1 VKGARVLVLEKYVIPGGSSGFYQRDGTYFDVGSSVMFGSDKGNLNLTQALEAVGCRMQ  
 Mtru 1 VKGA~~K~~VLVLEKYVIPGGSSGFYHR~~E~~GTYFDVGSSVMFGSDKGNLNLTQALEAVGCRMQ  
 Atha 1 VKE~~A~~RVLVLEKY~~I~~IPGGSSGFYERDGTYFDVGSSVMFGSDKGNLNLTQALK~~A~~AVGRKME

Lfil 61 VVPDPTTVHFHLPNNLSVRVHREYDKFIEELTCYFPHEKEGILKFYGECKWIFNALNSLE  
 Ljap 61 VVPDPTTVHFHLPNNLSVRVHREYDKFIEELTCYFPHEKEGILKFYGECKWIFNALNLE  
 Gmax 61 VVPDPTTVHFHLPNNLSVRVH~~K~~EYDKFIEELTSYFPHEKEGILKFYGECKWIFNALNSLE  
 Mtru 61 V~~T~~PDPTTVHFHLPNHLSVRVHREYDKFIEELTSYFPHEK~~G~~ILKFYGECKWIFNALNSLE  
 Atha 61 V~~T~~PDPTTVHFHLPNNLSVR~~I~~HREYDD~~F~~IAELTSK~~F~~PHEKEGILGFYGDCKWIFN~~S~~LNSLE

Lfil 121 LKSLEEPYLFQFFFQKPLECLTLAYYLQPQNAGAIARKYIQDPQLLSFIDAECFIVSTVN  
 Ljap 121 LKSLEEPYLFQFFFQKPLECLTLAYYLQPQNAGAIARKYIQDPQLLSFIDAECFIVSTVN  
 Gmax 121 LKSLEEPYLFQFFF~~R~~PLECLTLAYYLQPQNAGAIARKYIQDPQLLSFIDAECFIVSTVN  
 Mtru 121 LKSLEEPYLFQFFFQKPLECLTLAYYLQPQNAGAIARKYIQDPQLLSFIDAECFIVSTVN  
 Atha 121 LKSLEEPY~~I~~LFQFFFQKPLECLTLAYYLQPQNAGAIARKYIK~~K~~DPQLLSFIDAECFIVSTVN

Lfil 181 ALQTPMINAAVLCDRHFGGINYPLGGVGGIAKSLAKGLADQGSEILYKANVTS~~VII~~IEQG  
 Ljap 181 ALQTPMINAAVLCDRHFGGINYPLGGVGGIAKSLAKGLADQGSEILYKANVTS~~VII~~IEQG  
 Gmax 181 ALQTPMINAAVLCDRHFGGINYPLGGVGGIAKSLAKGLVDQGSEI~~V~~YKANVTS~~II~~IEQG  
 Mtru 181 ALQTPMINASMVLCDRHFGGINYPLGGVGGIAKSLAKGLVDQGSQ~~I~~LYKANVTS~~I~~TEQG  
 Atha 181 ALQTPMINASMVLCDRH~~Y~~GGINYP~~V~~GGVGGIAKSLAEGLVDQGSEI~~Q~~YKANV~~K~~~~S~~ILDHG

Lfil 241 KAVGVRLSDGREFFAKTIISNATRWDT-----  
 Ljap 241 KAVGVRLSDGREFFAKTIISNATRWDTFG-----  
 Gmax 241 KAVGVRLSNGREFFAKTIISNATRWDTFGKLLKGVP~~L~~PKEEENFQKVYVKAPSFLSIHMG  
 Mtru 241 KAVGVRLSDGREFFAKTIISNATRWDTFGKLVKGESLP~~K~~KEEENFQKVYVKAPSFLSIHMG  
 Atha 241 KAVGVRLADGREFFAKTIISNATRWDTFGKLLKG~~E~~KL~~P~~KEEENFQKVYVKAPSFLSIHMG

Lfil -----  
 Ljap 270 -----  
 Gmax 301 VKAEVLPPDTDCHHFVLESNWSKLEEPYGSIFLSIPTVLDSSLAPEGRHILHIFTSSME  
 Mtru 301 VKAEVLPPDTDCHHFVLENNWTSLEEPYGSIFLSIPTILDSSLAPEGRHILHIFTSSMD  
 Atha 301 VKAEVLPPDTDCHHFVLED~~D~~WKNLEEPYGSIFLSIPTILDSSLAPEGRHILHIFTSSIE

Lfil -----  
 Ljap 270 -----  
 Gmax 361 DWEGLSRVEYEAKKQLVADEITSRLENKLFPG~~L~~RSSIDE~~M~~EVGTPKTHRRYLARDEGTYG  
 Mtru 361 DWQGLSRIDYEAKKQVVADEIIISRLEKLFPG~~L~~RSSIDE~~I~~EVGTPKTHRRYLARDEGTYG  
 Atha 361 DWEGLPPKEYEAKKEDVAARIIQRLEKLFPG~~L~~SSSITEKEVGTP~~R~~THRRFLARDKGTYG

Lfil -----  
 Ljap 270 -----  
 Gmax 421 PMP~~R~~RIPKGLLGM~~P~~FNTT~~G~~IDGLYCVGDS~~C~~PGQGVIAVAFSGVMCAHRVAADIGLEKK~~S~~  
 Mtru 421 PMP~~R~~SIPKGLLGM~~P~~FNTT~~S~~IDGLYCVGDS~~C~~PGQGVIAVAFSGVMCAHRVAADIGLEKK~~S~~

Atha 421 PMPRGTPKGLLGMPFNTTAIDGLYCVGDSCFPQQGVIAVAFSGVMCAHVAADIGLEKKS

Lfil -----  
Ljap 270 -----WFR  
Gmax 481 PVLD~~S~~MLLRLLGWLR  
Mtru 481 PVLDGM~~S~~LLGWLR  
Atha 481 RVLDVG~~S~~LLGWLR

Figure S14. Amino acid alignment for E-CHY

Lfil 1 -----TLWWLVIPLPLPSMC-----SR  
 Ljap 1 -LSLSPLSIFPPSVKSSINKPSSWVSPDWLTSLSRSLTAGKNDDSGIPIASAKLDDVSD  
 Gmax 1 SLSLTPLSVFPPSVKSSINKPSSWSPDWLTSLSRSLTAGNDVSGIPVASAKLDDVSD  
 Mtru 1 SIPLSHLSISSLFTKSSTNKSTSWSVSPDWLTSLSKSLTTSKNDDSNPIASAKLDDVSD  
 Atha 1 SSSYSSLFTAKPSIRSSI~~E~~KQSQSWVSPDWLT~~T~~IRTLSGKND~~E~~SGIPIANAKLDDVAD

Lfil 17 IMG-----NMARVILLRFRSFC~~CL~~GLVLQLLRV  
 Ljap 60 LLGGALFLPLFKWM~~E~~YGPIYRLAAGPRNFVVVSDPAIAKHL~~N~~Y~~G~~KY~~G~~KLVAEVSEF  
 Gmax 60 LLGGALFLPLFKWM~~Q~~YGPIYRLAAGPRNFVVVSDPAIAKHL~~N~~Y~~G~~KY~~A~~KLVAEVSEF  
 Mtru 61 LLGGALFLPLFKWM~~N~~YE~~G~~PIYRLAAGPRNFVVVSDPAIAKHL~~N~~Y~~G~~KY~~G~~KLVAEVSEF  
 Atha 61 LLGGALFLPL~~Y~~KWM~~N~~YE~~G~~PIYRLAAGPRNFV~~T~~VSDPAIAKHL~~R~~NY~~P~~KY~~A~~KLVAEVSEF

Lfil 44 HSGGT~~C~~LL---XWIGY-----FVDV~~I~~RDXWRSYNLMH-----LM  
 Ljap 120 LF~~G~~GAIAEGPLWTARRAVVPSLHKRYLSVIVDRVFC~~R~~CAERLVEK~~L~~Q~~P~~DALNGTAVNM  
 Gmax 120 LF~~G~~GAIAEGPLWTARRAVVPSLHKRYLSVIVDRVFC~~R~~CAERLVEK~~L~~Q~~P~~DALNGTAVNM  
 Mtru 121 LF~~G~~GAIAEGPLWTARRAVVPSLHKRYLS~~I~~NDVDRVFC~~R~~CAERLVEK~~L~~Q~~A~~DAVNGTAVNM  
 Atha 121 LF~~G~~GAIAEGPLWTARRAVVPSL~~H~~RRYLSVIVE~~R~~VFC~~R~~CAERLVEK~~L~~Q~~P~~Y~~A~~EDGSAVNM

Lfil 75 ELQL~~T~~WFHSX~~L~~IMLLVY~~I~~YSTMT~~I~~TQ~~T~~VLLSVLF~~T~~LLXK~~R~~OARSTDLLPYWQ~~V~~B~~F~~LRKII  
 Ljap 180 EDKFSQLTLDVIGLSVFNYDFDSLNDSPV~~I~~GAVY~~T~~ALKEAE~~R~~STDLLPYWQ~~V~~B~~F~~LRKII  
 Gmax 180 EA~~K~~FSQLTLDVIGLSVFNYNFDSLNDSPVIEAVY~~T~~ALKEAE~~R~~STDLLPYWKF~~K~~FLCKII  
 Mtru 181 EDKFSQLTLDVIGLSVFNYNF~~D~~ALNDSPVIEAVY~~T~~ALKEAE~~R~~STDLLPYWKID~~F~~LC~~K~~II  
 Atha 181 EA~~K~~FSQM~~T~~LDVIGLS~~I~~FN~~Y~~NFDSL~~T~~DSPVIEAVY~~T~~ALKEAE~~L~~RSTDLLPYWKID~~A~~LC~~K~~IV

Lfil 135 PRQIKA~~E~~KAVSIIRKT~~V~~ENLIEKCKE~~I~~VESEGERIDG~~D~~EYVNDSDPSIILRF~~L~~LASREEVS  
 Ljap 240 PRQIKAENAVTIIIRKT~~V~~EDLIEKCKE~~I~~VESEGERIDG~~D~~EYVNDSDPSIILRF~~L~~LASREEVS  
 Gmax 240 PRQIKAEEAVSVIRKT~~V~~EDLIEKCKE~~I~~VESEGERID~~V~~EYVNDSDPSIILRF~~L~~LASREEVS  
 Mtru 241 PRQIKAENAVTVIRKT~~V~~EDLIEQCKE~~I~~VESEGERIDA~~D~~EYVNDADPSIILRF~~L~~LASREEVS  
 Atha 241 PRQ~~V~~KA~~E~~KAVT~~L~~IRE~~T~~VEDLIAKCKE~~I~~VEREGERIND~~E~~EYVNDADPSIILRF~~L~~LASREEVS

Lfil 195 SDQLRDDLLSMLVAGHETTG~~S~~VL~~T~~W~~T~~LY~~L~~LSKD~~S~~SLAKVQ~~Q~~EEVDRVLQ~~G~~RRPTFEDM~~K~~  
 Ljap 300 SDQLRDDLLSMLVAGHETTG~~S~~VL~~T~~W~~T~~LY~~L~~LSKD~~S~~SLAKVQ~~Q~~EEVDRVLQ~~G~~RRPTFEDM~~K~~  
 Gmax 300 SVQLRDDLLS~~I~~LVAGHETTG~~S~~VL~~T~~W~~T~~LY~~L~~LSKD~~S~~SLAKA~~Q~~EEVDRVLQ~~G~~RRPT~~M~~EDI~~K~~  
 Mtru 301 SVQLRDDLLSMLVAGHETTG~~S~~VL~~T~~W~~T~~LY~~L~~LSKD~~S~~SLAKA~~Q~~EEVDRVLQ~~G~~RRPT~~M~~EDI~~K~~  
 Atha 301 SVQLRDDLLSMLVAGHETTG~~S~~VL~~T~~W~~T~~LY~~L~~SKNS~~A~~LRKA~~Q~~EEVDRVL~~E~~GRNPA~~F~~EDI~~K~~

Lfil 255 LKFLTRC~~I~~TESLR~~L~~Y~~P~~PPV~~L~~IRRAQ~~V~~P~~D~~ELPGAY~~K~~VNAGQD~~I~~MISV~~Y~~NI~~H~~S~~S~~EVWDRA  
 Ljap 360 LKFLTRC~~I~~TESLR~~L~~Y~~P~~PPV~~L~~IRRAQ~~V~~P~~D~~ELPGAY~~K~~VNAGQD~~I~~MISV~~Y~~NI~~H~~S~~S~~EVWDRA  
 Gmax 360 LKFLTRC~~I~~TESLR~~L~~Y~~P~~PPV~~L~~IRRAQ~~V~~P~~D~~ELPG~~G~~Y~~K~~LDAGQD~~I~~MISV~~Y~~NI~~H~~R~~S~~EVWDRA  
 Mtru 361 LKFL~~N~~RC~~I~~TESLR~~L~~Y~~P~~PPV~~L~~IRRAQ~~V~~P~~D~~ELPG~~D~~Y~~K~~LDAGQD~~I~~MISV~~Y~~NI~~H~~S~~S~~K~~V~~WDRA  
 Atha 361 LKY~~I~~TRC~~I~~NE~~S~~MR~~L~~Y~~P~~PPV~~L~~IRRAQ~~V~~P~~D~~ILPG~~N~~Y~~K~~VNT~~G~~QD~~I~~MISV~~Y~~NI~~H~~R~~S~~EVWEKA

Lfil 315 EEF~~M~~PERFDLDGPMPNETNTDFRF~~I~~PFSGGPRKCVGDQF~~A~~LEATV~~S~~LAI~~F~~QHM~~N~~FELV  
 Ljap 420 EEFLPERFDLDGPMPNETNTDFRF~~I~~PFSGGPRKCVGDQF~~A~~LEATV~~S~~LAI~~F~~QHM~~N~~FELV  
 Gmax 420 EEF~~V~~PERFDLDGPVPNETNTDFRF~~I~~PFSGGPRKCVGDQF~~A~~LE~~M~~A~~I~~VALA~~I~~FLQHM~~N~~FELV  
 Mtru 421 EEFLPERFDLDGPVPNETNTDFRF~~I~~PFSGGPRKCVGDQF~~A~~LEATVALA~~V~~FLQHM~~N~~FELV

Atha 421 EEFLPERFDIDGAIPNETNTDFKFIPSGGPRKCVGDQFALMEATVALAVFLQRINVELV

Lfil 375 PDQNISM**T**GATIHTTNGLYMKLSQRVK  
Ljap 480 PDQNISM**T**GATIHTTNGLYMKLSQRVK  
Gmax 480 PDQNISM**T**GATIHTTNGLYMKLS**R**RLK  
Mtru 481 PDQNI**G**MTTGATIHTTNGLYMKLSQR**R**LK  
Atha 481 PDQ**T**ISM**T**GATIHTTNGLYMK**V**SQR--

Figure S15. Amino acid alignment for PSY

|      |     |   |  |
|------|-----|---|--|
| Lfil | 1   | VT <del>L</del> F <del>WV</del> AS <del>Y</del> P <del>N</del> LE <del>V</del> S <del>N</del> TG <del>S</del> LH <del>S</del> TRD <del>V</del> R <del>L</del> F <del>DSS</del> K <del>FML</del> P <del>D</del> E <del>R</del> S <del>I</del> R <del>A</del> K <del>K</del> D <del>R</del> K <del>K</del> R <del>W</del> R <del>F</del> C <del>S</del> L <del>S</del> T  |  |
| Ljap | 1   | VT <del>L</del> F <del>WV</del> AS <del>FP</del> N <del>Q</del> V <del>S</del> N <del>STR</del> L <del>LD</del> S <del>I</del> R <del>D</del> V <del>R</del> L <del>F</del> D <del>S</del> S <del>K</del> F <del>M</del> S <del>R</del> D <del>F</del> R <del>S</del> I <del>R</del> A <del>K</del> K <del>D</del> R <del>K</del> K <del>R</del> W <del>R</del> F <del>C</del> S <del>L</del> <del>S</del> T  |  |
| Gmax | 1   | VT <del>L</del> F <del>WV</del> F <del>VSSP</del> G <del>LEV</del> S <del>H</del> H <del>ST</del> G <del>L</del> L <del>D</del> S <del>V</del> R <del>H</del> V <del>K</del> F <del>L</del> D <del>S</del> S <del>K</del> V <del>M</del> S <del>R</del> D <del>F</del> G <del>S</del> I <del>R</del> V <del>E</del> K <del>D</del> K <del>E</del> G <del>W</del> R <del>L</del> C <del>S</del> L <del>S</del> T   |  |
| Mtru | 1   | VT <del>L</del> LR <del>V</del> A <del>SSPS</del> L <del>E</del> V <del>S</del> C <del>S</del> N <del>G</del> L <del>D</del> S <del>F</del> G <del>V</del> V <del>K</del> L <del>V</del> D <del>S</del> S <del>K</del> F <del>L</del> S <del>R</del> D <del>F</del> V <del>S</del> I <del>R</del> A <del>K</del> K <del>D</del> K <del>K</del> K <del>T</del> R <del>R</del> F <del>C</del> S <del>L</del> <del>S</del> T   |  |
| Atha | 1   | VA <del>V</del> LW <del>V</del> VAT <del>SS</del> -----LN <del>P</del> D <del>P</del> M <del>NN</del> C <del>G</del> L <del>V</del> R <del>V</del> L <del>E</del> S <del>S</del> R <del>L</del> F <del>S</del> P <del>-C</del> Q <del>N</del> Q <del>R</del> L <del>N</del> K <del>G</del> KK <del>K</del> Q <del>I</del> --PT <del>W</del> S <del>S</del>  |  |
| <br> |     |   |  |
| Lfil | 61  | GV <del>KYACI</del> G <del>QSG</del> L <del>E</del> S <del>A</del> S <del>S</del> F <del>P</del> M <del>I</del> S <del>V</del> LA <del>N</del> P <del>A</del> A <del>E</del> V <del>A</del> S <del>S</del> Q <del>K</del> V <del>Y</del> D <del>V</del> V <del>L</del> K <del>Q</del> A <del>S</del> L <del>I</del> I <del>K</del> R <del>K</del> L <del>S</del> A <del>G</del> D   |  |
| Ljap | 61  | GV <del>KYACV</del> G <del>QSG</del> L <del>E</del> S <del>A</del> S <del>S</del> F <del>P</del> M <del>I</del> S <del>V</del> LA <del>N</del> P <del>A</del> A <del>E</del> V <del>A</del> S <del>S</del> Q <del>K</del> V <del>Y</del> D <del>V</del> V <del>L</del> K <del>Q</del> A <del>S</del> L <del>I</del> I <del>K</del> R <del>K</del> L <del>S</del> A <del>G</del> D   |  |
| Gmax | 61  | DM <del>KYACV</del> G <del>R</del> S <del>G</del> L <del>E</del> S <del>A</del> S <del>N</del> F <del>P</del> L <del>I</del> A <del>N</del> LA <del>N</del> P <del>A</del> A <del>G</del> E <del>V</del> A <del>S</del> S <del>E</del> Q <del>K</del> V <del>Y</del> D <del>V</del> V <del>L</del> K <del>Q</del> A <del>S</del> L <del>V</del> K <del>R</del> K <del>L</del> G <del>A</del> E  |  |
| Mtru | 61  | D <del>I</del> KYACV <del>GQ</del> P <del>G</del> L <del>E</del> S <del>A</del> S <del>N</del> F <del>P</del> L <del>I</del> S <del>N</del> V <del>L</del> A <del>N</del> P <del>T</del> A <del>G</del> E <del>V</del> T <del>V</del> S <del>S</del> Q <del>K</del> V <del>Y</del> D <del>V</del> V <del>L</del> K <del>Q</del> A <del>S</del> L <del>V</del> K <del>R</del> K <del>L</del> S <del>S</del> G <del>E</del>   |  |
| Atha | 53  | SFVRNRSRR-----IG <del>V</del> V <del>S</del> S <del>S</del> LV <del>A</del> S <del>P</del> S <del>G</del> E <del>I</del> A <del>S</del> S <del>E</del> K <del>V</del> V <del>N</del> V <del>V</del> L <del>Q</del> A <del>A</del> L <del>V</del> N <del>K</del> QL <del>R</del> S <del>Y</del> D  |  |
| <br> |     |   |  |
| Lfil | 121 | LG <del>V</del> K <del>Q</del> N <del>V</del> FLPGNL <del>S</del> LL <del>S</del> E <del>A</del> Y <del>D</del> R <del>C</del> G <del>E</del> I <del>C</del> A <del>E</del> Y <del>A</del> K <del>T</del> F <del>Y</del> L <del>G</del> T <del>L</del> L <del>M</del> T <del>P</del> E <del>R</del> R <del>A</del> I <del>W</del> A <del>I</del> Y <del>V</del> W <del>C</del> R <del>R</del> T <del>D</del>  |  |
| Ljap | 121 | HG <del>V</del> K <del>Q</del> N <del>V</del> FLPGNL <del>S</del> LL <del>S</del> E <del>A</del> Y <del>D</del> R <del>C</del> G <del>E</del> I <del>C</del> A <del>E</del> Y <del>A</del> K <del>T</del> F <del>Y</del> L <del>G</del> T <del>L</del> L <del>M</del> T <del>P</del> E <del>R</del> R <del>A</del> I <del>W</del> A <del>I</del> Y <del>V</del> W <del>C</del> R <del>R</del> T <del>D</del>  |  |
| Gmax | 121 | LD <del>A</del> K <del>P</del> D <del>I</del> A <del>L</del> PGNL <del>S</del> LL <del>N</del> E <del>A</del> Y <del>D</del> R <del>C</del> G <del>E</del> I <del>C</del> A <del>E</del> Y <del>A</del> K <del>T</del> F <del>Y</del> L <del>G</del> T <del>L</del> L <del>M</del> T <del>P</del> E <del>R</del> R <del>A</del> I <del>W</del> A <del>I</del> Y <del>V</del> W <del>C</del> R <del>R</del> T <del>D</del>   |  |
| Mtru | 121 | LE <del>V</del> K <del>P</del> E <del>I</del> V <del>L</del> PGNL <del>S</del> LL <del>S</del> E <del>A</del> Y <del>D</del> R <del>C</del> G <del>E</del> I <del>C</del> A <del>E</del> Y <del>A</del> K <del>T</del> F <del>Y</del> L <del>G</del> T <del>L</del> L <del>M</del> T <del>P</del> E <del>R</del> R <del>A</del> I <del>W</del> A <del>I</del> Y <del>V</del> W <del>C</del> R <del>R</del> T <del>D</del>   |  |
| Atha | 105 | LD <del>V</del> K <del>Q</del> D <del>V</del> V <del>L</del> PG <del>S</del> L <del>S</del> LL <del>G</del> E <del>A</del> Y <del>D</del> R <del>C</del> G <del>E</del> V <del>C</del> A <del>E</del> Y <del>A</del> K <del>T</del> F <del>Y</del> L <del>G</del> T <del>L</del> L <del>M</del> T <del>P</del> E <del>R</del> R <del>A</del> I <del>W</del> A <del>I</del> Y <del>V</del> W <del>C</del> R <del>R</del> T <del>D</del>                            |  |
| <br> |     |   |  |
| Lfil | 181 | EL <del>V</del> D <del>G</del> P <del>N</del> A <del>SH</del> I <del>T</del> P <del>T</del> A <del>L</del> D <del>R</del> W <del>E</del> S <del>R</del> L <del>E</del> E <del>L</del> F <del>Q</del> G <del>R</del> P <del>F</del> D <del>M</del> L <del>D</del> A <del>A</del> V <del>S</del> D <del>T</del> V <del>T</del> K <del>F</del> P <del>V</del> D <del>I</del> Q <del>P</del> F <del>K</del> D <del>M</del> I <del>E</del> G <del>M</del>              |  |
| Ljap | 181 | EL <del>V</del> D <del>G</del> P <del>N</del> A <del>SH</del> I <del>T</del> P <del>T</del> A <del>L</del> D <del>R</del> W <del>E</del> S <del>R</del> L <del>E</del> E <del>L</del> F <del>Q</del> G <del>R</del> P <del>F</del> D <del>M</del> L <del>D</del> A <del>A</del> V <del>S</del> D <del>T</del> V <del>T</del> K <del>F</del> P <del>V</del> D <del>I</del> Q <del>P</del> F <del>K</del> D <del>M</del> I <del>E</del> G <del>M</del>              |  |
| Gmax | 181 | EL <del>V</del> D <del>G</del> P <del>N</del> A <del>SH</del> I <del>T</del> P <del>T</del> A <del>L</del> D <del>R</del> W <del>E</del> S <del>R</del> L <del>E</del> E <del>L</del> F <del>Q</del> G <del>R</del> P <del>F</del> D <del>M</del> L <del>D</del> A <del>A</del> V <del>D</del> T <del>V</del> A <del>K</del> F <del>P</del> V <del>D</del> I <del>Q</del> P <del>F</del> K <del>D</del> M <del>I</del> E <del>G</del> M                           |  |
| Mtru | 181 | EL <del>V</del> D <del>G</del> P <del>N</del> A <del>SH</del> I <del>T</del> A <del>A</del> M <del>D</del> R <del>W</del> E <del>S</del> R <del>L</del> E <del>L</del> F <del>Q</del> G <del>R</del> P <del>F</del> D <del>M</del> L <del>D</del> A <del>A</del> L <del>S</del> D <del>T</del> V <del>N</del> R <del>F</del> P <del>V</del> D <del>I</del> Q <del>P</del> F <del>K</del> D <del>M</del> I <del>E</del> G <del>M</del>                             |  |
| Atha | 165 | EL <del>V</del> D <del>G</del> P <del>N</del> A <del>SH</del> I <del>T</del> P <del>M</del> A <del>L</del> D <del>R</del> W <del>E</del> A <del>R</del> L <del>E</del> E <del>L</del> F <del>Q</del> G <del>R</del> P <del>F</del> D <del>M</del> L <del>D</del> A <del>A</del> L <del>D</del> T <del>V</del> A <del>R</del> Y <del>P</del> V <del>D</del> I <del>Q</del> P <del>F</del> E <del>D</del> M <del>I</del> E <del>G</del> M                           |  |
| <br> |     |   |  |
| Lfil | 241 | RT <del>D</del> L <del>R</del> K <del>S</del> R <del>Y</del> K <del>N</del> F <del>D</del> E <del>L</del> Y <del>L</del> Y <del>C</del> Y <del>V</del> A <del>G</del> T <del>V</del> G <del>L</del> M <del>S</del> V <del>P</del> I <del>M</del> G <del>I</del> S <del>P</del> D <del>S</del> QT <del>T</del> ST <del>E</del> S <del>V</del> Y <del>N</del> A <del>A</del> L <del>A</del> G <del>I</del> A <del>N</del> Q <del>L</del>                            |  |
| Ljap | 241 | RT <del>D</del> L <del>R</del> K <del>S</del> R <del>Y</del> K <del>N</del> F <del>D</del> E <del>L</del> Y <del>L</del> Y <del>C</del> Y <del>V</del> A <del>G</del> T <del>V</del> G <del>L</del> M <del>S</del> V <del>P</del> I <del>M</del> G <del>I</del> S <del>P</del> D <del>S</del> QT <del>T</del> ST <del>E</del> S <del>V</del> Y <del>N</del> A <del>A</del> L <del>A</del> G <del>I</del> A <del>N</del> Q <del>L</del>                            |  |
| Gmax | 241 | RL <del>D</del> L <del>K</del> K <del>P</del> R <del>Y</del> K <del>N</del> F <del>D</del> E <del>L</del> Y <del>L</del> Y <del>C</del> Y <del>V</del> A <del>G</del> T <del>V</del> G <del>L</del> M <del>S</del> V <del>P</del> I <del>M</del> G <del>I</del> S <del>P</del> N <del>E</del> AT <del>T</del> E <del>S</del> V <del>Y</del> N <del>A</del> A <del>L</del> A <del>G</del> I <del>N</del> Q <del>L</del>  |  |
| Mtru | 241 | RMDLKK <del>S</del> R <del>Y</del> K <del>S</del> F <del>D</del> E <del>L</del> Y <del>L</del> Y <del>C</del> Y <del>V</del> A <del>G</del> T <del>V</del> G <del>L</del> M <del>S</del> V <del>P</del> I <del>M</del> G <del>I</del> S <del>T</del> H <del>S</del> Q <del>A</del> T <del>T</del> E <del>S</del> V <del>Y</del> N <del>A</del> A <del>L</del> A <del>G</del> I <del>N</del> Q <del>L</del>  |  |
| Atha | 225 | RMDLKK <del>S</del> R <del>Y</del> Q <del>N</del> F <del>D</del> L <del>L</del> Y <del>L</del> Y <del>C</del> Y <del>V</del> A <del>G</del> T <del>V</del> G <del>L</del> M <del>S</del> V <del>P</del> I <del>M</del> G <del>I</del> D <del>P</del> K <del>S</del> K <del>A</del> T <del>T</del> E <del>S</del> V <del>Y</del> N <del>A</del> A <del>L</del> A <del>G</del> I <del>N</del> Q <del>L</del>  |  |
| <br> |     |   |  |
| Lfil | 301 | T <del>N</del> I <del>L</del> R <del>D</del> V <del>G</del> E <del>D</del> A <del>S</del> R <del>G</del> R <del>V</del> Y <del>L</del> P <del>Q</del> D <del>E</del> L <del>A</del> Q <del>A</del> G <del>L</del> S <del>E</del> D <del>D</del> I <del>F</del> A <del>G</del> K <del>V</del> T <del>D</del> K <del>W</del> R <del>N</del> F <del>M</del> K <del>S</del> Q <del>I</del> I <del>K</del> R <del>A</del> M <del>F</del> F <del>D</del> E <del>A</del> |  |
| Ljap | 301 | T <del>N</del> I <del>L</del> R <del>D</del> V <del>G</del> E <del>D</del> A <del>S</del> R <del>G</del> R <del>V</del> Y <del>L</del> P <del>Q</del> D <del>E</del> L <del>A</del> Q <del>A</del> G <del>L</del> S <del>E</del> D <del>D</del> I <del>F</del> A <del>G</del> K <del>V</del> T <del>D</del> K <del>W</del> R <del>N</del> F <del>M</del> K <del>S</del> Q <del>I</del> I <del>K</del> R <del>A</del> M <del>F</del> F <del>D</del> E <del>A</del> |  |
| Gmax | 301 | T <del>N</del> I <del>L</del> R <del>D</del> V <del>G</del> E <del>D</del> A <del>S</del> R <del>G</del> R <del>V</del> Y <del>L</del> P <del>Q</del> D <del>E</del> L <del>A</del> Q <del>A</del> G <del>L</del> S <del>E</del> D <del>D</del> I <del>F</del> A <del>G</del> K <del>V</del> T <del>D</del> K <del>W</del> R <del>N</del> F <del>M</del> K <del>S</del> Q <del>I</del> I <del>K</del> R <del>A</del> M <del>F</del> F <del>D</del> E <del>A</del> |  |
| Mtru | 301 | T <del>N</del> I <del>L</del> R <del>D</del> V <del>G</del> E <del>D</del> A <del>R</del> R <del>G</del> R <del>V</del> Y <del>L</del> P <del>Q</del> D <del>E</del> L <del>T</del> I <del>A</del> G <del>L</del> S <del>D</del> D <del>D</del> I <del>F</del> A <del>G</del> K <del>V</del> T <del>D</del> K <del>W</del> R <del>N</del> F <del>M</del> K <del>S</del> Q <del>I</del> I <del>K</del> R <del>A</del> M <del>F</del> F <del>D</del> E <del>A</del> |  |
| Atha | 285 | T <del>N</del> I <del>L</del> R <del>D</del> V <del>G</del> E <del>D</del> A <del>R</del> R <del>G</del> R <del>V</del> Y <del>L</del> P <del>Q</del> D <del>E</del> L <del>A</del> Q <del>A</del> G <del>L</del> S <del>E</del> D <del>I</del> F <del>A</del> G <del>K</del> V <del>T</del> D <del>K</del> W <del>R</del> N <del>F</del> M <del>K</del> Q <del>I</del> I <del>K</del> R <del>A</del> M <del>F</del> F <del>D</del> E <del>A</del>                |  |
| <br> |     |   |  |
| Lfil | 361 | E <del>K</del> G <del>V</del> M <del>E</del> L <del>N</del> K <del>A</del> S <del>R</del> W <del>P</del> V <del>L</del> A <del>S</del> L <del>L</del> Y <del>R</del> Q <del>I</del> D <del>E</del> I <del>E</del> A <del>N</del> D <del>Y</del> N <del>N</del> F <del>T</del> K <del>R</del> A <del>V</del> S <del>T</del> K <del>K</del> L <del>L</del> S <del>L</del> P <del>A</del> A <del>Y</del> A <del>R</del> T <del>M</del>                               |  |
| Ljap | 361 | E <del>K</del> G <del>V</del> M <del>E</del> L <del>N</del> K <del>A</del> S <del>R</del> W <del>P</del> V <del>L</del> A <del>S</del> L <del>L</del> Y <del>R</del> Q <del>I</del> D <del>E</del> I <del>E</del> A <del>N</del> D <del>Y</del> N <del>N</del> F <del>T</del> K <del>R</del> A <del>V</del> S <del>T</del> K <del>K</del> L <del>L</del> S <del>L</del> P <del>A</del> A <del>Y</del> A <del>R</del> T <del>M</del>                               |  |
| Gmax | 361 | E <del>K</del> G <del>V</del> T <del>E</del> L <del>N</del> E <del>A</del> S <del>R</del> W <del>P</del> V <del>W</del> A <del>S</del> L <del>L</del> Y <del>R</del> Q <del>I</del> D <del>E</del> I <del>E</del> A <del>N</del> D <del>Y</del> N <del>N</del> F <del>T</del> K <del>R</del> A <del>V</del> S <del>T</del> K <del>K</del> L <del>L</del> S <del>L</del> P <del>A</del> A <del>Y</del> A <del>R</del> S <del>I</del>                               |  |
| Mtru | 361 | E <del>K</del> G <del>V</del> T <del>E</del> L <del>N</del> E <del>Q</del> S <del>R</del> W <del>P</del> V <del>W</del> A <del>S</del> L <del>L</del> Y <del>R</del> Q <del>I</del> D <del>E</del> I <del>E</del> A <del>N</del> D <del>Y</del> N <del>N</del> F <del>T</del> K <del>R</del> A <del>V</del> S <del>T</del> K <del>K</del> L <del>L</del> S <del>L</del> P <del>I</del> A <del>Y</del> A <del>R</del> S <del>M</del>                               |  |
| Atha | 345 | E <del>K</del> G <del>V</del> T <del>E</del> L <del>S</del> A <del>A</del> S <del>R</del> W <del>P</del> V <del>W</del> A <del>S</del> L <del>L</del> Y <del>R</del> Q <del>I</del> D <del>E</del> I <del>E</del> A <del>N</del> D <del>Y</del> N <del>N</del> F <del>T</del> K <del>R</del> A <del>V</del> G <del>K</del> V <del>K</del> I <del>A</del> A <del>L</del> P <del>I</del> A <del>Y</del> A <del>K</del> S <del>V</del>                               |  |
| <br> |     |   |  |
| Lfil | 421 | V <del>A</del> P <del>P</del> R <del>K</del> L <del>SH</del> V  |  |
| Ljap | 421 | V <del>A</del> P <del>P</del> R <del>K</del> L <del>SH</del> V  |  |
| Gmax | 421 | V <del>F</del> P <del>S</del> R <del>K</del> L <del>S</del> E <del>V</del>  |  |
| Mtru | 421 | V <del>P</del> P <del>S</del> K <del>K</del> L <del>SH</del> V  |  |
| Atha | 405 | L <del>K</del> T <del>S</del> S <del>S</del> R <del>L</del> S <del>I</del>  |  |

Figure S16. Amino acid alignment for ZEP-1

|      |     |   |    |
|------|-----|---|----|
| Lfil | 1   | -VX <del>I</del> CLVILSMPCSQIWAKED-   |    |
| Ljap | 1   | GV <del>X</del> CLVILSMPCSQIWAKED-  |    |
| Gmax | 1   | GLPVTRVISRMLQ <del>E</del> ILARAVGEDIIMNASNVVN <del>F</del> VDDGNKVTVELENGQKYEGDVLVGA   |    |
| Mtru | 1   | GLPVTRVISRMLQ <del>E</del> ILARAVGDDVIMNGSNVVDFIDHETKVTVVLDNGQKYDGDLLVG   | A  |
| Atha | 1   | GLPVTRVISRMLQ <del>E</del> ILARAVGEDVIRNESNVVD <del>F</del> EDSGDKVTVVLENGQRYEGD <del>L</del> VG  | A  |
| <br> |     |   |    |
| Lfil | 21  | -----   |    |
| Ljap | 22  | -----   |    |
| Gmax | 61  | DGIWSKVRKQ <del>L</del> FG <del>L</del> TEA <del>V</del> SGYTCYTGIADF <del>V</del> PADIE <del>T</del> VGYRVFLGHKQYFVSSDVAG  | AK |
| Mtru | 61  | DGIWSKVRTKLF <del>G</del> STEAT <del>Y</del> SGYTCYTGIADF <del>V</del> PPDIESVGYRVFLGHKQYFVSSDVAG   | AK |
| Atha | 61  | DGIWSKVRNNL <del>F</del> GRSEAT <del>Y</del> SGYTCYTGIADFTPADIESVGYRVFLGHKQYFVSSDVGG  | AK |
| <br> |     |   |    |
| Lfil | 21  | -----AW-----  |    |
| Ljap | 22  | -----AW-----  |    |
| Gmax | 121 | MQWYAFHKE <del>P</del> PGGV <del>D</del> E <del>P</del> NGKKER <del>L</del> R <del>I</del> FEGWCDNA <del>V</del> DL <del>I</del> LATEEEAILRRDIYDR <del>I</del> PTLT |    |
| Mtru | 121 | MQWYAFHQEPAGGVDT <del>P</del> NGKKER <del>L</del> K <del>I</del> FEGWCDNA <del>I</del> DL <del>I</del> LATEEEAILRRDIYDR <del>I</del> PTLT                           |    |
| Atha | 121 | MQWYAFHEEPAGGADAPNGMKKRLFEIFDGWCDNV <del>I</del> DL <del>I</del> LATEEEAILRRDIYDRSPGFT  |    |
| <br> |     |   |    |
| Lfil | 23  | -----PLDSYQLASELDNAWEQS <del>I</del> KSGSP <del>I</del> EIDSSLRS  |    |
| Ljap | 24  | -----PLDSYQLASELDNAWEQS <del>I</del> SGSP <del>I</del> EIDSSLRS   |    |
| Gmax | 181 | WGKGRVTLLGDS <del>V</del> HAMQP <del>N</del> MQGGCMAIEDSYQLAWELEN <del>E</del> NAWEQS <del>I</del> KSGSP <del>I</del> IDSSLRS                                       |    |
| Mtru | 181 | WGKGRVTLLGDS <del>V</del> HAMQP <del>N</del> MQGGCMAIEDGYQLAFELDN <del>A</del> WQOSAKSGSTIDIASLKS   |    |
| Atha | 181 | WGKGRVTLLGDS <del>T</del> HAMQP <del>N</del> MQGGCMAIEDS <del>H</del> QLALELDEAWKQS <del>V</del> ETTPV <del>D</del> VSSLKR  |    |
| <br> |     |   |    |
| Lfil | 56  | YERERRLRVAIIHGMARMAALMASTYKAYLGVLGPLF <del>L</del> TKFRI <del>H</del> PG <del>R</del> VG  |    |
| Ljap | 57  | YERERRLRVAIIHGMARMAALMASTYKAYLGVLGPLF <del>L</del> TKFRI <del>H</del> PG <del>R</del> VG  |    |
| Gmax | 241 | YERERRLRVAIIHGMARMAALMASTYKAYLGVLGPLF <del>L</del> TKFRI <del>H</del> PG <del>R</del> VG  |    |
| Mtru | 241 | YERERRLRVTFVHGMARMAALMASTYKAYLGVLGPLF <del>E</del> FLTKFRI <del>H</del> PG <del>R</del> VG  |    |
| Atha | 241 | YEESRRLRVAIIH <del>A</del> MARMAA <del>I</del> MASTYKAYLGVLGPLS <del>F</del> LT <del>K</del> FRVPH <del>P</del> GRVG  |    |

Figure S17. Amino acid alignment for ZEP-3

Lfil 1 KKNLRLILVAGGGIGGLVFALAAKRKGFEVVVFEKDM~~S~~AIRGE~~G~~KYRGPIQIQSNALAALE  
 Ljap 1 -----  
 Gmax 1 KK~~K~~LRVLVAGGGIGGLVFALAAKRKGFEVVVFEKDM~~S~~AIRGE~~G~~QYRGPIQIQSNALAALE  
 Mtru 1 KKQLKVLVAGGGIGGLVFALAAKRKGFEVVVFEKDL~~S~~AIRGE~~G~~QYRGPIQIQSNALAALE  
 Atha 1 KKKS~~R~~VLVAGGGIGGLVFALAAKKGF~~D~~V~~I~~VFEKDL~~S~~AIRGE~~G~~KYRGPIQIQSNALAALE

Lfil 61 AIDP~~D~~VADEV~~M~~RLGCITGDRINGLVDG~~V~~SGSWYVKFDTFTPAAERGLPVTRVISRMVLQG  
 Ljap 1 -----  
 Gmax 61 AIDLEVAEEV~~T~~RVGCITGDRINGLVDG~~I~~SGSWYIKFDTFTPAAERGLPVTRVISRMALQE  
 Mtru 61 AIDMNVADEV~~M~~RVGCITGDRINGLVDG~~I~~SGSWYIKFDTFTPAAERGLPVTRVISRMALQE  
 Atha 61 AIDIFVAE~~Q~~VM~~E~~AGCITGDRINGLVDG~~I~~SGT~~W~~YVKFDTFTPAAASRGLPVTRVISRMLTQQ

Lfil 121 ILARAVGDD~~T~~IMN~~A~~SNVV~~S~~FVDDGNKVTVELEN~~E~~KYEGDLLVGADGIWSKVRKQLFGLT  
 Ljap 1 -----  
 Gmax 121 ILA~~H~~AVG~~E~~DVIMN~~D~~SNVVDFV~~D~~HGD~~K~~V~~T~~VELEN~~G~~QKYLD~~G~~LLVGADGIWSKVRKKLFGQT  
 Mtru 121 ILARAVGDDVIMNGSNVVDF~~I~~DHET~~K~~V~~T~~V~~V~~LENGQKYD~~G~~LLVGADGIWSKVR~~T~~KLFGST  
 Atha 121 ILARAVG~~E~~DVIR~~N~~ESNVVDF~~E~~DSG~~D~~K~~V~~T~~V~~LENGQ~~R~~YE~~G~~LLVGADGIWSKVRNNLFGRS

Lfil 181 EAVYSGYTCYTGIADFVPADIESVGYRVFLGHKQYFVSSDVGAGKMQWYAFHQEP~~P~~PGGV~~D~~  
 Ljap 1 -----  
 Gmax 181 EATYSGYTCYTGIADFVPADIESVGYRVFLGHKQYFVSSDVGAGKMQWYGFHQEPAGGAD  
 Mtru 181 EATYSGYTCYTGIADFVP~~P~~DIESVGYRVFLGHKQYFVSSDVGAGKMQWYAFHQEPAGGVD  
 Atha 181 EATYSGYTCYTGIADFT~~P~~DIESVGYRVFLGHKQYFVSSDVG~~G~~GMQWYAFHEEPAGGAD

Lfil 241 IPNGRK~~E~~RLK~~I~~FE~~G~~WCDNVIELIV~~A~~TEEEAILRRDIYDRTPTLTWGKGCVTLLGDSVHA  
 Ljap 1 -----  
 Gmax 241 IPNGKKERLLK~~I~~FK~~G~~WCDNV~~I~~DLI~~H~~ATEEEAILRRDIYDRTPTFTWGKGHV~~T~~LLGDSIHA  
 Mtru 241 TPN~~G~~KKERLLK~~I~~FE~~G~~WCDNA~~A~~IDLIV~~A~~TEEEAILRRDIYDRTPTLTWGKG~~R~~V~~T~~LLGDSVHA  
 Atha 241 APNGM~~K~~KR~~I~~FE~~I~~FDG~~W~~CDNV~~I~~DLI~~H~~ATEEEAILRRDIYDRSPGFTWGKG~~R~~V~~T~~LLGDSIHA

Lfil 301 MQPNL~~G~~QGGCMAIEDG~~Y~~QLALELDNAWQES~~I~~KSGSPIDIDSSLKSYERERRLRV~~A~~I~~H~~AM  
 Ljap 1 -----  
 Gmax 301 MQPNMGQGGCMAIEDS~~Y~~QLALELDNAWQQS~~I~~KSGSPIDIDSSLKSYERERRLRV~~A~~I~~V~~HGM  
 Mtru 301 MQPNMGQGGCMAIEDG~~Y~~QLAFELDNAWQQS~~A~~KSG~~S~~TIDIAS~~L~~KSYERERRLRV~~T~~FVHGM  
 Atha 301 MQPNMGQGGCMAIEDS~~E~~QLALELDEAWKQS~~V~~ETTPV~~D~~VSSLKRYEE~~S~~RRLRV~~A~~I~~H~~AM

Lfil 361 ARMAALMASTYKP~~Y~~LGVLGPLF~~L~~TKFRV~~P~~HPGRVGG~~R~~FFIDKMMPLMLNWVLGGN~~S~~K  
 Ljap 1 -----  
 Gmax 361 ARMAAMMASTYKAYLGVLGPLF~~L~~TKFR~~I~~PHPGRVGG~~R~~FFIDKMMPLMLNWVLGGN~~S~~K  
 Mtru 361 ARMAALMASTYKAYLGVLGPLF~~E~~FLTKFR~~I~~PHPGRVGG~~R~~FFI~~Q~~KSMPLMLNWVLGGN~~S~~K  
 Atha 361 ARMAA~~I~~MASTYKAYLGVLGPLS~~F~~LTKFR~~V~~PHPGRVGG~~R~~FF~~V~~DIAMP~~S~~MLDWVLGGN~~E~~K

Lfil 421 LEGRPLCCRLSDKANDQ~~L~~H~~R~~WFEDDDALERT~~I~~TGEWILLPCGDEAGLSK~~P~~C~~I~~LRHDET~~K~~P  
 Ljap 1 -----  
 Gmax 421 LEGRPVCCRLSDKANDQ~~L~~H~~R~~WFEDNDALERA~~I~~NGEWILLPCGDEAGP~~T~~K~~P~~C~~I~~LTQDEM~~K~~P  
 Mtru 421 LEGRPLCCRLSDKASDQ~~L~~H~~T~~WFEDDDALERT~~I~~NGEWILLPCGDVP~~G~~HVK~~P~~I~~S~~LNQ~~D~~DT~~K~~P  
 Atha 421 LQGRPPSCRLTDKADDRLREWFEDDDALERT~~I~~KGEWYL~~I~~PHGDDCCVSETLC~~I~~TKDEDQ~~P~~

|      |     |   |
|------|-----|---|
| Lfil | 481 | CIIGQEDYPGSSITITLPQVSQMHAQINYKDGAFFLTDIQSQHGTWITDNEGRRNRVPPN        |
| Ljap | 1   | ----QEDYPGSSITITLPQVSQMHAQINYKDGAFFLTDIQSQHGTWITDNEGRRNRVPPN        |
| Gmax | 481 | CIIGO[KDH]PGSSI[TIPLPQVSQMHA[RIN]YKDGAFFLTDI[RSL]HGTWITDNEGRRYRVPPN |
| Mtru | 481 | YIIGQEDYPGSQLITIPLPQVSQI[HARIN]FKDGAF[FLTDI]RSQHGTWITDNEGRRYMVSPN   |
| Atha | 481 | CIV[GQDFPGM]RIVVIPSSQVYKLYACSCDLQRSSFLIDGSSKRTRNC-----              |

|      |     |                  |
|------|-----|------------------|
| Lfil | 541 | CPARVRPSDIIEFG   |
| Ljap | 57  | CPARVRPSDIIEFG   |
| Gmax | 541 | YPARVRPSDVVEFG   |
| Mtru | 541 | YPAR[TRPSH]VIEFG |
| Atha |     | -----            |

## Appendix 6

Supplementary Figures S18 – S19 Amino acid alignments for non-differentially expressed flavonoid genes in *Lotus filicaulis*

Figure S18. Amino acid alignment for F3H-2

|      |     |  |        |
|------|-----|--|--------|
| Lfil | 1   | MEVSGKPIRVQSLAHSGLSRVPPE-----                                  | YIQPPQ |
| Ljap | 1   | MEVSGEPIRVQSLAHSGLSRVPPE-----                                  | YIQPPQ |
| Gmax | 1   | MT-----TRVQSLAQSGLSRVPPE-----                                  | YIQPPQ |
| Mtru | 1   | MELTGEPIRVQSITQSGLSQVPPE-----                                  | YIQPPQ |
| Atha | 1   | MY-----LMSSFSSSLPPYPFHSKISTIKTSSLGELESKTTSDSHKGFLSTSS          |        |
| <br> |     |  |        |
| Lfil | 31  | NR----PVHHNQPDPSTNIPTINLSGFDPNHR-----ESIRRACREWGAFHVTNHGVPTTLL |        |
| Ljap | 31  | NR----PVHHNQPDPSTNIPTINLSGFDPNHRDSTRESIRRACREWGAFHVTNHGVPTTLL  |        |
| Gmax | 26  | TR----PVRHTAPEP-DSIPVIDLSSFDPTQRASRDRDSIARACREWGAFHVTNHGVPPSLL |        |
| Mtru | 31  | NR----PIN---TDPTATIPAIIDLNFNTNHRNSTLESISHACRGWGAFHVTNHGPPSLL   |        |
| Atha | 51  | HRKLDLISH-YPGDAASIPTVDLSSD-----SAREAIGDACRDWGAFHVINHGVPIHLL    |        |
| <br> |     |  |        |
| Lfil | 84  | DSLRRMGLIAFFNECPMPEKLRYAGAAGSAASEGYGSRMLVSD--ENNDGAQVLDWRDYFD  |        |
| Ljap | 88  | DSLRRSGLAFFNECPMPEKLHYAGAAGSAASEGYGSRMLVSD--ENNDGAQVLDWRDYFD   |        |
| Gmax | 82  | ASLRRAGLSFFSDTPIDOKLRYSCSA-AASEEGYGSKMLATTSDQNDAVQVLDWRDYFD    |        |
| Mtru | 85  | DAVRNSGLTFFNNCPMSEKLYSCTAGTAASEEGYGSRMLVSS-ND---HEVLDWRDYFD    |        |
| Atha | 105 | DRMRSLIGLSFFQDSPMEEKLYACDSTAASEEGYGSRMLVGA--KD---DVVLDWRDYFD   |        |
| <br> |     |  |        |
| Lfil | 142 | HHTLPLSRRNPWRPEFSSGYRELVARYSDEMKA LAQKLLS LISESLGLRPSCIEDVVGE  |        |
| Ljap | 146 | HHTLPLSRRNPWRPEFSSGYRELVARYSDEMKA LAQKLLS LISESLGLRPSCIEDVVGE  |        |
| Gmax | 140 | HHTLPLSRRNPWRPEFPADYRELVATYSDEMKA LAQKLLALISESLGLRASCIEDAVGE   |        |
| Mtru | 140 | HHTFPLSRRNPINWPDTSDYREINVNYSDEMKA LAQKLLALISESLGLQSSCIEDAVGD   |        |
| Atha | 160 | HHTFPPSRRNPSHWPITHPSDYRQVGEYGDDEMKKLAQMLLGLISESLGLPCSSIEAVGE   |        |
| <br> |     |  |        |
| Lfil | 202 | FYQNITISYYPPCPQPDLTGLQSHSDFGAITLLIQDDVGGLQVLKNGDGCGGGGDSWVTV   |        |
| Ljap | 206 | FYQNITISYYPPCPQPDLTGLQSHSDFGAITLLIQDDVGGLQVLKNGDG--GGDSWITV    |        |
| Gmax | 200 | FYQNITISYYPPCPEPDLTLGLQSHSDMGAITLLIQDDVGGLQVLKG-----GNKWVTV    |        |
| Mtru | 200 | IYQNITVSYYPPCPQPDLTGLQSHSDFGAVTLLIQDDVGGLQVLKDGD--GGDKWVTV     |        |
| Atha | 220 | IYQNITVSYYPPCPQPELTGLQSHSDFGAITLLIQDDVEGLQLYKDA-----QWLTV      |        |
| <br> |     |  |        |
| Lfil | 262 | QPLSDAILVLLADQTEIITNGKYRSCVHRAITNPDRARLSVATFHDPAKTVRISPASELI   |        |
| Ljap | 264 | QPLSDAILVLLADQTEVNKKT--L-----                                  |        |
| Gmax | 254 | QPLSDAILVLLADQTEIITNGKYRSCVHRAITNPDRARLSVATFHDPAKTVKISPASELI   |        |
| Mtru | 257 | KPLSDAILVLLGDQTEIITNGKYRSCVHRAVTNPDRARLSVATFHDPAKTVKIFPVSELI   |        |
| Atha | 273 | PPISDAILTLIADQTEIITNGRYKSAQHRAVTNANRARLSVATFHDPSKTARIAPVSQL-   |        |
| <br> |     |  |        |
| Lfil | 322 | DESSPAKF RDV VYGDYVTSWYTKGP EGKRNIDALVLES*                     |        |
| Ljap | 286 | -----P*  |        |
| Gmax | 314 | NDSSLA KYRDV VYGDYVSSWYTKGP CGKRNIDALLDP*                      |        |
| Mtru | 317 | NDSSPAKYRGV VYGDYVSSWYTKGP DGKRNIDALLLES*                      |        |
| Atha | 332 | SPPSYKEV VYGDYVSSWYSKGP EGKRNIDALLY--*                         |        |

Figure S19. Amino acid alignment for F3H

Lfil 1 MGEVDPAFIQDPEHRPKLSI-----IKAEGIPVIDLSPSI[SQ]TVSDPSAIEGLVKEIGE  
 Ljap 1 MGEVDPAFIQEPEHRP[N]LSI-----I[Q]AEGIPVIDLSPSI[NKT]ISDPSAIEGLVKKVGR  
 Gmax 1 MGEVD[T]AFIQEPEHRPKLSF-----NQAEGIPIDLSPITNHAVSDPSAIENLVKEIES  
 Mtru 1 MGEVDPAFVQE[Q]EHRPKLSI-----IEAKGIP[E]IDLSPILHHAVPNPSDIENLVKEIGS  
 Atha 1 MGE[DE]AFIQAPEHRPNT[H]LTNSGDFIFSDEIPTIDLSS[E-DTHHDKTA---IAKEIAE

Lfil 55 ACKEWGFFQVTNHGVP[LSLRQ]SIDEASRKFFAQSI[E]EKRKIYRDESTISGYYDTEHTKNV  
 Ljap 55 ACKEWGFFQVTNHGVP[LSLRQ]SIDEASRKFFAQSMEEKRKIYRDESTISGYYDTEHTKNV  
 Gmax 55 ACKEWGFFQVTNHGVP[L]LRONIEKASRLFFEQ[Q]EEKKKVKSRDEKSTISGYYDTEHTKNV  
 Mtru 55 ASKEWGFFQVTNHGVP[SLRQ]LEEASRLFFAQSL[E]EKKKVKARDEVNP[IG]YYDTEHTKNV  
 Atha 57 ACKRWGFFQV[INHGI]PSALRHRVEKTAEEFNLT[EE]KRVKRDEVNP[PMGYH]DEEHTKNV

Lfil 115 RDWKEVFDFVAREPTVVPLTSDD[ED]DDR[LT]QWTNKSPEYPPNFRVILQ[EYI]QEL[E]KLAFK  
 Ljap 115 RDWKEVFDFVATEPTVVPLTSDD[ED]DDR[LT]QWTNKSPEYPPNFSVILQ[EYI]QEM[E]KLAFK  
 Gmax 115 RDWKEVFDFIAKDPFT[IP]VTSD-EHDDRVT[H]WTNVSPEYPPNFRDIME[EYI]QEV[E]KLSFK  
 Mtru 115 RDWKEVFDF[ISQDPT]IVP[RS]SD-EHDDGVIQWTNQS[PQ]YPP[FR]TILE[EYI]QEV[E]KLAIR  
 Atha 117 RDWKE[I]FDFFLQDSTIVP[AS]PE-P[ED]TEL[R]KLT[N]QWPQNP[SH]REV[C]Q[EY]AREVEKLAFR

Lfil 175 LLELIALS[IG]LEAKRFEQFFT[KD]QTSFIRLNHYPPCPFPHLALGVGRHKDAGGLTILAQD  
 Ljap 174 LLELIALSLGLEAKRFEQFFT[KD]QTSFIRLNHYPPCPFPHLALGVGRHKDAGGLTILAQD  
 Gmax 174 LLELIALSLGLEAKRFEFFMKDQTSFIRLNHYPPCPYPFH[ALGVGRHKDGGALT]VLAQD  
 Mtru 174 LLELIALSLGLEAKRFEFFF-KYQTSFIRENHYPPCPYPFH[ALGVGRHKDAGALT]ILAQD  
 Atha 176 LLEL[VS]ISLGLPGDRLTGFF-N[E]QTSFIRFNHYPPCPNPFLALGVGRHKDGGALT[VLAQD

Lfil 235 QVGGLEVKRKADQQWVRVEPTPDAFIINVCD[SI]QVWSNDAYESVEHRVMVNSEKERFSIP  
 Ljap 234 EVGGLEVVKRKADQQWVRVEPTSDAFIINVCD[SI]QF-----  
 Gmax 234 EVGGLEVVKRKADQEWTRVKPTPDAYIIINVGD[LI]QVWSNDAYESVEHRVMVNSEKERFSIP  
 Mtru 233 EVGGLEVVKRKSDQQWVIVKPTPDAYIIINVGD[LI]QVWSNDAYESVEHRVMVNSEKERFSIP  
 Atha 235 SVGG[Q]VS[RE]SDGQWIPVKPISDALIINMGNCI[Q]VWTNDEYW[SAE]HRV[V]NTSKERFSIP

Lfil 295 YFFNPAHDCEVKPLDELTNEQNPSKYRPYEWGKFVNRMGGNLKKNVENIQIYHY----  
 Ljap 269 -----FVCREGAM-----  
 Gmax 294 FFFNPAHDIEVKPLEELTNEHNPSKYRPYKWGKF[LVHRKGNSFKQ]VENIQIYHYKIT\*  
 Mtru 293 FFFFPAHDTVVKPLEELTNEENPPKYRPYNWGKF[LVNRKSSNFE]KKKVENIQIYHYKIA\*  
 Atha 295 FFFFPSHEAN[E]PLEELISEENPPCYKYNWGKFVSENRSDFKKLEVENIQIDHEK-A\*

## Appendix 7

Supplementary Figures S20 – S28 Amino acid alignments for red-differentially expressed flavonoid genes in *Lotus filicaulis*

Figure S20. Amino acid alignment for ANS

|      |     |   |
|------|-----|---|
| Lfil | 1   | M A P T V V E R V E S L S G S G I Q S I P K E Y V R P K E E L A N I G D V F E E E K K - V G P Q V P T I D L K E I D S P   |
| Ljap | 1   | M A P T V V E R V E S L S G S G I Q S I P K E Y V R P K E E L A N I G D V F E E E K K - V G P Q V P T I D L K D I D S P   |
| Gmax | 1   | M G - T V A P R V E S L A S S G I K C I P K E Y V R P Q E E L K S I G N V F E E E K K - E G I Q V P T I D L K E I D S E   |
| Mtru | 1   | M G - T V A Q R V E S L A L S G I S S I P K E Y V R P K E E L A N I G N I F D E E K K - E G P Q V P T I D L K E I N S S   |
| Atha | 1   | M V -- A V E R V E S L A K S G I I S I P K E Y I R P K E E L E S I N D V F L E E K K E D G P Q V P T I D L K N I E S D    |
| <br> |     |   |
| Lfil | 60  | D E F V R A K C R E K L R K A A E E W G V M H L V N H G I P D E L L N Q L K S A G A E F F S L P V E E K E K Y A N D Q A   |
| Ljap | 60  | D E F V R A K C R E K L R K A A E E W G V M H L V N H G I P D E L L N Q L K S A G A E F F S L P V E E K E K Y A N D Q A   |
| Gmax | 59  | D E V V R G K C R E K L K K A A E E W G V M H L V N H G I P D E L I E R V K K A G E T F F G L A V E E K E K Y A N D L E   |
| Mtru | 59  | D E I V R G K C R E K L K K A A E E W G V M H L V N H G I S D D L I N R L K K A G E T F F E L P V E E K E K Y A N D Q S   |
| Atha | 59  | D E K I R E N C I E E L K K A S L D W G V M H L I N H G I P A D I M E R V K K A G E F F S L S V E E K E K Y A N D Q A     |
| <br> |     |   |
| Lfil | 120 | A G N V Q G Y G S K L A N N A S G Q L E W E D Y F F H L I F P E D K R D L S I W P K T P S Y Y T E V T S D Y A R R L R V   |
| Ljap | 120 | A G N V Q G Y G S K L A N N A S G Q L E W E D Y F F H L I F P E D K R D L S I W P K T P S Y Y T E V T S D Y A R R L R V   |
| Gmax | 119 | S G K I Q G Y G S K L A N N A S G Q L E W E D Y F F H L A F P E D K R D L S E W P K K P A D Y I E V T S E Y A K R L R G   |
| Mtru | 119 | S G K I Q G Y G S K L A N N A S G Q L E W E D Y F F H C I F P E D K R D L S I W P K T P A D Y T K V T S E Y A K E L R V   |
| Atha | 119 | T G K I Q G Y G S K L A N N A S G Q L E W E D Y F F H L A Y P E E F K R D L S I W P K T P S D Y I E A T S E Y A K C L R L |
| <br> |     |   |
| Lfil | 180 | L A S K I L E V L S L E L G L E E G R L E K E V G G M E E L L L Q M K I N Y Y P K C P Q P E L A L G V E A H T D I S A L   |
| Ljap | 180 | L A S K I L E V L S L E L G L E E G R L E K E V G G M E E L L L Q M K I N Y Y P K C P Q P E L A L G V E A H T D I S A L   |
| Gmax | 179 | L A T K I L E A L S I G L G L E G R R L E K E V G G M E E L L L Q I K I N Y Y P I C P Q P E L A L G V E A H T D V S S L   |
| Mtru | 179 | L A S K I M E V L S L E L G L E G R R L E K E A G G M E E L L L Q M K I N Y Y P I C P Q P E L A L G V E A H T D V S S L   |
| Atha | 179 | L A T K V F K A L S V G L G L E P D R L E K E V G G I E E L L L Q M K I N Y Y P K C P Q P E L A L G V E A H T D V S A L   |
| <br> |     |   |
| Lfil | 240 | T F L L H N M V P G L Q L F Y E G K W V T A K C V P D S I L M H I G D T T E I L S N G K F K S I L H R G L V N K E K V R   |
| Ljap | 240 | T F L L H N M V P G L Q L F Y E G K W V T A K C V P D S I L M H I G D T T E I L S N G K F K S I L H R G L V N K E K V R   |
| Gmax | 239 | T F L L H N M V P G L Q L F Y E G Q W V T A K C V P D S I L M H I G D T I E I L S N G K Y K S I L H R G L V N K E K V R   |
| Mtru | 239 | T F L L H N M V P G L Q L F Y E G K W V T A K C V P D S I L M H I G D T I E I L S N G K Y K S I L H R G L V N K E K V R   |
| Atha | 239 | T F I L H N M V P G L Q L F Y E G K W V T A K C V P D S I V M H I G D T I E I L S N G K Y K S I L H R G L V N K E K V R   |
| <br> |     |   |
| Lfil | 300 | I S W A V F C E P P K E K I I I L K P L P E L V T E T E P A R F P P R T F A Q H I H H K L F R K D Q E A S A Q -- S K *    |
| Ljap | 300 | I S W A V F C E P P K E K I I I L K P L P E L V T E T E P A R F P P R T F A Q H I H H K L F R K D Q E A S A Q -- S K *    |
| Gmax | 299 | I S W A V F C E P P K E K I I I L Q P L P E L V T E T E P A R F P P R T F A Q H I H H K L F R K D Q E G L P N ----- *     |
| Mtru | 299 | I S W A V F C E P P K E K I I I L K P L P E L V T E K E P A R F P P R T F A Q H I H H K L F R K D E E E K K D D P K K *   |
| Atha | 299 | I S W A V F C E P P K D K I V I L K P L P E M V S V E S P A K F P P R T F A Q H I E H K L F G K E Q E E L V S E K N D *   |

Figure S21. Amino acid alignment for CHS-2

|      |     |   |
|------|-----|---|
| Lfil | 1   | MV-----SVAEIRKAQRAEGPATILAIGTANPPNCVDQSTYPDFYFKITNSEHMTTELKEK           |
| Ljap | 1   | MV-----SVAEIRKAQRAEGPATILAIGTANPPNCVDQSTYPDFYFKITNSEHMTTELKEK           |
| Gmax | 1   | MV-----SVAEIRQAQRAEGPATILAIGTANPPN[R]VDQSTYPDFYF[R]ITNS[Q]HMTTELKEK     |
| Mtru | 1   | MV-----SVSEIRKAQRAEGPATILAIGTANPANCVEQSTYPDFYFKITNSEHKTELKEK            |
| Atha | 1   | MVMAGASSIDEIRQAQRADGPAGILAIGTANPENHVLQAEYPDYYFRITNSEHMTDLKEK            |
| <br> |     |   |
| Lfil | 56  | FQRMCDSMIKKRYMYLNEEILKENPNLCAYMAPSLDARQDMVVVEVPRGLGKEAA[T]KAIK          |
| Ljap | 56  | FQRMCDSMIKKRYMYLNEEILKENPNLCAYMAPSLDARQDMVVVEVPRGLGKEAA[T]KAIK          |
| Gmax | 56  | FQRMCDSMIK[T]RYMYLNEEILKENPNMCAYMAPSLDARQDMVVVEVPRGLGKEAAVKAIK          |
| Mtru | 56  | FQRMCDSMIK[R]RYMYL[T]EEILKENPSVCEYMAPSLDARQDMVVVEVPRGLGKEAAVKAIK        |
| Atha | 61  | [F]KRMCDKSTIRKRHMHLTEEF[FLKENPH]MCAYMAPSLDTRQDI[V]VVEVP[K]LGKEAAVKAIK   |
| <br> |     |   |
| Lfil | 116 | EWGQPKSKITHLIFCTTSGVDMPGADYQLTKLLGLRPSVKRYMMYQQGCFAGGTVLRLAK            |
| Ljap | 116 | EWGQPKSKITHLIFCTTSGVDMPGADYQLTKLLGLRPSVKRYMMYQQGCFAGGTVLRLAK            |
| Gmax | 116 | EWGQPKSKITHLIFCTTSGVDMPGADYQLTK[Q]LGLRPYVKRYMMYQQGCFAGGTVLRLAK          |
| Mtru | 116 | EWGQPKSKITHLIVCTTSGVDMPGADYQLTKLLGLRPYVKRYMMYQQGCFAGGTVLRLAK            |
| Atha | 121 | EWGQPKSKITH[V]VFC TTSGVDMPGADYQLTKLLGLRPSVKRLMMYQQGCFAGGTVLRIAK         |
| <br> |     |   |
| Lfil | 176 | DLAENNKGARVLVVCSEITAVTFRGPNDTHLDLSLVGQALFGDGAAAVIVGSDPVPEI-EK           |
| Ljap | 176 | DLAENNKGARVLVVCSEITAVTFRGPNDTHLDLSLVGQALFGDGAAAVIVGSDPVPEI-EK           |
| Gmax | 176 | DLAENNKGARVLVVCSEITAVTFRGPSDTHLDLSLVGQALFGDGAAAVIVGSDP[P]QV-EK          |
| Mtru | 176 | DLAENNKGARVLVVCSEITAVTFRGPSDTHLDLSLVGQALFGDGAAAVIVGSDPVPEI-EK           |
| Atha | 181 | DLAENN[R]GARVLVVCSEITAVTFRGPSDTHLDLSLVGQALFSDGAALIVGSDPDT[SV]GEK        |
| <br> |     |   |
| Lfil | 235 | PLFELVWTAQTIAPDSGAI[D]GHLREVGLTFHLLKDVP[G]IVSKNIEKALIEAFQPLGISD         |
| Ljap | 235 | PLFELVWTAQTIAPDSGAI[D]GHLREVGLTFHLLKDVP[G]IVSKNIDKALVEAFQPLNISD         |
| Gmax | 235 | PLFELVWTAQTIAPDS[G]AI[D]GHLREVGLTFHLLKDVP[G]IVSKNIDKALFEAFNP[N]LISD     |
| Mtru | 235 | PIFEM[V]WTAQTIAPDS[G]AI[D]GHLREAGLT[F]HLLKDVP[G]IVSKNITKALVEAFEPPLGISD  |
| Atha | 241 | PIFEM[V]SAAQ[TL]PDSDGAI[D]GHLREVGLTFHLLKDVPGLISKNIVKSLDEAFKPLGISD       |
| <br> |     |   |
| Lfil | 295 | YNSIFWIAHPGGPAILDQVEQKL[LS]LKPEKM[KATR]EV[LV]SEYGNMSSACVLFILDEM[R]KKSA  |
| Ljap | 295 | YNSIFWIAHPGGPAILDQVEQKLGLKPEKM[KATR]NVLS[D]YGNMSSACVLFILDEM[R]KKSA      |
| Gmax | 295 | YNSIFWIAHPGGPAILDQVEQKLGLKPEKM[KATR]DV[LV]SEYGNMSSACVLFILDEM[R]RKSA     |
| Mtru | 295 | YNSIFWIAHPGGPAILDQVEQKLALKPEKM[NATR]EV[LV]SEYGNMSSACVLFILDEM[R]KKST     |
| Atha | 301 | WNS[L]FWIAHPGGPAILDQVEI[KLGLK]E[KM][ATR]HV[LV]SEYGNMSSACVLFILDEM[R]RKSA |
| <br> |     |   |
| Lfil | 355 | Q[D]GLKT[G]EGLEWGVLFGFGPGLTIETVVLRSVA-[I]*                              |
| Ljap | 355 | ENGLKT[G]EGLEWGVLFGFGPGLTIETVVLRSVA-[I]-                                |
| Gmax | 355 | ENG[H]KT[G]EGLEWGVLFGFGPGLTIETVVLHSVA-[I]*                              |
| Mtru | 355 | I[D]GLKT[G]EGLEWGVLFGFGPGLTIETVVLRSVA-[I]*                              |
| Atha | 361 | KDG[V]ATT[G]EGLEWGVLFGFGPGLT[V]TVVLHSVP-[L]*                            |

Figure S22. Amino acid alignment for CHS-4

Lfil 1 MV-----SVAEIRKAQRAEGPATIFIAIGTANPPNCVDQSTYPDFYFRVTNSEHKTELKEK  
Ljap 1 MV-----SVAEIRKAQRAEGPATIFIAIGTANPPNCVDQSTYPDFYFRVTNSEHKTELKEK  
Gmax 1 MV-----SVAEIRQAQRAEGPATILAIGTANPPNRVDQSTYPDFYFRITNSHMTTELKEK  
Mtru 1 MV-----SVSEIRNAQRAEGPATILAIGTANPANCVEQSTYPDFYFKITNSEHKTELKEK  
Atha 1 MV MAGASSIDEIRQAQRAADGPAGILAIGTANPENHVLQAEYPDYYFRITNSEHMTDLKEK

Lfil 56 FQRMCDSKSMIKKRYMHLTEDLILKENPNMCAYMAPSLDARQDMVVVEVPRLGKEAAVKAI-  
Ljap 56 FQRMCDSKSMIKKRYMHLTEDLILKENPNMCAYMAPSLDARQDMVVVEVPRLGKEAAIKAIC  
Gmax 56 FQRMCDSKSMIKTRYMYLNEEILILKENPNMCAYMAPSLDARQDMVVVEVPKLGKEAAVKAIK  
Mtru 56 FQRMCDSKSMIKRRYMYLTEEFLILKENPSVCCEYMAPSLDARQDMVVVEVPRLGKEAAVKAIK  
Atha 61 FKRMCDSKSTIRKRHMHLTEEFILKENPHMCAYMAPSLDTRQDIVVVVEVPKLGKEAAVKAIK

Lfil 114 -----  
Ljap 116 EWGQPQSKITHLIFCTTSGVDMPGADYQLTKLLGLRPYVKRYMMYQQGCFAGGTVLRLAK  
Gmax 116 EWGQPQSKITHLIFCTTSGVDMPGADYQLTKQLGLRPYVKRYMMYQQGCFAGGTVLRLAK  
Mtru 116 EWGQPQSKITHLIVCTTSGVDMPGADYQLTKLLGLRPYVKRYMMYQQGCFAGGTVLRLAK  
Atha 121 EWGQPQSKITHVVFC TTSGVDMPGADYQLTKLLGLRPSVKRLMMYQQGCFAGGTVLRIAK

Lfil 114 -----  
Ljap 176 DLAENNKGARVLVVCSEITAVTFRGSDTHLDLSLVGQALFGDGAAALIVGSDPIPEI-EK  
Gmax 176 DLAENNKGARVLVVCSEITAVTFRGSDTHLDLSLVGQALFGDGAAAVIVGSDPIPQV-EK  
Mtru 176 DLAENNKGARVLVVCSEVTAVTFRGSDTHLDLSLVGQALFGDGAAALIVGSDPVPEI-EK  
Atha 181 DLAENNKGARVLVVCSEITAVTFRGSDTHLDLSLVGQALFSDGAAALIVGSDPDTSVGEEK

Lfil 114 -----  
Ljap 235 PIFEMVWTAQTIAPDSEGAIDGHLREVGLTFHLLKDVPGLIVSKNIEKALIEAFQPLGISD  
Gmax 235 PLYELWVTAQTIAPDSEGAIDGHLREVGLTFHLLKDVPGLIVSKNIDKALFEAFNPLNISD  
Mtru 235 PIFEMVWTAQTIAPDSEGAIDGHLREAGLTTFHLLKDVPGLIVSKNINKALVEAFEPPLGISD  
Atha 241 PIFEMVSAAQTLIPDSGAIIDGHLREVGLTFHLLKDVPGLISKNIVKSLDEAFKPLGISD

Lfil 114 -----  
Ljap 295 YNSIFWIAHPGGPAILDQVEQKLGLKPEKMNATRDVLSNYGNMSSACVLFILDEMRRKSA  
Gmax 295 YNSIFWIAHPGGPAILDQVEQKLGLKPEKMKATRDVLSYEYGNMSSACVLFILDEMRRKSA  
Mtru 295 YNSIFWIAHPGGPAILDQVEQKLALKPEKMKATREVLSEYGNMSSACVLFILDEMRRKSA  
Atha 301 WNSLFWIAHPGGPAILDQVEIKLGLKEKMFATRHVLSEYGNMSSACVLFILDEMRRKSA

Lfil 114 -----  
Ljap 355 QDGKTTGEGLIKWGVLFGFPGPLTIETVVVLRSVAI\*  
Gmax 355 ENGHKTGEGLEWGVLFGFGPGLTIETVVVLHSVAI\*  
Mtru 355 QDGKTTGEGLEWGVLFGFGPGLTIETVVVLRSVAI\*  
Atha 361 KDGWATTGEGLEWGVLFGFGPGLTETVVVLHSVPL\*

Figure S23. Amino acid alignment for CHS-5

Lfil 1 -----  
Ljap 1 MV-----SVAEIRKAQRAEGPATIFAI GTANPPNCVDQSTYPDFYFRVTNSEHKTTELKEK  
Gmax 1 MV-----SVAEIRQAQRAEGPATILAIGTANPPNRVDQSTYPDYYFRITNSIHMTTELKEK  
Mtru 1 MV-----SVSEIRKAQRAEGPATILAIGTANPPNCVEQSTYPDFYFRITNSEHKTTELKEK  
Atha 1 MVMAGASSIDEIRQAQRAADGPAGILAIGTANPENHVLIQAEYPDYYFRITNSEHMTDLKEK

Lfil 1 -----  
Ljap 56 FQRMCDKSMIKKRYMHLTEDLILKENPNMCAYMAPSLDARQDMVVVEVPRLGKEAAVKAIK  
Gmax 56 FQRMCDKSMIKTRYMYLNNEEILKENPNMCAYMAPSLDARQDMVVVEVPKLGKEAAVKAIK  
Mtru 56 FQRMCDKSMIKRRYMYLTEEILKENPSVCYMAPSLDARQDMVVVEVPRLGKEAAVKAIK  
Atha 61 FKRMCDKSTIRKRHMHLTEEFLKENPHMCAYMAPSLDTRQDIVVVVEVPKLGKEAAVKAIK

Lfil 1 -----  
Ljap 116 EWGQPQSKITHLIFCTTSGVDMPGADYQLTKLLGLRPYVKRYMMYQQGCFAGGTVLRLAK  
Gmax 116 EWGQPQSKITHLIFCTTSGVDMPGADYQLTKQLGLRPYVKRYMMYQQGCFAGGTVLRLAK  
Mtru 116 EWGQPQSKITHLIVCTTSGVDMPGADYQLTKLLGLRPYVKRYMMYQQGCFAGGTVLRLAK  
Atha 121 EWGQPQSKITHVVFC TTSGVDMPGADYQLTKLLGLRPSVKRLMMYQQGCFAGGTVLRIAK

Lfil 1 -MAENNKGARVLVVCSEITAVTFRGSDTHLDLSLVGQALFGDGAAALIVGSDPVPEV-EK  
Ljap 176 DLAENNKGARVLVVCSEITAVTFRGSDTHLDLSLVGQALFGDGAAALIVGSDPVPEV-EK  
Gmax 176 DLAENNKGARVLVVCSEITAVTFRGSDTHLDLSLVGQALFGDGAAAVIVGSDPITPQV-EK  
Mtru 176 DLAENNKGARVLVVCSEITAVTFRGSDTHLDLSLVGQALFGDGAAALIVGSDPITPEI-EK  
Atha 181 DLAENNKGARVLVVCSEITAVTFRGSDTHLDLSLVGQALFSDGAAALIVGSDPDTSVGEEK

Lfil 59 PLFELVWTAQTIAPDSEGAIDGHLREVGLTFHLLKDVPG-----  
Ljap 235 PLFELVWTAQTIAPDSEGAIDGHLREVGLTFHLLKDVPGIVSKNIEKALIEAFQPLGISD  
Gmax 235 PLYELVWTAQTIAPDSEGAIDGHLREVGLTFHLLKDVPGIVSKNIDKALFEAFNPLNISD  
Mtru 235 PIFEMVWTAQTIAPDSEGAIDGHLREAGLTFHLLKDVPGEIVSKNINKALVDAFQPLGVSD  
Atha 241 PIFEMVSAAQTILPDSGAIDGHLREVGLTFHLLKDVPGLISKIVKSLDEAFKPLGISD

Lfil 97 -----  
Ljap 295 YNSIFWIAHPGGPAILDQVEQKLISLKPEKMFATREVLSEYGNMSSACVLFILDEMRRKSA  
Gmax 295 YNSIFWIAHPGGPAILDQVEQKLGLKPEKMKATRDVLSEYGNMSSACVLFILDEMRRKSA  
Mtru 295 YNSIFWIAHPGGPAILDQVEQKLALKPEKMKATREVLSEYGNMSSACVLFILDEMRRKSA  
Atha 301 WNSIFWIAHPGGPAILDQVEIKLGLKEKMFATRHVLSEYGNMSSACVLFILDEMRRKSA

Lfil 97 -----  
Ljap 355 QDGKTTGEGLEWGVLFGFGPGLTIETVVVLRSVTI\*  
Gmax 355 ENGHKTGEGLEWGVLFGFGPGLTIETVVVLHSVAI\*  
Mtru 355 QDGKTTGEGHEWGVLFGFGPGLTIETVVVLRSVAI\*  
Atha 361 KDGWATTGEGLEWGVLFGFGPGLTETVVVLHSVPL\*

Figure S24. Amino acid alignment for DFR

Lfil 1 MGSVPETVCVTGAAGFIGSFLVMRLMERYMVRATVRDPANMKVKHLLPEAKTKLTL  
 Ljap 1 MGSVPETVCVTGAAGFIGSFLVMRLMERYMVRATVRDPANMKVKHLLPEAKTKLTL  
 Gmax 1 MGSASESVCVTGASGFIGSFLVMRLMERYYTVRATVRDPVNMKVKHLLPEAKGKLSL  
 Mtru 1 MGSMAETVCVTGASGFIGSFLVMRLMERYMVRATVRDPENLKKVSHLLPEAKGKLSL  
 Atha 1 MVSQKEETVCVTGASGFIGSFLVMRLMERYFVRATVRDPGNLKKVQHLLDLPNAKTLTL

Lfil 61 WKADLAEGSFDEAIKGCTGVFHATPMDFESKDPENEVIKPTINGVLIDIMKASQKAKTV  
 Ljap 61 WKADLAEGSFDEAIKGCTGVFHATPMDFESKDPENEVIKPTINGVLIDIMKACQKAKTV  
 Gmax 61 WKADLAEGSFDEAIKGCTGVFHATPMDFESKDPENEVIKPTINGVLIDIMKACIKAKTV  
 Mtru 61 WKADLGEEGSFDEAIKGCTGVFHATPMDFESKDPENEVIKPTIKGVLDIMKACIKAKTV  
 Atha 61 WKADLSEEGSYDDAINGCDGVFHATPMDFESKDPENEVIKPTVNGMLGIMKACVKAKTV

Lfil 121 RRLVFTSSAGTLNVIEHQKQMFDESCWSDVEFCRRVKMTGWMYFVSKTLAEQEAWKFAKE  
 Ljap 121 RRLVFTSSAGTLNVIEHQKQMFDESCWSDVEFCRRVKMTGWMYFVSKTLAEQEAWKFAKE  
 Gmax 121 RRLIFTSSAGTLNVIERQKPVFDDTCWSDVEFCRRVKMTGWMYFVSKTLAEKEAWKFAKE  
 Mtru 121 RRFITFTSSAGTLNVTEEDQKPLWDESCWSDVEFCRRVKMTGWMYFVSKTLAEQEAWKFAKE  
 Atha 121 RRFVFTSSAGTVNVEEHQKNVYDENWDSDIEFTIMSKKMTGWMYFVSKTLAEKAADFEE

Lfil 181 HGIDFITIIPPLVVGSGFLMPTMPPSLITALSPITGNEAHYSIIKQGQYVHLDDLCAHIF  
 Ljap 181 HGIDFITIIPPLVVGSGFLMPTMPPSLITALSPITGNEAHYSIIKQGQYVHLDDLCAHIF  
 Gmax 181 QGLDFITIIPPLVVGPFMLMPTMPPSLITALSPITGNEAHYSIIKQGQFVHLDDLCAHIF  
 Mtru 181 HNMDFITIIPPLVVGPFITPTMPPSLITALSPITGNEAHYSIIKQGQFVHLDDLCAHIF  
 Atha 181 KGDIFISIIPTLVVGPFITTSMPPSLITALSPITRNEAHYSIIKQGQYVHLDDLCAHIF

Lfil 241 LFEHPESEGRYICSASEATIHDIAKLINSKYPEYNIPTKFKNIPDDELELVRFSSKKIKDM  
 Ljap 241 LFEHPESEGRYICSASEATIHDIAKLINSKYPEYNIPTKFKNIPDDELELVRFSSKKIKDM  
 Gmax 241 LFEPEVEGRYICSACDATIHDIAKLINQKYPEYKVPTKFKNIPDQLELVRFSSKKITDL  
 Mtru 241 LFEHMEVEGRYICSACEANIHDIAKINTKYPEYNIPTKFENNIPDDELELVRFSSKKIKDL  
 Atha 241 LYEQAAAKGRYICSSHDAITLTISKFLRPKYPEYNVPSTFEGVDENLKSIEFSSKKLTDM

Lfil 301 GFEFKYSLEDMYTGAIDTCKEKGLLPKAAENPSN-----  
 Ljap 301 GFEFKYSLEDMYTGAIDTCKEKGLLPKAAENPSN-----  
 Gmax 301 GFKFKYSLEDMYTGAIDTCRKGLLPKPAEKGLF-----  
 Mtru 301 GFEFKYSLEDMYTEAIDTCIEKGLLPKFVK--ST-----  
 Atha 301 GENFKYSLEEMFIESIFTCRQKGFLPVSLSYQSISEIKTKNENIDVKTDGGLTDGMKPCN

Lfil 335 -----G-----K\*  
 Ljap 335 -----G-----K\*  
 Gmax 335 -----TKPAETPVNAI-MHK\*  
 Mtru 333 -----N-----K\*  
 Atha 361 KTETGITGERTDAPMLAQQMCA\*

Figure S25. Amino acid alignment for F3H

|      |     |  |
|------|-----|--|
| Lfil | 1   | MASFKPKTLTTLAQQNTELESSFVRDEDERPKVAYNNFSNEIPVISLAGIDEVDGRRSEIC  |
| Ljap | 1   | MASFKPKTLTTLAQQNTELESSFVRDEDERPKVAYNNFSNEIPVISLAGIDEVDGRRSEIC  |
| Gmax | 1   | MAPT-AKTLTYLAQEKTLESSFVRDEIPRKVAYNFSDEIPVISLAGIDEVDGRRREIC     |
| Mtru | 1   | MAP--AQTLTYLAQEKTLESSFVRDEIPRKVAYNNFSNEIPISLDGIDDAGGRRAEIC     |
| Atha | 1   | -----  |
| <br> |     |  |
| Lfil | 61  | NKIVEACENWGIFQVVDHGVDTELVSHTT LAKEFFALPPEEKLRFDMTGGKKGGFIVSS   |
| Ljap | 61  | NKIVEACENWGIFQVVDHGVDTELVSHTT LAKEFFALPPEEKLRFDMTGGKKGGFIVSS   |
| Gmax | 60  | EKIVEACENWGIFQVVDHGVDQQLVAEMTRLAKEFFALPPDEKLRFDMMSGAKKGGFIVSS  |
| Mtru | 59  | NKIVEACENWGIFQVVDHGVDSKLISEMTRALEAKGFFDLPPEEKLRFDMSGGKKGGFIVSS |
| Atha | 1   | ----- MTRLARDFFALPPEDKLRFDMMSGKKGGFIVSS                        |
| <br> |     |  |
| Lfil | 121 | HLQGESVQDWREIVTYFSYPIRNRDYSRWPDTPAGWKAVTEYESEKLMGLACKLLEVLS    |
| Ljap | 121 | HLQGESVQDWREIVTYFSYPIRNRDYSRWPDTPAGWKAVTEYESEKLMGLACKLLEVLS    |
| Gmax | 120 | HLQGESVQDWREIVTYFSYPIRKERDYSRWPDTPEGWRSVTEEYSDKVMLACKLLEVLS    |
| Mtru | 119 | HLQGEAVKDWRLETVTYFSYPIRQRDYSRWPDKPEGWKEVTEQYSEKLMNLACKLLEVLS   |
| Atha | 34  | HLQGEAVQDWREIVTYFSYPIRNRDYSRWPDKPEGWVKVTEEYSEFLMSLACKLLEVLS    |
| <br> |     |  |
| Lfil | 181 | AMGLEKEALTKACVDMHQKVVNYYPKCPQPDLTGLKRHTDPGTITLLLQDVGGQAT       |
| Ljap | 181 | AMGLEKEALTKACVDMQKVVNYYPKCPQPDLTGLKRHTDPGTITLLLQDVGGQAT        |
| Gmax | 180 | AMGLEKEGLSKACVDMQKVVNYYPKCPQPDLTGLKRHTDPGTITLLLQDVGGQAT        |
| Mtru | 179 | AMGLEKDALTAKACVDMQKVVIYYPKCPQPDLTGLKRHTDPGTITLLLQDVGGQAT       |
| Atha | 94  | AMGLEKESLTNACVDMQKIVVNYYPKCPQPDLTGLKRHTDPGTITLLLQDVGGQAT       |
| <br> |     |  |
| Lfil | 241 | RDNGKTWITVQPLEGAFVVNLGDGHYLSNGRFKNADHQAVVNSNSSRLSIATFQNPAPD    |
| Ljap | 241 | RDNGKTWITVQPVEGAFVVNLGDGHYLSNGRFKNADHQAVVNSNSSRLSIATFQNPAPD    |
| Gmax | 240 | RDNGKTWITVQPVEAAFFVVNLGDHAHYLSNGRFKNADHQAVVNSNHRLSIATFQNPAPN   |
| Mtru | 239 | KDNGKTWITVQPVEGAFVVNLGDGHYLSNGRFKNADHQAVVNSNYSRRLSIATFQNPAPD   |
| Atha | 154 | RDNGKTWITVQPVEGAFVVNLGDGHYLSNGRFKNADHQAVVNSNSSRLSIATFQNPAPD    |
| <br> |     |  |
| Lfil | 301 | ATVYPLKVREGEKSVMEEPITFAEMYRRKMSKDIELARMKKLAKEK-KLQDLE-----     |
| Ljap | 301 | ATVYPLKVREGEKSVMEEPITFAEMYRRKMSKDIELARMKKLAKEK-KLQDLE-----     |
| Gmax | 300 | ATVYPLKIREGEKPVMEEPITFAEMYRRKMSKDIETARMKKLAKEK-HLQDLENEKHLQE   |
| Mtru | 299 | ATVYPLKIRGEKSVMEEPITFAEMYRRKMSKDIETARMKKLAKEEKELRDLE-----      |
| Atha | 214 | ATVYPLKVREGEKAILEEPITFAEMYRKMGEDDELARIKKLAKEE-RD-----          |
| <br> |     |  |
| Lfil | 353 | ---KAKLEPKPMNEIFA*   |
| Ljap | 353 | ---KAKLEPKPMNEIFA*   |
| Gmax | 359 | LDQKAKLEAKPLKEILIA*  |
| Mtru | 352 | ---KAKIEAKPKNEILIA*  |
| Atha | 262 | ---HKEVDPKPVDDQIFIA*   |

Figure S26. Amino acid alignment for F3'H

Lfil 1 M-EFPWMIIGFATITFLIFIHRRVVF-STR-PSLPLPPGPKWPPIIGNFPHMGPVPHSLA  
 Ljap 1 M-EFPWMIIGFATITFLIFIHRRVVF-STR-PSLPLPPGPKWPPIIGNFPHMGPVPHSLA  
 Gmax 1 M-SP-LIVALATIAAAIILYRIIKF-ITR-PSLPLPPGPKWPPIGNFPHMGPVPHSLA  
 Mtru 1 M-SLWFIAIASFTLCILIIYRFMKF-AKRSSSLPPLPPGPKWPPIIGNMPHLGPAPHQSTIA  
 Atha 1 MATLFLTILLATVLFIL--RIFSHRRNRSHNNRLPPGPNPWPPIIGNLPHMGTKPHRTLS

Lfil 58 ALARAHGPLMHLKLGFVDVVAASA AVAEQFLKVDANFSSRPPNAGAKYIAYNYQDLVF  
 Ljap 58 ALARAHGPLMHLKLGFVDVVAASA AVAEQFLKVDANFSSRPPNAGAKYIAYNYQDLVF  
 Gmax 57 ALARIHGPLMHLRLGFVDVVAASASVAEQFLKTHDSNFSSRPPNAGAKYIAYNYQDLVF  
 Mtru 58 ALAKIHGPLMHLKLGFVDVTVAAASCVAEQFLKVDANFSSRPPNTGAKYIAYNYQDLVF  
 Atha 59 AMVTTYGPTIHLRLGFVDVVAASKSVAEQFLKTHDANFASRPPNSGAKHMAINYQDLVF

Lfil 118 APYGARWRYLRKITNLHLFSGKALDNFKHLRQEEVSRLTRNISK--SN SKAVNLGQLLNV  
 Ljap 118 APYGARWRYLRKITNLHLFSGKALDNFKHLRQEEVSRLTRNISK--SN SKVNLGQLLNV  
 Gmax 117 APYGPRWRMLRKISSVHLFSNKVMEEFKHLRQEEVARLTCLAS--SDTKAVNLGQLLNV  
 Mtru 118 APYGPRWRMLRKISSVHLFSNKVMEEFKHLRQEEVARLTCLASNSYSDTKAVNLGQLLNV  
 Atha 119 APYGHHRWRLLRKISSVHLFSAKALEDFKHVRQEEVGTLTRELVR--VGTKPVNLGQLVNM

Lfil 176 CTTNALSRVMIGRRVFNDGGCDPRADEFKAMVVELMVLAGVFNI GDFIPSLEWLDLQG  
 Ljap 176 CTTNALSRVMIGRRVFNDGGCDPRADEFKAMVVELMVLAGVFNI GDFIPSLEWLDLQG  
 Gmax 175 CTTNALARAMIGRRVFNDGGCDPRADEFKAMVMEVVLAGVFNI GDFIPSLEWLDLQG  
 Mtru 178 CTTNALARVMI GRRVFNDGGCDPRADEFKEMVLELMVLAGVFNI GDFIPSLEWLDLQG  
 Atha 177 CVVNALGREMIGRRIFG---ADADHKADEFESMVTEMALAGVFNI GDFVPSLWLDLQG

Lfil 236 VQAKMKKLHNRFDEFLTSIIIEH-NTSSKSENHKDLLSTLLSLKDVP-DDDGNRINDIEI  
 Ljap 236 VQAKMKKLHNRFDEFLTSIIIEH-NTSSKSENHKDLLSTLLSLKDVP-DDDGNKINDIEI  
 Gmax 235 VQAKMKKLHKRFDALTSIIIEHHNNSSSKNENHKNFLSILLSLKDVR-DDHGNHLDTEI  
 Mtru 238 VQAKMKKLHKKFDALTNIIDERENSNFKEKHKDLLSTLLLKEET-DVDGNKLTYIEI  
 Atha 234 VACKMKRLHKRFDALSSILKEH-EMNGQDQKHTDMLSTLISLGTDLDGDGGSLTDI

Lfil 294 KALLNMFTAGTDTSASTTEWAIAELIRSPRILAQQELEDTVVGRENVRDDLPHL PY  
 Ljap 294 KALLNMFTAGTDTSASTTEVIAELIRSPRILAQQELEDTVVGRENVRDDLPHL PY  
 Gmax 294 KALLNMFTAGTDSSSTTEWAIAELIKNPQILAKLQQELEDTVVGDRRSVKEEDIAHL PY  
 Mtru 297 KALLNMFAAGTDSSSTTEWAIAELIRNPRLAQVQQELEDNVVGRDRNVKEEDI PNLPY  
 Atha 293 KALLNMFTAGTDTSASTVWDWAIAELIRHPDIMVKAQEELDIIVVGRDRPVNESDIAQLPY

Lfil 354 LQAVVKETFRLHPSTPLSLPRVASESCEVLGYHIPKGSTLLNVWAIARDPKEWAEPLEF  
 Ljap 354 LQAVVKETFRLHPSTPLSLPRVASESCEVLGYHIPKGSTLLNVWAIARDPKEWAEPLEF  
 Gmax 354 LQAVIKETFRLHPSTPLSPRAAAESCEIFGYHIPKGATLLVNWIWAIARDPKEWNDPLEF  
 Mtru 357 LQAVIKETFRLHPSTPLSLPRIASESCEIFGYHIPKGSTLLNVWAIARDPKEWVDPLEF  
 Atha 353 LQAVIKNFRLHPPTPLSLPHIASESCEINGYHIPKGSTLLTNIWAIARDPDQWSDPLAF

Lfil 414 KPERF-LEGDKVDVKGNDFKVIPFGAGRRICAGMSLGLRMVQLLTATLVHSFNWELEN  
 Ljap 414 KPERF-LEGDKVDVKGNDFKVIPFGAGRRICAGMSLGLRMVQLLTATLVHSFNWELEN  
 Gmax 414 RPERFLLGGEKADVDVFGNDFEVIPFGAGRRICAGISLGLQMVQLLTAALAHSDWELED  
 Mtru 417 KPERFLPGGEKCDVDVKGNDFEVIPFGAGRRICPGMSLGLRMVQLLTATLAHSFDWELEN  
 Atha 413 KPERFLPGGEKSGVDVKGSDFELIPFGAGRRICAGISLGLRTIQFLTATLVQGFDWEAG

|      |     |   |
|------|-----|---|
| Lfil | 473 | GLNHEKLNMD <b>E</b> AYGLTLQRAVPLSV <b>S</b> RPRLS <b>H</b> VYA <b>A</b> <b>S</b> <b>H</b> -*  |
| Ljap | 473 | GLNHEKLNMD <b>E</b> AYGLTLQRAVPLSV <b>S</b> RPRLS <b>H</b> VYA <b>A</b> <b>S</b> <b>H</b> -*  |
| Gmax | 474 | C <b>M</b> P <b>E</b> KLNMD <b>E</b> AYGLTLQRAVPLSVH <b>P</b> P <b>R</b> <b>R</b> <b>I</b> <b>A</b> <b>P</b> <b>H</b> V <b>S</b> <b>M</b> <b>S</b> -*   |
| Mtru | 477 | GLNAG <b>K</b> M <b>N</b> <b>M</b> <b>D</b> <b>E</b> <b>G</b> YGLTLQRAVPLSV <b>H</b> <b>P</b> <b>K</b> <b>P</b> <b>R</b> <b>L</b> <b>S</b> <b>H</b> V <b>Y</b> <b>S</b> <b>S</b> <b>C</b> <b>F</b> -*   |
| Atha | 473 | G <b>V</b> T <b>P</b> <b>E</b> KLN <b>M</b> <b>F</b> <b>E</b> <b>S</b> <b>Y</b> GLTLQRAVPL <b>V</b> <b>V</b> <b>H</b> <b>P</b> <b>K</b> <b>P</b> <b>R</b> <b>L</b> <b>A</b> <b>P</b> <b>N</b> <b>V</b> <b>Y</b> <b>G</b> <b>L</b> <b>G</b> <b>S</b> * |

Figure S27. Amino acid alignment for MAX1

Lfil 1 MVFMDFEWLFQIIPS-VPWSSAMFTLLATIG-GFSVLYGPyWGVRKVPGPPSVPLIGHLP  
Ljap 1 MVFMDFEWLFQIIPS-VPWSSAMFTLLATIG-GFLVLYGPyWGVRKVPGPPSVPLIGHLP  
Gmax 1 MVSIVIEWLFPFPC-VAMF---TTLLMLIG-GLLGYLYGPyWGLRKVPGPPIIPLVGHLH  
Mtru 1 MVFMDFEWLFQIIPS-VAMF---TTLLMLIG-GWLIIYLYEPYWRVRKVPGPPSLPLVGHLH  
Atha 1 MKTQHQWWEVLDPF-LTQHEALIAFLTFAAVVIVIYLYRPSWSVCNVPGPTAMPLVGHLH

Lfil 59 LLAKYGPDVFSVLAKQYGPIYRFHMGRQPLIIIADAEELCKEAGIKKKFDITNRSPSPIS  
Ljap 59 LLAKYGPDVFSVLAKQYGPIYRFHMGRQPLIIIADAEELCKEAGIKKKFDITNRSPSPIS  
Gmax 56 LLAKYGPDVFSLLAKQYGPIYRFHMGRQPLILVADPELCKEVGIKKKFDIPNRSIPSPIS  
Mtru 57 LLAKHGPDVFSVLAKQYGPIYRFHMGRQPLIIIVADAELCKEVGIKKKFDIPNRSTPSPIK  
Atha 60 LMAKYGPDVFSVLAKQYGPIFRQGMGRQPLIIIAEELCDEVGIKKKFDIPNRSIPSPIS

Lfil 119 ASPLHQKGLFFT[KDSQWSTMRTNTILSLYQPSHLSRLVPTMQSFIESATQNLDQKEDFIF  
Ljap 119 ASPLHQKGLFFT[KDSQWSTMRTNTILSLYQPSHLSRLVPTMQSFIESATQNLDQNEDFIF  
Gmax 116 ASPLHQKGLFFT[RDSDRWSTMRTNTILS[VQPSHLSASLVPTMQSFIESATQNLDTPNEDIIF  
Mtru 117 ASPLHQKGLFFS[RDSDQWSTMRTNTILS[VQPSHLSRLVPTMQSFIESATQNLDQKEDEIFF  
Atha 120 ASPLHKKGLFFT[RDKRWSKMRNTNTILSLYQPSHLSLTIPTMHSFITSATHNLDSKPRDIVE

Lfil 179 SNLSLSSLATDVIGQAAFGVDFGLSKPQPVCDEIKSVN---KEVRDSSTGNEVSDFINQHI  
Ljap 179 SNLSLSSLATDVIGQAAFGVDFGLSRPQSVRDESG---N---KEVRGSGAGNEVSDFINQHI  
Gmax 176 SNLSLSSLATDVIGEAAGVNFGLSKPHSVCESIKSVSV---NNVR---NDDEVSDFINQHI  
Mtru 177 SNLSLSSLATDVIGQAAFGVNFGLSQSHSVHESKNVATDNKLMNASGSNEVTDFINQHI  
Atha 180 SNLFLKLTTDTIIGQAAFGVDFGLSGKKPIKD-----VEVTDFINQHV

Lfil 236 YSTTQLKMDLSGSFSIILGLLVPILQEPFRQVLKRIPGTMDWKIERTNRKL[G]SRLDEIVE  
Ljap 234 YSTTQLKMDLSGSFSIILGLLVPILQEPFRQILKRIPGTMDWKIERCTNRKL[G]SRLDEIVE  
Gmax 232 YSTTQLKMDLSGSFSIILGLLAPILQEPFRQILKRIPGTMD[SKIESTNEKLSG]PLDEIVK  
Mtru 237 YSTTQLKMDLSGSFSIILGLLVPILQEPFRQILKRIPGTMDWKIERTN[EKG]GRLDEIVE  
Atha 222 YSTTQLKMDLSGSLSIILGLLIPILQEPFRQVLKRIPGTMDWRVEKTNARLSGQLEIVS

Lfil 296 KRMKDRVRSSKDFLSLILNARESKTVSENVFTPDYISAVTYEHLLAGSATTSFTLSSIVY  
Ljap 294 KRMKDKVRSSKDFLSLILNARESKTVSENVFTPDYISAVTYEHLLAGSATTSFTLSSIVY  
Gmax 292 RRMEDK[NR]TSK[N]FLSLILNARESKKVSENVFSPDYISAVTYEHLLAGSATTAFTLSSIVY  
Mtru 297 KRTKDRTRSSKDFLSLILNARESKAVSENVFTP[Y]ISAVTYEHLLAGSATTSFTLSSVVY  
Atha 282 KRAKEAETDSKDFLSLILKARESDPFAKNIFTSDYISAVTYEHLLAGSATTAFTLSSVLY

Lfil 356 LVAGHPEVEKKMLQEIDGFGPV[DQT]PTSQDLQEKFPYLDQVIKEAMRYTVSPLVARETS  
Ljap 354 LVAGHPEVEKKMLQEIDGFGPV[DQT]PTSQDLQEKFPYLDQVIKEAMRYTVSPLVARETS  
Gmax 352 LVAGHREVEKKL[L]QEIDGFGPPDRIPAQDLHDSPYLDQVIKEAMRFYTVSPLVARETS  
Mtru 357 LVAAHPEVEKKML[E]IDG[G]SLSLDQIPTSQDLHDQFPYLDQVIKEAMRFYIVSPLVARETS  
Atha 342 LVSGHLDVEKRL[L]QEIDGFGNRDLIPTAHDLOQHFPYLDQVIKEAMRFYMVSPVARETA

Lfil 416 NEVEIGGYLLPKGTWVWLALGVVAKDPRNFPEPEKF[K]PERFD[P]KCEEMKRRHPYAFIPFG  
Ljap 414 NEVEIGGYLLPKGTWVWLALGVVAKDPRNFPEPEKF[K]PERFD[P]KCEEMKRRHPYAFIPFG  
Gmax 412 NEVEIGGYLLPKGTWVWLALGVLA[K]DPRNFPEPEKF[K]PERFD[P]KCEEMKRRHPYAFIPFG  
Mtru 417 NEVEIGGYLLPKGTWVWLALGVLA[K]DHNFP[E]PEKF[K]PERFD[P]NCEEMKQRHPYAFIPFG  
Atha 402 KEVEIGGYLLPKGTWVWLALGVLA[K]DPK[N]FPEPEKF[K]PERFD[P]NGEEEKHRHPYAFIPFG

|      |     |  |
|------|-----|--|
| Lfil | 476 | IGPRACIGQKFSLQEIKLSLIHLYRKYLFRHSPNMENPLELEYGIVLNFKHGVKVRAlKR |
| Ljap | 474 | IGPRACIGQKFSLQEIKLSLIHLYRKYLFRHSPNMENPLELEYGIVLNFKHGVKVRAlKR |
| Gmax | 472 | IGPRACIGQKFSLQEIKLTIHLYRKYLFRHSPNMENPLELEYGIVLNFKHGVKVRAlKR  |
| Mtru | 477 | IGPRACIGQKFSMQEIKLSLIHLYRKYLFRHSADMESPLELEYGIVLNFKHGVKFsvIKR |
| Atha | 462 | IGPRACVGQFALQEIKLTIHLYRNYIFRHSLEMEIPLQDYGITLSFKNGVKERTIKR    |

|      |     |        |
|------|-----|--------|
| Lfil | 536 | TERSC* |
| Ljap | 534 | TERSC* |
| Gmax | 532 | T----* |
| Mtru | 537 | TEMSC* |
| Atha | 522 | F----* |

Figure S28. Amino acid alignment for UFGT73C5

Lfil 1 MVFQEN---QPHFILFPLMAQGHIIIPMVDIARLLAQRGVIVTIFTTPKNASRFTSVLSRA  
Ljap 1 ---  
Gmax 1 MVFQTN--NNPHFILFPLMAQGHIIIPMMDIARLLAHRGVIVTIFTTPKNASREN SVLSRA  
Mtru 1 MVL PANINDVPHFVLFPLIAQGHIIIPMIDIAKLLAQRGVIVTIFTTPKNASRFTSVLSRA  
Atha 1 MVSETTKSSPLHVFPLMAQGHMIPMVDIARLLAQRGVITIVTTPHNAAREKNVLNRA

Lfil 58 ISSGLQIRLVLEQFPYKQAGLPEGCENFD MATSKGMFSKIFRGITMLQNSAEELFEKLSP  
Ljap 1 -----EELFEKLSP  
Gmax 59 ISSGLQIRLVOLHFPSKEAGLPEGCENFD MVTSDIMVYKMFNVINMLHKQAEFFEA LTP  
Mtru 61 VSSGLQIKIVTLNFPTKQAGLPEGCENFD MVDSDIMRMNLFAITLQKPAEELFDALTP  
Atha 61 IESGLPINLVQVKFPYLEAGLQE QENIDSIDTMRMIPFFKAVNFLEEPVQKLIEEMNP

Lfil 118 RPSCIISDFCIPWTAQVAAKYNIPRISHGFSCFCLHCILKVQT-SKVTESITSETEYFT  
Ljap 10 RPSCIISDFCIPWTAQVAAKYNIPRISHGFSCFCLHCILKVQT-SKVTESITSETEYFT  
Gmax 119 KPSCIISDFCIPWTAQVAOKHCIPRISHGFACFCFLHCMIMVHT-SNVCESTASESEYFT  
Mtru 121 KPSCIISDFHIPWTDQIAENHKIPRISHGFSCFCLHCMLKQT-SKILERVDSESEYFT  
Atha 121 RPSCLISDFCIPYPTSKIAKKFNIPKILFHGMGCFCLLCMHVLRKNREILDNLKSDKELFT

Lfil 177 VPGIPDQIQVNKSQIPGPM---S---DDLKD YGEQMYEAEKSSYGVINTFEELENAYVK  
Ljap 69 VPGIPDQIQVNKSQIPGPM---S---DDLKD YGEQMYEAEKSSYGVINTFEELENAYVK  
Gmax 178 IPGIPDQIQVTKEQIPMMI---SNSDEEMKHFR EQMRDADIKSYGVINTFEELEKAYVR  
Mtru 180 VPGIPDQIQVTKEQIPGIL---K---GELKEEGEKMHDAMKSYGEINTFEELEKAYVK  
Atha 181 VPDFPDRVEFTRTQPVETVYVPA---GDWKDIFDGMVEANETS YGVIVNSFQELEPAYAK

Lfil 231 DYKKERNDKVWCIGPVSLCNKDGLDKAQRGNKASINEHHCKWL DLQQPKSVVYVCL GSL  
Ljap 123 DYKKERNDKVWCIGPVSLCNKDGLDKAQRGNKASINEHHCKWL DLQQPKSVVYVCL GSL  
Gmax 235 DYKKV RNDKVWCIGPVSLCNQDNLDKVQRGNHASINEHHCKWL DLQQPKSAVYVCFGSL  
Mtru 234 DYKKEKNGKVWFVGPVSLCNKDGLDKAQRGIIASI SEHHCKWL DLIHQPKSVVYACLGSL  
Atha 238 DYKEVRSGKAWTIGPVSLCNKGADKAERGNKSDIDQDECLKWL DSKKHGSVLYVCL GSI

Lfil 291 CNLIPSQLMELALALEATERPFIWVIREGNQF-QELE-KWMSEE GFEERTKGRGLVIRGW  
Ljap 183 CNLIPSQLMELALALEATERPFIWVIREGNQF-QELE-KWMGEKGFEERTKGRGLVIRGW  
Gmax 295 CNLIPSQLVELALALEDTKKPFVWVIREGNKF-QELEKKWI SEE GFEERTKGRGLIIRGW  
Mtru 294 CNLIPSQLMELALALEATNRPFIWVIREGNKSSE ELE-KWIS E---ERNKGRGLIIRGW  
Atha 298 CNLPLSQLKELGLGLEESQRPFIWVIRGWEKY-KELV-EWES ESGFEDRIQDRGLLIK GW

Lfil 349 APQVLILSHPAIGGFLTHCGWNSTLEGISAGVPMVTWPLFADQFLNEKLV TQVLRIGVSL  
Ljap 241 APQVLILSHPAIGGFLTHCGWNSTLEGISAGVPMVTWPLFADSF-EENLSRPPGRCPVSX  
Gmax 354 APQVLILSHPSIGGFLTHCGWNSTLEGISAGVPMITWPLFADQFLNEKLV TQVLRIGVSL  
Mtru 349 APQVLILSHPSIGGFLTHCGWNSTLEGISAGIPLVTWPLFADQFLNEKLV TQVLRIGVSL  
Atha 356 SPQMLILSHPSVGGFLTHCGWNSTLEGITAGIPLITWPLFADQFCNEKLV VEVLKAGVRS

Lfil 409 GVEVPLKWGEEEKTGVLVKKEDIKKGICLIMDDESKESEERRQKARELSEIAKKA VENG  
Ljap 300 RHMLPLSIRKIE-----  
Gmax 414 GMEVPMKFGE EEEKTGVLVKKEDIKKAICIVMDDDGEESKDRRERATKLSEIAKFAVEKEG  
Mtru 409 GVEVPMRIGVEESSLGVLVKKEGIKEAICIVMD-EGEESKERRERASKLSEMAKEAVEKGG  
Atha 416 GVEOPMKWGE EEEKIGVLVDKEGVKKAVEEIMG-ESDDAKERRRAKELGDSAHKAVEEGG

|      |     |  |
|------|-----|--|
| Lfil | 469 | SSYHNMTLLIQDIMQ-----                           |
| Ljap | 311 | -----  |
| Gmax | 474 | SSHLD <b>MT</b> LLIQDIMQQSSSKEEVRTSLKESQFSCNA* |
| Mtru | 468 | SSH <b>LNIN</b> LLIQDIMEQ-----*                |
| Atha | 475 | SSH <b>SNISFLI</b> QDIMELAEP-----NN*           |

## Appendix 8

Supplementary Figures S29 – S30 Amino acid alignments for yellow-differentially expressed flavonoid genes in *Lotus filicaulis*

Figure S29. Amino acid alignment for CHS-1

|      |     |  |
|------|-----|--|
| Lfil | 1   | MV-----TVEEIRNAQRSSGPATILAFGTATPSHCVMQADYPDYYFRITNSEHMTDLKEK   |
| Ljap | 1   | MV-----TVEEIRNAQRSSGPATILAFGTATPSHCVMQADYPDYYFRITNSEHMTDLKEK   |
| Gmax | 1   | MV-----TVEEIRNAQRSHGPATILAFGTATPSNCVSQADYPDYYFRITNSEHMTDLKEK   |
| Mtru | 1   | MV-----TVEEIRKAQRSSGPATILAFGTATPSHCVTQAEPYDYYFRITNSEHMTDLKEK   |
| Atha | 1   | MVMAGASSLDEIRQAQRADGPAGILAI GTANPENHVLQAEYPDYYFRITNSEHMTDLKEK  |
| <br> |     |  |
| Lfil | 56  | FKRMCEKSMIRKRYMHLTEEILKENPAMCAYMAPSLDARQDLVVVEVPKLGKEAAAKAIK   |
| Ljap | 56  | FKRMCEKSMIRKRYMHLTEEILKENPAMCAYMAPSLDARQDLVVVEVPKLGKEAAAKAIK   |
| Gmax | 56  | FKRMCEKSMIKKRYMHLTEEEFLKENPNMCYMAPSLDVRQDVVVMEVPKLGKQAAAKAIK   |
| Mtru | 56  | FKRMCEKSMIKKRYMHLTEEEFLKENPNMCAYMAPSLDARQDLVVVEVPKLGKDAAKKAIA  |
| Atha | 61  | FKRMCDKSTIRKRHMHLTEEFLKENCNPHMCAYMAPSLDTRQDIVVVEVPKLGKEAAVKAIK |
| <br> |     |  |
| Lfil | 116 | EWGQPDKSKITHLVFCTTSGVDMPGADYQLTKLLGLKPSVKRLMMYQQGCFAGGTVLRLAK  |
| Ljap | 116 | EWGQPDKSKITHLVFCTTSGVDMPGADYQLTKLLGLKPSVKRLMMYQQGCFAGGTVLRLAK  |
| Gmax | 116 | EWGQPDKSKITHLVFCTTSGVDMPGADYQLTKLLGLRPSVKRLMMYQQGCFAGGTVLRLAK  |
| Mtru | 116 | EWGQPDKSKITHVVFCCTTSGVDMPGADYQLTKLLGLKPSVKRLMMYQQGCFAGGTVLRLAK |
| Atha | 121 | EWGQPDKSKITHVVFCCTTSGVDMPGADYQLTKLLGLRPSVKRLMMYQQGCFAGGTVLRIAK |
| <br> |     |  |
| Lfil | 176 | DLAENNKGARVLVVCSEITAVTFRGSDTHLDLSLVGQALFGDGAAAIIVGADPDIAV-ER   |
| Ljap | 176 | DLAENNKGARVLVVCSEITAVTFRGSDTHLDLSLVGQALFGDGAAAIIVGADPDIAV-ER   |
| Gmax | 176 | DLAENNKGARVLVVCSEITAVTFRGSDTHLDLSLVGQALFGDGAAAIIGSDPDPAV-ER    |
| Mtru | 176 | DLAENNKNARVLVVCSEITAVTFRGSDTHLDLSLVGQALFGDGAAAMIIGADPDIAV-ER   |
| Atha | 181 | DLAENNKGARVLVVCSEITAVTFRGSDTHLDLSLVGQALFSDGAAALIVGSDPDTSVGEK   |
| <br> |     |  |
| Lfil | 235 | PIFOLVSAGQTILPDSDGAIDGHLREVGLTFHLLKDVPGIISKHIEKSLSEAFAPIGISD   |
| Ljap | 235 | PIFOLVSAGQTILPDSDGAIDGHLREVGLTFHLLKDVPGIISKHIEKSLSEAFAPIGISD   |
| Gmax | 235 | PIFEMISAAQQTILPDSDGAIDGHLREVGLTFHLLKDVPGIISKNIEKSLVEAFEPIGISD  |
| Mtru | 235 | PIFEITVSAAQQTILPDSDGAIDGHLREVGLTFHLLKDVPGIISKNIEKSLVEAFAPIGISD |
| Atha | 241 | PIFEMVSAAQQTILPDSDGAIDGHLREVGLTFHLLKDVPGLISKIVKSLDEAFKPLGISD   |
| <br> |     |  |
| Lfil | 295 | WNSLFWIAHPGGPAILDQVEAKLRLKEEKLRSTRHVLGEYGNMSSACVLFILDEVRRRSK   |
| Ljap | 295 | WNSLFWIAHPGGPAILDQVEAKLRLKEEKLRSTRHVLGEYGNMSSACVLFILDEVRRRSK   |
| Gmax | 295 | WNSLFWIAHPGGPAILDQVEKLRLKPEKLQSTRHVLSEYGNMSSACVLFILDEMRKKS     |
| Mtru | 295 | WNSLFWIAHPGGPAILDQVEKLRLKEEKLRSTRHVLSEYGNMSSACVLFILDEMRRSK     |
| Atha | 301 | WNSLFWIAHPGGPAILDQVEIKLGLKEEKMRATHVLSEYGNMSSACVLFILDEMRRSA     |
| <br> |     |  |
| Lfil | 355 | EEGKETTGIDGLEWGVLFGFGPGLTVETVVLHSVPLEAX                        |
| Ljap | 355 | EEGKETTGIDGLEWGVLFGFGPGLTVETVVLHSVPLEAX                        |
| Gmax | 355 | EEGKSTTGEGLEWGVLFGFGPGLTVETVVLHSVPLEGX                         |
| Mtru | 355 | EEGKITTGEGLEWGVLFGFGPGLTVETVVLHSVPMQGX                         |
| Atha | 361 | KICGVATTGEGLEWGVLFGFGPGLTVETVVLHSVPLX--                        |

Figure S30. Amino acid alignment for PAP-1

Lfil 1 MDLETLYSPCFM---SNSNWFVQESAHNT**EWSREDNKRFESALAIY**-DKDTPDRWLNVAA  
Ljap 1 MDLETLYSPCFM---PNSNWFVQESAH**HSTEWSREDNKRFESALAIY**-DKDTPDRWLNVAA  
Gmax 1 M**ELETIYPPCFM**---PNSNWFVQES-H**STEWIREDNKKFESALAIY**-D**DTPDRWF**KVA  
Mtru 1 MDLETLYSPYFM---LD**NWLVEES**-Y**QTEWSREDNKKFESALAIY**-DKDTPDRWLKVAE  
Atha 1 MSSST**MYRGVNMFSPAN**TNWIFQEV-REATW**AENKRFEK**ALAYLDDKDNL**EWSWSKIAD**

Lfil 57 MIPGKTVL**DVIKQYRELEEDV**G**EIEAGH**V**PVPGYHSSS**-----FTFEVVENQNFD----  
Ljap 57 MIPGKTVL**DVIKQYRELEEDV**G**EIEAGH**V**PVPGYHSSS**-----FTFEVVENQNFD----  
Gmax 56 MIPGKTVF**DVIKQYRELEEDV**S**EIEAGR**V**PIPGYLASS**-----FTFE**LVDNHNYD**----  
Mtru 56 MIPGKTVF**DVIKQYRELVEDV**S**EIEAGN**V**PIPGYLASS**-----FTFEVVE**KQNYD**----  
Atha 60 I**IIPGKTVADVIKRYKELEDVSDIEAGLIP**IPPGY**GGDASSAANS**DYFF**GLENSSYGYDYV**

Lfil 107 --GL**KRKPGTT**--LR--GSDHERKKGV**PWTEEEHKRFLMGLLK**YGKGDWRNIARNFVMTK  
Ljap 107 --GL**KRKPGTT**--LR--GSDHERKKGV**PWTEEEHKRFLMGLLK**YGKGDWRNIARNFVMTK  
Gmax 106 --GCRRRL-AP--VR--GSD**QERKKGV**PWTE**DEHRRFLMGLLK**YGKGDWRNI**SRNFVVTK**  
Mtru 106 --GNRRRH-VT--VR--GSDHERKKGV**PWTEEEHRRFLMGLLK**YGKGDWRNI**SRNFVVTK**  
Atha 120 VGGKRSSPA**MTCFRSPMPE**KERKKGV**PWTEDEHLRFLMGLKK**YGKGDWRNI**AKSFVTTR**

Lfil 161 TPTQVASHAQ**KYYIRQKV**-SGGKD**KRRPSIHDITTVNL**TETSTT**SENNKPLSFK**----  
Ljap 161 TPTQVASHAQ**KYYIRQKV**-SGGKD**KRRPSIHDITTVNL**TETST**SENNKPLSFK**----  
Gmax 159 TPTQVASHAQ**KYYIRQKV**-SGGKD**KRRPSIHDITTVNL**TET**SASDKN-KPQLENASPVLA**  
Mtru 159 TPTQVASHAQ**KYYIRQKVSSGGKDKRRPSIHDITTVNL**TET**SSPSEN-KSLLVNVS**PMQQ  
Atha 180 TPTQVASHAQ**KYFLRQL**--TDGKD**KRRSSIHDITTVNIP**ADASATA-TTADVALSP---

Lfil 214 -----**STS**NYNSGS**LMVF-NPNCD**DL**IMMPSSSDIITS**KTL**LQGQDLYD**  
Ljap 214 -----**STS**NYNSGS**LMVF-NPNCD**DL**IMMPSSSDIITS**KTL**LQGQDLYD**  
Gmax 217 PQQKLNSIS**KVQLGWTS**SHYND**GSMVF-NPNSD**ALF**V-SSSPDVTS**MALK**MQGQDLYD**  
Mtru 218 -----KMGWST**SHYNDGS**-----PQGQDLYD  
Atha 234 -----**T**PAN--SF**DVFLQP**N-----\*

Lfil 258 CSLHEAYAK**IKIPS**FRA**APRN**FNKEAVFGI**HVL\***  
Ljap 258 CSLHEAYAK**SKIPS**FRA**APRN**FNKEAVFGI**HVL\***  
Gmax 274 CALHEAYAK**VKVGFSMAPRD**FNNE**EA**VFGI**HAL\***  
Mtru 239 CSFHEAYAK**IKVSGFATASRDF**FNKG**AVFGI**HAL\*  
Atha 247 --**PHYSF**A**SASAS**S**YYNAFPQWS**-----\*

Appendix 9

## Supplementary Figures S31 – S34 Amino acid alignments for non-differentially expressed lignin genes in *Lotus filicaulis*

Figure S31. Amino acid alignment for CCR

```

Lfil 1 -----
Ljap 1 -----
Gmax 1 MPT--DTSSVSG--EIVCVTGAGGIASWLVKILLLEKGYTVRGTVRNPDDPKNGHLKELE
Mtru 1 MPAYDNTSSVGGDQTVCVTGAGGIASWLVKILLERGYTVRGTVRNPDDPKNGHLKELE
Atha 1 MPV--DVASPAG--KTCVVTGAGGYIASWIVKILLERGYTVKGTVRNPDDPKNTTHLRELE

Lfil 1 -----
Ljap 1 -----
Gmax 57 GGKERLTLHKVDLFDIASIKAALHGCHGVFHTASPVTDNPEEMVEPAVKGTKNVIIAAAE
Mtru 61 GARERLTLHKVDLLLDLQSIQSVVHGCHGVFHTASPVTDNPDEMLEPAVNGBTKNVIIASAE
Atha 57 GGKERLILCKADLQDYEAALKAAIDGCDGVFHTASPVTDDPEOMVEPAVNAGAKFVINAAAAE

Lfil 1 -----
Ljap 1 -----
Gmax 117 AKVRRVVFTSISIGTVYMDPNTSRDALVDESFWSDLEYCKNTKNWYCYGKTVAEQAAWDVA
Mtru 121 AKVRRVVFTSISIGTVYMDPNTSRDVVDESYWSDLEHCKNTKNWYCYGKTVAEQAASWDIA
Atha 117 AKVKRVVIITSSISIGAVYMDPNRDPEAVDESCWSDLDFCKNTKNWYCYGKMVAEQAAWETA

Lfil 1 -----
Ljap 1 -----
Gmax 177 KERGVDLVVNPVLVIGPLLQOPTINASTIHIILKYLTGSAKTYVNATQAYVHVVDVALAH
Mtru 181 KENQVDLVVNPVVIIGPLLQOPTINASTIHIILKYLNGAAKTYVNATQSYYHVVDVALAH
Atha 177 KEKGVDLVVNPVLVIGPLLQOPTINASLYHVILKYLTGSAKTYANLTQAYVHVVDVALAHV

Lfil 1 -----
Ljap 1 -----
Gmax 237 LVYETPSASGRFICAESSLHRGEVVEILAKFFPEYPIPTKCSDEKNPRVKPYIFSNQKLK
Mtru 241 LVYETNSASGRYICCETALHRGEVVEILAKYFPEYPLPTKCSDEKNPRVKPYIFSNQKLK
Atha 237 LVYEAPSASGRYLLAESARHRGEVVEILAKLFPEYPLPTKCKDEKNPRAKPYKFTNQKLK

Lfil 15 DLGLEFTPVKQCLYETVKNLQEKGHLPPVLIN-----QQUEI-----I*
Ljap 21 DLGLEFTPVKQCLYETVMNLQEKGHLPPVLIN-----QQUEI-----I-
Gmax 297 DLGLEFTPVKQCLYDTVKNLQEKGHLPPVPPK-----QKDS-----Y*
Mtru 301 DLGLEFTPVKQCLYDTVRSLOQEKGHLPIPPM-----QEDS-----A*
Atha 297 DLGLEFTSTKOSLYDTVKSLQEKGHLAPPSSASQESVENGKIGS*
```

Figure S32. Amino acid alignment for F5H

Lfil 1 MDSLPTWQTIANLRHEQLPMAFLFMVPMILLLGLASRIRKRPYPPGPKGLPIIGNMLMM  
 Ljap 1 MDSLPTWQTIANLRHEQLPMAFLFMVPMILLLGLASRIRKRPYPPGPKGLPIIGNMLMM  
 Gmax 1 M-----ANLDLDPFQTTSILILVPIALLVALLSRTTRAPYPPGPKGLPIIGNMLMM  
 Mtru 1 MDSLKKSPIMENLKEEPFLMAIMFTVPLILLLGLVSRILKPRYPPGPIIGLPIIGNMLMM  
 Atha 1 MESSIONTQLSKLSDP--TTSLVIVVSIFITR-RFRPPYPPGPRGWPIIGNMLMM

Lfil 61 DQLTHRGLATLAKQYGGIFHRLRMGFTHMVAVSDPDAARQVLQVHDNIFSNRPATIAISYL  
 Ljap 61 DQLTHRGLATLAKQYGGIFHRLRMGFTHMVAVSDPDAARQVLQVHDNIFSNRPATIAISYL  
 Gmax 52 EQLTHRGLANLAKHYGGIFHRLRMGFTHMVAISDPAARQVLQVQDNIFSNRPATIAISYL  
 Mtru 61 DQLTHRGLANLAKKYGGIFHRLRMGFTHMVAISDADAARQVLQVQDNIFSNRPATIAISYL  
 Atha 57 DQLTHRGLANLAKKYGGIChLRMGFLHMYAVSSPEVARQVLQVQDSVFSNRPATIAISYL

Lfil 121 TYDRADMAFAHYGPFWRQMRKLCVMKLFSRKRAESWQSVRDEVDAAVRDVDQNTGT[PVN]  
 Ljap 121 TYDRADMAFAHYGPFWRQMRKLCVMKLFSRKRAESWQSVRDEVDAAVRDVDQNTGT[PVN]  
 Gmax 112 TYDRADMAFAHYGPFWRQMRKLCVMKLFSRKRAESWQSVRDEVDAAVRAVASSVGKPVN  
 Mtru 121 TYDRADMAFAHYGPFWRQMRKLCVMKLFSRKHAESWQSVRDEVDYAVRTVSDNIGNPVNI  
 Atha 117 TYDRADMAFAHYGPFWRQMRKVCMKVFSRKRAESWASVRDEVDKMVRSSCNVGKPINV

Lfil 181 GEVVFNLTKNIIYRAAFSSSKEGQDEFIGILQEFSKLFGAFNIADFIPYIGAIDPQGLN  
 Ljap 181 GEVVFNLTKNIIYRAAFSSSKEGQDEFIGILQEFSK[H]FGAFNIADFIPYIGAIDPQGLN  
 Gmax 172 GEIVFNLTKNIIYRAAFSSSQEGQDE-----LN  
 Mtru 181 GEIVFNLTKNIIYRAAFSSSREGQDEFIGILQEFSKLFGAFNISDFVPCFGAIDPQGLN  
 Atha 177 GEQIFALTENITYRAAFGSACEKGQDEFIRILQEFSKLFGAFNVADFIPYFGWIDPQGIN

Lfil 241 ARLVKARAALDSFIGKIIDEHVQKKRGGGGVGDGETDMVDELLAFYTEEEVAGSKGS  
 Ljap 241 ARLVKARAALDSFIGKIIDEHVQKKRGGGGVGDGETDMVDELLAFYTEEEVAGSKGS  
 Gmax 201 SRLARARGALDSFSDKIIDEHVHKMKNDKSSEIVDGETDMVDELLAFYSEEAK-----L  
 Mtru 241 ARLVKARKEELDSFIIDKIIDEHVQKKKS----VVD-EETDMVDELLAFYSEEAK-----L  
 Atha 237 KRLVKARNDLDGFIDDIIDEHMKKKENQNAVDGDVVDTDMVDDLLAFYSEEAK-----L

Lfil 300 SNESEDLQNSIKLTKDNKAIIMDVMFGGTETVASAIEWAISELMKTPPEELKRVQQELAD  
 Ljap 300 SNESEDLQNSIKLTKDNKAIIMDVMFGGTETVASAIEWAMSELMKTPPEELKRVQQELAD  
 Gmax 255 NNESDDLQNSIRLTKDNKAIIMDVMFGGTETVASAIEWAMAELMRSPEDQKRVQQELAD  
 Mtru 290 NNESDDLNNSIKLTKDNKAIIMDVMFGGTETVASAIEWAMAELMKSPEDLKKVQQELAE  
 Atha 292 VSETADLQNSIKLTDNIKAIIMDVMFGGTETVASAIEWALTELLSPEDLKRVQQELAE

Lfil 360 VVGLDRVVEESDFEKLTHLKCALKETLRLHPPIPLLLHESAEDTVGGYFIPKKARVMIN  
 Ljap 360 VVGLDRVVEESDFEKLTHLKCALKETLRLHPPIPLLLHETAEDVTGGYFIPKKARVMIN  
 Gmax 315 VVGLDRRAEESDFEKLTYLKCALKETLRLHPPIPLLLHETAEDATVGGYLVPKKARVMIN  
 Mtru 350 VVGLSRQVEEESDFEKLTYLKCALKETLRLHPPIPLLLHETAEEATVNGYFIPKQARVMIN  
 Atha 352 VVGLDRRVEESEDIKLTLYKCTLKETLRMHPPIPPLLHETAEDTSIDGEFIPKKSARVMIN

Lfil 420 VWAIGRDKNCWEEPDSFKPSRFLKPGVPDFKGSNFEFIPFGSGRRSCPGMQLGLYALDLA  
 Ljap 420 VWAIGRDKNCWEEPNSFKPSRFLKPGVPNFKGSNFEFIPFGSGRRSCPGMQLGLYALDLA  
 Gmax 375 AWAIGRDKNSWEEPESFKPARFLKPGVPDFKGSNFEFIPFGSGRRSCPGMVLGLYALELT  
 Mtru 410 AWAIGRDKANCWEEPQSFKPSRFLKPGVPDFKGSNFEFIPFGSGRRSCPGMQLGLYALDLA  
 Atha 412 AFAIGRDPSTSWTDPDTFRPSRFLEPGVPDFKGSNFEFIPFGSGRRSCPGMQLGLYALDLA

|      |     |  |
|------|-----|--|
| Lfil | 480 | VAHLLHCFTWELPDGMKPSEMDMSDVFGLTAPRASRLVAIPTKRVICPLS*  |
| Ljap | 480 | VAHLLHCFTWELPDGMKPSEMDMSDVFGLTAPRASRLVAIPTKRVVCPLS*  |
| Gmax | 435 | VAHLLHCFTWELPDGMKPSEMDM <b>G</b> DVFGLTAPR <b>S</b> IRL <b>I</b> A <b>V</b> PTKRVVCPLF*                            |
| Mtru | 470 | VAHLLHCFTWELPDGMKPSEMDMSDVFGLTAPRASRLVAIPTKRVICPLD*  |
| Atha | 472 | VAH <b>T</b> LHCFTW <b>K</b> LPDGMKPSE <b>I</b> DMNDVFGLTAP <b>K</b> ATRL <b>F</b> AVPT <b>T</b> RL <b>I</b> CAL-* |

Figure S33. Amino acid alignment for POD-2

Lfil 1 -----  
Ljap 1 MANSMSLLM-LLSLLAFAPLCCLCHKK--MGGYLYPQFYDYSCPQAKEIVKSIVANAVARE  
Gmax 1 MANSMSFFL-LLSLLAFAPLCCLCHYN--QE~~G~~GYLYPQFYDYSCPQVCHIVKSVI~~A~~KYVAEQ  
Mtru 1 MANSLSFLM-LLSLLAFAPFCCLCHKK--MGSYLYPQFYDYSCPQAQNIVKSILANAVAKE  
Atha 1 MAKSLNILIAALSLIAFSPFCLOSKAYGSGGYLF~~P~~QFYDQSCPKAQEIVQSIVAKAFEHD

Lfil 1 -----K  
Ljap 58 TRTAASILRLHFHDCFVKGCDAS~~I~~LLDGSGSFTSEKVSNPNRNSARGFEVIDEIKQALEK  
Gmax 58 PR~~I~~AASILRLHFHDCFVKGCDAS~~I~~LLDSSVNI~~I~~SEKG~~S~~NPNRNSARGFEVVDAIKAELER  
Mtru 58 PR~~I~~AASLLRLHFHDCFVKGCDAS~~I~~LLDN~~S~~GS~~I~~SEKG~~S~~NPNRNSARGFEVIDEIKYALEK  
Atha 61 PR~~M~~PASL~~L~~RHFHDCFVKGCDAS~~I~~LLDSSGT~~I~~SEKR~~S~~NPNRNSARGFELIFEIKH~~A~~LEQ

Lfil 2 ECPQVSCADILALAARDSTVLTGGPSWEVPLGRDSRS~~A~~SLSGSNNNI PAPNNTFQTIL  
Ljap 118 ECPQVSCADILALAARDSTVLTGGPSWEVPLGRDSRS~~A~~SLSGSNNNI PAPNNTFQTIL  
Gmax 118 KCPSTVSCADIL~~T~~LAARDS~~V~~L~~T~~GGPSWEVPLGRDS~~I~~LGAS~~I~~SGSNNNI PAPNNTFQTIL  
Mtru 118 ECPHTVSCADIL~~A~~ARDSTV~~L~~AGGP~~N~~WEVPLGRDS~~I~~LGASLSGSNNNI PAPNNTFQTIL  
Atha 121 ECPE~~T~~VSCADILALAARDSTV~~I~~TGGPSWEVPLGRD~~A~~RGASLSGSND~~I~~PAPNNTFQTIL

Lfil 62 TKFKLQGLNIVDLVALSGSHT-----  
Ljap 178 TKFKLQGLNIVDLVALSGSHTIGNSRC~~A~~SFRQRLYNQ~~T~~GNGKADFTLD~~P~~NYAAE~~L~~R~~T~~QCP  
Gmax 178 TKE~~N~~LQGLD~~I~~VDLVALSG~~G~~H~~T~~IGNARCT~~F~~K~~Q~~RLYNQ~~S~~GNG~~E~~PD~~S~~TLDQYYAA~~A~~TLRNRC~~P~~  
Mtru 178 TKFKLQGLDIVDLVALSGSHTIGK~~S~~RCTSFRQRLYNQ~~T~~GNGKQDF~~T~~LDQYYAAE~~L~~R~~T~~QCP  
Atha 181 TKFKRQGLD~~I~~VDLV~~S~~LSGSHTIGNSRCTSFRQRLYNQ~~S~~GNGKPD~~M~~TLSQYYAT~~I~~LR~~Q~~CP

Lfil 82 -----  
Ljap 238 KSGGDQNLVFLDPVTPTKFDNTYFKNL~~L~~AYKGL~~S~~DE~~I~~LLTANQESAQLVKLYAERNDL  
Gmax 238 SSGGDQNLFFLDYATPYKFDNSYET~~N~~L~~L~~AYKGL~~S~~SDQ~~V~~LFTMNQESAELVKLYAERNDI  
Mtru 238 RS~~G~~GDQNLFFLDY~~V~~TP~~T~~KFDNNYFKNL~~L~~AYKGL~~S~~DE~~I~~LLTKNQESAELVKLYAERNDL  
Atha 241 RSGGDQTLFFLD~~F~~ATE~~F~~KFDNHYFKNL~~I~~MYKGL~~S~~DE~~I~~LF~~T~~KNQSKELVELYAENQEA

Lfil 82 -----  
Ljap 298 FFEQFAKSMIMMGNISPLTGSKGEIRKNCRVIN---\*  
Gmax 298 FFEQFAKSMIKMGNISPLTN~~S~~KG~~E~~IRENCRRINA---\*  
Mtru 298 FFEQFAKSMIKMGNISPLTGSRGNIRTNCRVINTW-\*  
Atha 301 FFEQFAKSM~~I~~MGNISPLTGAKGEIRR~~I~~CRRVN~~H~~AY\*

Figure S34. Amino acid alignment for POD-1

|      |     |   |
|------|-----|---|
| Lfil | 1   | MVNSIKCFL-LLS-LAFVPFCHCKKEQ--GVNLYPQFYDQSCPKAQEIVKSIVAKAFAKD    |
| Ljap | 1   | MVNSIKCFL-LLS-LAFVPFCHCKKEQ--GVNLYPQFYDQSCPKAQEIVKSIVAKAFAKD    |
| Gmax | 1   | MVKSIINFLL-LLSLLAFVPSCHCKKIKI--GGYLYPQFYDGSCPRAQEIVQSIVAKAVAKE  |
| Mtru | 1   | MVNSINFLL-LFSLLVFAAPCCCHCKTKL--GDYLYPQFYDESCPKEEIVKSIVVAKAVTKE  |
| Atha | 1   | MAKSINILIAALSLIAFSPFCLOSKAYGSGGYLF PQFYDQSCPKAQEIVQSIVAKAFEHD   |
| <br> |     |   |
| Lfil | 57  | PRMAASLLRLHFHDCFVKGCDGSVLLDSSGTIISEKRSPNPNRNSARGFEVIDEIKSAIEK   |
| Ljap | 57  | PRMAASLLRLHFHDCFVKGCDGSVLLDSSGTIISEKRSPNPNRNSARGFEVIDEIKSAIEK   |
| Gmax | 58  | PRMAASLLRLHFHDCFVKGCDASVLLDSSGTIISEKRSPNPNRDSARGFEVIDEIKSALEK   |
| Mtru | 58  | PRMAASLLRLHFHDCFVKGCDASVLLDSSGTIISEKRSPNPNRNSARGFEVIDEIKSAVEK   |
| Atha | 61  | PRMPASLLRLHFHDCFVKGCDASILLDSSGTIISEKRSPNPNRNSARGFELIFEIKHALEQ   |
| <br> |     |   |
| Lfil | 117 | ECPHTVSCADILAIAARDSTVLTGGPSWGVPLGRDSLGAISGSNQNI PAPNNTFQTIL     |
| Ljap | 117 | ECPHTVSCADILAIAARDSTVLTGGPSWGVPLGRDSLGAISGSNQNI PAPNNTFQTIL     |
| Gmax | 118 | ECPHTVSCADILAALAAARDSTVLTGGPSWGVPLGRDSLGAISGSNNNI PAPNNTFQTIL   |
| Mtru | 118 | ECPQTIVSCADILITLAARDSTVLTGGPSWDVPLGRDSLGAISGSNNNI PAPNNTFQTIL   |
| Atha | 121 | ECPETVSCADILALAARDSTVITGGPSWEVPLGRDARGASLSGSNDIPAPNNTFQTIL      |
| <br> |     |   |
| Lfil | 177 | TKFKLKGLDIVDLVALSGSHТИGDSRCTSFRQRLYNQTGNGKADFTLEQSYAAKLRTQCP    |
| Ljap | 177 | TKFKLKGLDIVDLVALSGSHТИGDSRCTSFRQRLYNQTGNGKADFTLDQSYAAKLRTQCP    |
| Gmax | 178 | TKFKLKGLDIVDLVALSGSHТИGNSRCTSFRQRLYNQTGNGKADFTLDQVYAAELRTRCP    |
| Mtru | 178 | TKFKLKGLNIVDLVALSGSHТИGDSRCTSFRQRLYNQTGNGKSDFTLDQVYAAQLRTRCP    |
| Atha | 181 | TKFKRQGLDIVDLVSLSGSHТИGNSRCTSFRQRLYNQSGNGKPDMTLSQYYATILRQRC     |
| <br> |     |   |
| Lfil | 237 | RSGGDQNLVFVLDFTPVKFDDNNYFKNLLAFKGGLSSDEILLTDNKESADLVKKYAE RNDL  |
| Ljap | 237 | RSGGDQNLVFVLDFTPVKFDDNNYFKNLLAFKGGLSSDEILLTDNKESADLVKKYAE RNDL  |
| Gmax | 238 | RSGGDQNLVFVLDFTPVTFKFDNFYYKNLLANKGGLSSDEILLTKNQVSADLVKQYAE NNDL |
| Mtru | 238 | RSGGDQNLVFVLDFTPVKFDDNNYFKNLLANKGGLSSDEILLTKNQVSADLVKKYAE SNDL  |
| Atha | 241 | RSGGDQTLFELDFATPEFKFDNHYFKNLI MYKGGLSSDEILFTKNQSKELVELYAENQEA   |
| <br> |     |   |
| Lfil | 297 | FFEQFAKSMVKMGNITPLTGSRGEIRRICRRINN--*                           |
| Ljap | 297 | FFEQFAKSMVKMGNITPLTGSRGEIRRICRRINN--*                           |
| Gmax | 298 | FFEQFAKSMVKMGNITPLTGSRGEIRKNCRGINK--*                           |
| Mtru | 298 | FFEQFAKSMVKMGNITPLTGSRGEIRKRCRKINN--*                           |
| Atha | 301 | FFEQFAKSMVKMGNISPLTGAKGEIRRICRRVNHAY*                           |

## Appendix 10

Supplementary Figures S35 Amino acid alignments for red-differentially expressed lignin genes in *Lotus filicaulis*

Figure S35. Amino acid alignment for CAD

|      |     |  |   |
|------|-----|--|---|
| Lfil | 1   | MSNE-EGKLVCVTGASGYIASWIVKFLLEHGYTVRATVRDL                    | SNPNKVEHLVKLDVAKERL                                 |
| Ljap | 1   | MSNE-EGKLVCVTGASGYIASWIVKFLLEHGYTVRATVRDL                    | SNPNKVEHLVKLDVAKERL                                 |
| Gmax | 1   | MSNNAGKVVCVTGASGFIASWIVKFLLQRGYTVRATVRVYPSNLKKV              | HLVKLGAKERL   |
| Mtru | 1   | MMGG-EGKVVVCVTGANGFIASWIVKFLLQC                              | GYTVRATVRHPSNSEKVDHLVKLDGAKERL                      |
| Atha | 1   | MSSE-EKTVCVTGASGYIASWIVKLLLRGYTVA                            | KASVRDPNDPRKTEHLLALEGAERL                           |
| <br> |     |  |   |
| Lfil | 60  | QLFKADLLEEGSFDSVIQGCHGVFHVASPVLMFVEDPQAEILDPAVKGTLNVLKSCAKSP |   |
| Ljap | 60  | QLFKADLLEEGSFDSVIQGCHGVFHVASPVLMFVEDPQAEILDPAVKGTLNVLKSCAKSP |   |
| Gmax | 61  | QLFKADLLEEGSFDSVVEGCHGVFHTASPVRFV                            | VNDPQAEILDPAVKGTLNVLQSCAKSP                         |
| Mtru | 60  | QLFKADLLEEGSFDSVVEGCDG                                       | GVFHTASPVRFVNDPQAEILDPAVKGTLNVLQSCAKSP              |
| Atha | 60  | KLFKANLLEEGSFDSAIDGCEGVFHTASPFYHDVKDPQAEILDPAVKGTINVLSSCLKTS |   |
| <br> |     |  |   |
| Lfil | 120 | SVKRVVLTSSTSAVLFNGRPKSPEVVVDETWFSDPDFL                       | RRESKFFHKWY-----                                    |
| Ljap | 120 | SVKRVVLTSSTSAVLFNGRPKSPEVVVDETWFSDPDFL                       | RRESKV-----CV                                       |
| Gmax | 121 | SVKRVVLTSSISAVAFNRRPKTPQVVVDETWFSDPDFCRE                     | LEL---WYTLSKTLAEDAAW                                |
| Mtru | 120 | SVKRVILTSSISAVVFDTRPKNPVGIVVDETWFSDPDFCRESKL                 | LEL---WYTLSKTLAEEAAW                                |
| Atha | 120 | SVKRVVLTSSIAAVAFNGMPRTPETIVDETWFADPDYCRASKL                  | LEL---WYVLSKTLAENAAW                                |
| <br> |     |  |   |
| Lfil | 168 | -----  | TLSKTMA--E <del>DAA</del> --                        |
| Ljap | 165 | -----  | PFPNL <del>SIG</del> GWVDVKDVAKA                    |
| Gmax | 178 | KFVNENSIDMISINPTMVAGPLLQPE                                   | TNESVEPILNLINGK-PFPNKSFGWVDVKDVANA                  |
| Mtru | 177 | KFVNENSIDMVAINPTMVAGPLLQPE                                   | ELINGSVEPILNLISGI-PFPNKAYGWCNVKDvana                |
| Atha | 177 | KFAKENNLQI   | VSINPAMVIGPLLQPTLNTSAAAVALSLIKGAQTFPNATFGWVNVKDVANA |
| <br> |     |  |   |
| Lfil | 179 | -----  | WKFAK-  |
| Ljap | 183 | HIQAYEITASASGRYCLVERVAHF                                     | SELLEFYMICIQHCKEQISVWTMNHSQH--FNFHKK                |
| Gmax | 237 | HILAYEIASASGRYCLVERVIHYSELATILR                              | --GLYPTIQIPDKCEVDEPYIPTYQISTE                       |
| Mtru | 236 | HILAYETASASGRYCLAERVVHYSELAMILR                              | --DLYPTIQISDKCEDDGPYMPYQISKE                        |
| Atha | 237 | HIQAFENPDADGRYCLVERVAHYSE                                    | VNILH--DLYPDFQLEPKCADEKIYIPTYKVSKE                  |
| <br> |     |  |   |
| Lfil | 184 | -EN-KIDMVVILPSMVVGPLLRLPEVNFS-VEPV                           | LNIINGVPF-P-  |
| Ljap | 241 | EPR-AXESSLLXXXF  | -----RKPXKAS-EKFR-----LST--                         |
| Gmax | 295 | KAKKDLGIEFTPLEVSI  | -----RETVESFREKKI-----VNFPN*                        |
| Mtru | 294 | KAK-SLGIEFTSLEVTL  | -----KETVESFREKKI-----VNFP---*                      |
| Atha | 295 | KAE-SLGVEFVPLEVSI  | -----KETVESLRDKGF-----TREF---*                      |

## Appendix 11

Supplementary Figures S36 – S40 Amino acid alignments for yellow-differentially expressed lignin genes in *Lotus filicaulis*

Figure S36. Amino acid alignment for C3'H

|      |     |  |
|------|-----|--|
| Lfil | 1   | MSLLLAIPISLITLLLSYTLYQ---RLKFKLPPGPRPWPVVGPNLYDIKPVRFRCFAEWAQ  |
| Ljap | 1   | MSLLLAIPISLITLLLSYTLYQ---RLKFKLPPGPRPWPVVGPNLYDIKPVRFRCFAEWAQ  |
| Gmax | 1   | MALLLIVPISLVTLWLGYTLYQ---RLRFKLPPGPRPWPVVGPNLYDIKPVRFRCFAEWAQ  |
| Mtru | 1   | MSPLIIVFATIAAAIIYRLLNLITKPSLPPLPPGPSPWPIVGNLPHMGPVPHHALAVLAL   |
| Atha | 1   | MSWFILIA-VATIAAVVSYKLIQ---RLRYKFPPGPSPKPIVGNLHYDIKPVRFCYWEAQ   |
| Lfil | 58  | SYGPIISVWFGSTLNVVVSNSELAKEVLKEKDQQQLADRHSRSAAKFSRDGQDLIWADYG   |
| Ljap | 58  | SYGPIISVWFGSTLNVVVSNSELAKEVLKEKDQQQLADRHSRSAAKFSRDGQDLIWADYG   |
| Gmax | 58  | SYGPIISVWFGSTLNIVSNELAKEVLKEHDQQLADRHSRSAAKFSRDGKDQDLIWADYG  |
| Mtru | 61  | KHGP <del>LMH</del> RLGYVDVIVAAASASVAEQFLKVHD <del>T</del> NFSSRPTNSGAKYLAYNYQDLVEAPYG   |
| Atha | 57  | SYGPIISVWIGSI <del>LNVVVS</del> SSELAKEVLKEHDQKLADRHRNRSTEAFSRNGQDLIWADYG  |
| Lfil | 118 | PHYVKVRKVCTLELFSPKRLEALRPPIREDEVTAMVESVF <del>H</del> DCTNSENLGKGLLVKKYLGT   |
| Ljap | 118 | PHYVKVRKVCTLELFSPKRLEALRPPIREDEVTAMVESVF <del>H</del> DCTNSENLGKGLLVKKYLGT   |
| Gmax | 118 | PHYVKVRKVCTLEFTPKRLESLRPPIREDEVT <del>T</del> MVESV <del>N</del> HCTT <del>T</del> GNLGKAILVRKHLGS   |
| Mtru | 121 | PR <del>W</del> RLLRK <del>I</del> SYVHM <del>F</del> SSKALDEFRH <del>I</del> RQEVARLIRN <del>I</del> ASSGSKAVNLGQ-----MLNV  |
| Atha | 117 | PHYVKVRKVCTLEFTPKRLESLRPPIREDEVTAMVESVF <del>R</del> DCNLPE <del>N</del> RAKGLQLRKYLGA   |
| Lfil | 178 | VAFNNITRLA <del>F</del> GKRFVNSE---GV <del>I</del> DEQGVEFKAI <del>V</del> ANGLKLGASLAMA <del>E</del> HIPWL <del>R</del> WMFP  |
| Ljap | 178 | VAFNNITRQ <del>A</del> FGKRFVNSE---GV <del>I</del> DEQGVEFKAI <del>V</del> ANGLKLGASLAMA <del>E</del> HIPWL <del>R</del> WMFP  |
| Gmax | 178 | VAFNNITRLA <del>F</del> GKRFVNSE---GV <del>M</del> DEQGVEFKAI <del>V</del> ENGLKLGASLAMA <del>E</del> HIPWL <del>R</del> WMFP  |
| Mtru | 175 | CTTNALARVMIGR <del>V</del> NEGNGGCECDPRADEFKS <del>M</del> VVELMVLAGVFNI <del>GDFL</del> PAFE <del>W</del> LDL   |
| Atha | 177 | VAFNNITRLA <del>F</del> GKRFMNAE---GV <del>V</del> DEQGLEFKAI <del>V</del> S <del>N</del> GLKLGASL <del>S</del> IAE <del>H</del> IPWL <del>R</del> WMFP  |
| Lfil | 235 | L-EEE <del>E</del> FAKHGARRDRLTRAIMEEHTQARQKSGGAKQHFVDALLTLQDKYDLSEDT----  |
| Ljap | 235 | L-EEE <del>E</del> FAKHGARRDRLTRAIMEEHTQARQKSGGAKQHFVDALLTLQDKYDLSEDT----  |
| Gmax | 235 | L-EEGAFAKHGARRDRLTRAIMTEHTEAR <del>K</del> SGGAKQHFVDALLTLQDKYDLSEDT----   |
| Mtru | 235 | QGVQGKMKKLHKRFDTFLTS <del>I</del> E <del>H</del> RISKSE---KHNDL <del>I</del> STLL <del>S</del> KE <del>K</del> LPED <del>E</del> DTLNDT  |
| Atha | 234 | A-DEKAFAEHGARRDRLTRAIMEEHTLARQKSSGAKQHFVDALLTLKDQYDLSEDT----   |
| Lfil | 290 | -IIGLLWDMITAGMDTTAISVEWAMAE <del>L</del> IKNPRVQQKAQEE <del>L</del> DRVIGF <del>E</del> RVLTETDFSNL  |
| Ljap | 290 | -IIGLLWDMITAGMDTTAISVEWAMAE <del>L</del> IKNPRVQQKAQEE <del>L</del> DRVIGF <del>E</del> RVLTETDFSNL  |
| Gmax | 290 | -IIGLLWDMITAGMDTTAISVEWAMAE <del>L</del> IRNPRVQQKVQEELDRVIGL <del>E</del> RVMTB <del>A</del> DFSSL  |
| Mtru | 292 | EIK <del>A</del> LLN <del>I</del> FTAGTD <del>T</del> TA <del>S</del> TEWAI <del>S</del> ELIKNP <del>K</del> IMAC <del>Q</del> ELDTV <del>V</del> GRDRLV <del>T</del> EQDL <del>T</del> NL   |
| Atha | 289 | -IIGLLWDMITAGMDTTA <del>I</del> TA <del>E</del> WAMAE <del>M</del> IKNPRVQQKVQE <del>E</del> DRV <del>V</del> GLD <del>R</del> ILTEADFSRL  |
| Lfil | 349 | PYLQCV <del>A</del> KEAMRLHPPTPLMLPHRS <del>N</del> ANVKIGGYDIPKG <del>S</del> NVHVNVWA <del>V</del> ARDPAWKNPL  |
| Ljap | 349 | PYLQCV <del>A</del> KEAMRLHPPTPLMLPHRS <del>N</del> ANVKIGGYDIPKG <del>S</del> NVHVNVWA <del>V</del> ARDPAWKNPL  |
| Gmax | 349 | PYLQCV <del>I</del> KEAMRLHPPTPLMLPHR <del>A</del> ANVK <del>V</del> GGYDIPKG <del>S</del> NVHVNVWA <del>V</del> ARDPAW <del>K</del> DPL   |
| Mtru | 352 | PYL <del>E</del> AVV <del>K</del> E <del>T</del> RLHP <del>S</del> TPL <del>S</del> LP <del>R</del> VATQS <del>C</del> E <del>I</del> FN <del>Y</del> HIPKG <del>A</del> TL <del>V</del> NV <del>W</del> AT <del>S</del> RDP <del>K</del> EWSNPL |

Atha 348 PYLQCVVKESFRLHPPTPLMLPHRSNADVKIGGYDIPKGSNVHVNVAVARDPAWKNPF

|      |     |   |      |  |
|------|-----|---|------|--|
| Lfil | 409 | EFRPERFLE   | ---- | EDVDMKGHDFRLLPFGAGRRVCPGAQLGINLVTSMLGHLLHHFCWAP  |
| Ljap | 409 | EFRPERFLE   | ---- | EDVDMKGHDFRLLPFGAGRRVCPGAQLGINLVTSMLGHLLHHFCWAP  |
| Gmax | 409 | EFRPERFLE   | ---- | EDVDMKGHDFRLLPFGAGRRVCPGAQLGINLVTSMLGHLLHHFCWTP  |
| Mtru | 412 | EF <span style="background-color: black; color: black;">K</span> PERFLPGGEKF <span style="background-color: black; color: black;">D</span> V <span style="background-color: black; color: black;">V</span> R <span style="background-color: black; color: black;">G</span> NDFE <span style="background-color: black; color: black;">V</span> TFPGAGRR <span style="background-color: black; color: black;">I</span> CAGMSL <span style="background-color: black; color: black;">G</span> LRMV <span style="background-color: black; color: black;">Q</span> LTAT <span style="background-color: black; color: black;">I</span> A <span style="background-color: black; color: black;">A</span> YDWEL |      |  |
| Atha | 408 | EFRPERFLE   | ---- | EDVDMKGHDFRLLPFGAGRRVCPGAQLGINLVTSMM <span style="background-color: black; color: black;">S</span> HLLHHFWTP |

|      |     |  |
|------|-----|--|
| Lfil | 465 | PEGVKPEEIDMVENPGLVTYMRTPVQAVATPRLPSHLYKRV <span style="background-color: black; color: black;">P</span> ADI*   |
| Ljap | 465 | PEGVKPEEIDMVENPGLVTYMRTPVQAVATPRLPSHLYKRV <span style="background-color: black; color: black;">P</span> ADI*   |
| Gmax | 465 | PEGVKPEEIDMGENPGLVTYMRTP <span style="background-color: black; color: black;">I</span> QALASPRLPSHLYKRV <span style="background-color: black; color: black;">P</span> AEI*   |
| Mtru | 472 | ENGVSPEK <span style="background-color: black; color: black;">I</span> NMDEAYGLTLQR <span style="background-color: black; color: black;">R</span> AVPILAHPRPRLS <span style="background-color: black; color: black;">P</span> HLYL-----*   |
| Atha | 464 | PQGT <span style="background-color: black; color: black;">K</span> PEEIDMSEN <span style="background-color: black; color: black;">P</span> GLVTYMRTPVQAVATPRLPS <span style="background-color: black; color: black;">D</span> LYKRV <span style="background-color: black; color: black;">P</span> YDM* |

Figure S37. Amino acid alignment for CAD

|      |     |   |
|------|-----|---|
| Lfil | 1   | MGSLE-AERTTVGWAARDPSGILSPYTFTLRNTGPDDVYIKVHYCGVCHTDVHQVKNDLG  |
| Ljap | 1   | MGSLE-AERTTVGWAARDPSGILSPYTFTLRNTGPDDVYIKVHYCGVCHTDVHQVKNDLG  |
| Gmax | 1   | MGSLE-AERTTVGLAARDPSGILSPYTNYLRNTGPDDVYIKVHYCGICHSDLHQIKNDLG  |
| Mtru | 1   | MGSLEVAERTTVGLAARDPSGILTPYTTLRNTGPDDVYIKIHYCGVCHSDLHQIKNDLG   |
| Atha | 1   | MGIME-AERKTTGWAARDPSGILSPYTYTTLRETGPEDVNIRIICCGICHTDLHQTKNDLG |
| <br> |     |   |
| Lfil | 60  | MSNYPMVPGEVVGEVLEVGSVDVTRFTVGEIVGAAGLLVGCCCKCTACQSDIEQYCKKKIW |
| Ljap | 60  | MSNYPMVPGEVVGEVLEVGSVDVTRFTVGEIVGAAGLLVGCCCKCTACQSDIEQYCKKKIW |
| Gmax | 60  | MSNYPMVPGEVVGEVLEVGSVDNSRFRVGEIVGVGLLVGCCCKNCQPCQODIENYCSKKIW |
| Mtru | 61  | MSNYPMVPGEVVGEVLEVGSNVTRFKVGEIVGVGLLVGCCCKSCRACDSEIEQYCNKKIW  |
| Atha | 60  | MSNYPMVPGEVVGEVVEVGSDVSKFTVGDIVGVGCLVGCCGGCSPCERDLEQYCPKKIW   |
| <br> |     |   |
| Lfil | 120 | NYNDVYVDGKPTQGGFAETIVVEQKFVVKIPEGMAPEQVAPLLCAAVTVYSPLSHFGLKE  |
| Ljap | 120 | NYNDVYVDGKPTQGGFAETIVVEQKFVVKIPEGMAPEQVAPLLCAAVTVYSPLSHFGLKE  |
| Gmax | 120 | SYNDVYVDGKPTQGGFAETMVEQKFVVKIPEGLAPEQVAPLLCAGVTVYSPLVHFGLKE   |
| Mtru | 121 | SYNDVYTDGKTTQGGFAESTVVEQKFVVKIPEGLAPEQVAPLLCAGVTVYSPLSHFGLKT  |
| Atha | 120 | SYNDVYINGQOPTQGGFAKATVVHQKFVVKIPEGMAVEQAAPLLCAGVTVYSPLSHFGLKQ |
| <br> |     |   |
| Lfil | 180 | SGLRGGILGLGGVGHMGVIIIAKAMGHHTVISSSDRKKEAIEDLGADAYLVSSDTTSMQ   |
| Ljap | 180 | SGLRGGILGLGGVGHMGVIIIAKAMGHHTVISSSDRKKEAIEDLGADAYLVSSDTTSMQ   |
| Gmax | 180 | SGLRGGILGLGGVGHMGVKIAKALGHHTVISSSDKKKEALEHHLGADQYLVSSDTVTAMQ  |
| Mtru | 181 | PGLRGGILGLGGVGHMGVKVAKAFGHHTVISSSDKKKEALEDLGADSYLVSSDTVGMO    |
| Atha | 180 | PGLRGGILGLGGVGHMGVKIAKAMGHHTVISSSNKKREALQDLGADDYVIGSDQAKMS    |
| <br> |     |   |
| Lfil | 240 | EAADSLDYIIDTVPVGHPLEPYLSLLKLDGKLILMGVINTPLQFITPMVMLGRRSITGSF  |
| Ljap | 240 | EAADSLDYIIDTVPVGHPLEPYLSLLKLDGKLILMGVINTPLQFITPMVMLGRRSITGSF  |
| Gmax | 240 | EAADSLDYIIDTVPVGHPLEPYLSLLKLDGKLILMGVINTPLQFVSPMVMLGRRSITGSF  |
| Mtru | 241 | EAADSLDYIIDTVPVGHPLEPYLSLLKDQKLIIMGVINTPLQFVTPMVMLGRRSITGSF   |
| Atha | 240 | EIADSLDYVIDTPVPHALEPYLSLLKLDGKLILMGVINNPLQFITPLIMLGEKVITGSF   |
| <br> |     |   |
| Lfil | 300 | IGSIKETEEMLEFWKEKGLTSMIEIVNMDYINKAFERLEKNDVRYRFVVDVKGSKLIDQ*  |
| Ljap | 300 | IGSIKETEEMLEFWKEKGLTSMIEIVNMDYINKAFERLEKNDVRYRFVVDVKGSKLIDQ*  |
| Gmax | 300 | IGSMKETEEMLEFWKEKGLSSMIEIVNMDYINKAFERLEKNDVRYRFVVDVKGSKLVD-*  |
| Mtru | 301 | VGSVKETEEMLEFWKEKGLSSMIEIVTMDYINKAFERLEKNDVRYRFVVDVKGSKFED-*  |
| Atha | 300 | IGSMKETEEMLEFCKEKGLSIIIEVKMDYNTAFLERLEKNDVRYRFVVDVEGSNLDA-*   |

Figure S38. Amino acid alignment for CCoA-OMT

|      |     |   |  |
|------|-----|---|--|
| Lfil | 1   | -----   |  |
| Ljap | 1   | -----   |  |
| Gmax | 1   | MTL-----IKELEQQPNQIAGHKELAHKSLLQSDALYQYILETSVYPREHESLKE   |  |
| Mtru | 1   | MAI-----NNG-BEQQNQLIGDADLAHKTLQSDALYQYILDTSVFPREHPCLKE  |  |
| Atha | 1   | MATTTTEATKTSSTNGEDQKQSQNLRHQEVGHKSSLQSDLYQYILETSVYPREPESMKE   |  |
| <br> |     |   |  |
| Lfil | 1   | -----   |  |
| Ljap | 1   | -----   |  |
| Gmax | 51  | LRELTEKHPWNLMATPPDEGQLLGMLLKLI <sub>N</sub> AKNTMEIGVFTGYSLLSTALALPSDG---   |  |
| Mtru | 50  | LREMTEKHPRNHMAIPGDEGQLISMLVKLI <sub>N</sub> AKNTLEIGVYTGYSLLSTALALPSDGKVL   |  |
| Atha | 61  | LREVTAKHPWNIMTSADEGQFLNMLIKLVNAKNTMEIGVYTGYSLLATALALPEDGKIL   |  |
| <br> |     |   |  |
| Lfil | 1   | -----ILGLPIIEKAGVAHKIDFREGPALPVLDLLEDENNKGTTFDFVFVDADKDN  |  |
| Ljap | 1   | -----SFLWML-----I   |  |
| Gmax | 108 | -----KAGVAHKIDFREGPALPLLDQLIKDEKNKGAFDFIYVDADKDN  |  |
| Mtru | 110 | ALDKSREYYE <sub>L</sub> GLP <sub>M</sub> IEKAGVAHKIDFRQG <sub>I</sub> ALPLLDELIQDENNKGTTFDFVFVDADKNN                                      |  |
| Atha | 121 | AMDVNREN <sub>Y</sub> E <sub>L</sub> GLP <sub>I</sub> IEKAGVAHKIDFREGPALPVLD <sub>I</sub> VADEKNHGT <sub>Y</sub> DF <sub>I</sub> FVDADKDN |  |
| <br> |     |   |  |
| Lfil | 52  | YLNYHKRVIELVKVGG <sub>I</sub> GEDNTLWNGSVVSAPDAPMMMDYLHYRGYVIELNKYLAQDSR  |  |
| Ljap | 8   | RMNYHKRVIELVKVGG <sub>I</sub> GEDNTLWNGSVVSAPDAPMMMDYLHYRAYVIELNKYLAQDSR  |  |
| Gmax | 151 | YLNYHKRVIELVKVGG <sub>I</sub> GYDNTLWNGSVVAPPDAPLMDYVKYYRD <sub>E</sub> VMELNKALALDSR   |  |
| Mtru | 170 | YLNYHVRMIDL <sub>D</sub> LKVGG <sub>I</sub> GYDNTLWSGSVAAPPDAPMMDFIRNLRCGYVIEFNKHIAQDSR   |  |
| Atha | 181 | Y <sub>T</sub> INYHKRLIDLVKIGG <sub>I</sub> GYDNTLWNGSVVAPPDAPMRKYVRYYRD <sub>E</sub> VLELNKAADPR   |  |
| <br> |     |   |  |
| Lfil | 112 | IEICQLPVGDGITLCRRRI*  |  |
| Ljap | 68  | IEICQLPVGDGITLCRRRI-  |  |
| Gmax | 211 | VEICQLPVGDGITLCRRRI*  |  |
| Mtru | 230 | IEICQLSIA <sub>D</sub> GITLCRRRIK*  |  |
| Atha | 241 | IEICMLPVGDGITICRRRI*  |  |

Figure S39. Amino acid alignment for CCR-2

|      |     |   |
|------|-----|---|
| Lfil | 1   | MPAAAES---PSPTGETICVTGAGGGFIASWMVKLLLQKGYTVRGTVRNPDDPKNGHLR     |
| Ljap | 1   | MPAA-----AGETICVTGAGGGFIASWMVKLLLQKGYTVRGTVRNPDDPKNGHLR         |
| Gmax | 1   | MPSAEA-----SSETICVTGAGGGFIASWMVKLLLQKGYTVRGTVRNPDDPKNGHLK       |
| Mtru | 1   | MPAAATAAAAESSSVSGETICVTGAGGGFIASWMVKLLLQKGYTVRGTVRNPDDPKNGHLK   |
| Atha | 1   | MPVDVA-----SPAGKTVCTGAGGYIASWIVKILLERGYTVKGTVRNPDDPKNTHLR       |
| <br> |     |   |
| Lfil | 58  | ELEGASDRLTLIKV DLLNSVRAAVHGS HGVFHTASPVTDNPEEMVEPAVNGAKN VIIA   |
| Ljap | 50  | ELEGASDRLTLIKV DLLNSVRAAVHGS HGVFHTASPVTDNPEEMVEPAVNGAKN VIIA   |
| Gmax | 52  | ELEGASERLTLHKV DLLNSVRAAVHGS HGVFHTASPVTDNPEEMVEPAVNGAKN VIIA   |
| Mtru | 61  | KLEGAKERLTLVKV DLLNSVRAAVHGS HGVFHTASPVTDNPEEMVEPAVNGAKN VIIA   |
| Atha | 54  | ELEGGKERLILCKADLQDYEAALKAAIDGCDGVFHTASPVTDPEDPEQMVEPAVNGAKFVINA |
| <br> |     |   |
| Lfil | 118 | AAEAKVRRVVFTSSIGAVYMDPKRSVDSVVDESCWSDEFCKD TKNWYCYGKAVAEQAAW    |
| Ljap | 110 | AAEAKVRRVVFTSSIGAVYMDPKRSVDSVVDESCWSDEFCKD TKNWYCYGKAVAEQAAW    |
| Gmax | 112 | AAEAKVRRVVFTSSIGAVYMDPSRSIDLVVDESCWSDEFCKD TKNWYCYGKAVAEQAAW    |
| Mtru | 121 | GAEAKVRRVVFTSSIGAVYMDPNRSV DVEVDESCWSDEFCKKTKNWYCYGKAVAEAAAW    |
| Atha | 114 | AAEAKVKR VVITSSIGAVYMDPNRDPEAVVDESCWSLDIFCKNTKNWYCYGKMVAEQAAW   |
| <br> |     |   |
| Lfil | 178 | DTAKEKGVDLVVVNPVLVLGPLLQOPTINASTHILKYLTGSAKTYANATQAYADVRDVAL    |
| Ljap | 155 | -----ANATQAYADVRDVAL  |
| Gmax | 172 | DTAKE NGVDLVVVNPVLVLGPLLQOPTINASTHILKYLTGSAKTYANATQAYVHVRDVAL   |
| Mtru | 181 | DVAKEKGVDLVVVNPVLVLGPLLQOPTINASTHILKYLTGSAKTYANATQAYVHVRDVAL    |
| Atha | 174 | ETAKEKGVDLVVNPVLVLGPPLQOPTINASLYHVLKYLTGSAKTYANLTQAYVDVRDVAL    |
| <br> |     |   |
| Lfil | 238 | AHILVYEKPEASGRYICSESSLHRGEELVEILAKHFPEYPMPTKCKDEKNPRAKPYIFSNO   |
| Ljap | 170 | AHILVYERPEASGRYICSESSLHRGEELVEILAKHFPEYPMPTKCKDEKNPRAKPYIFSNO   |
| Gmax | 232 | AHILVYEKPSASGRYLCAESSSLHRGEELVEILAKYFPEYPMPTKCSDEKNPRAKPYTFSNO  |
| Mtru | 241 | AHILVYEKPSASGRYLCAETSLHRGEELVEILAKYFPEYPMPTKCSDEKNPRAKPYHIFSNI  |
| Atha | 234 | AHV LVYEAPSASGRYLLAESARHRGEVVEILAKLFPEYPMPTKCKDEKNPRAKPYKFTNQ   |
| <br> |     |   |
| Lfil | 298 | KLKDLGLEFTPVS HSLYETVKSLQEKGHLT--IPK---QEDSI AVKS*              |
| Ljap | 230 | KLKDLGLEFTPVS HSLYETVKSLQEKGHLT--IPK---QEDSI AVKS*              |
| Gmax | 292 | KLKDLGLEFTPVS QCLYETVKSLQEKGHLP--VPAKQQEEDSTTVKS*               |
| Mtru | 301 | KLKDLGLEFTPVS ECLYETVKSLQDQGHLS--IPN---KEDSLAVKS*               |
| Atha | 294 | KTKDLGLEFTSTKQSLYTHRKNPW KMLRSGLEK---LINSLKYP*                  |

Figure S40. Amino acid alignment for CCR-1

Lfil 1 -----  
Ljap 1 -----  
Gmax 1 MPT--DTSSVSG-E IVCVTGAGGFIAASWLVKILLKGYTVRGTVRNPDDPKNGHLKELE  
Mtru 1 MPAYDNTSSVSGGDQTVCVTGAGGFIAASWLVKILLERGYTVRGTVRNPEDPKNGHLKELE  
Atha 1 MPV--DVASPAG-KTVCVTGAGGYIAASWIVKILLERGYTVKGTVRNPDDPKNTHLRELE

Lfil 1 -----  
Ljap 1 -----  
Gmax 57 GGKERLTLHKVDLFDIASIKAALHGCHGVFHTASPVTDNPEEMVEPAVKGTKNVIIAAAE  
Mtru 61 GAFERLTLHKVDLLDLQSIQSVVHGCHGVFHTASPVTDNPEMLEPAVNGTKNVIIASAE  
Atha 57 GGKERLILCKADLQDYEAALKAAIDGCDGVFHTASPVTDDPEQMVEPAVNGAKFVINAAAE

Lfil 1 -----  
Ljap 19 TKVQRVVFVTSSIGTVYMDPNRSRDEVVDESCWSDLEYCKNTKNWYCYGKTVAEQVAWEAA  
Gmax 117 AKVRRVVFVTSSIGTVYMDPNTSRDAVDESFWSDLEYCKNTKNWYCYGKTVAEQAADVA  
Mtru 121 AKVRRVVFVTSSIGTVYMDPNTSRDVVDESYSWSDEHCKNTKNWYCYGKTVAEQSAWDIA  
Atha 117 AKVKRKKVITSSIGAVYMDPNRDPFAVVDESCWSDLDFCKNTKNWYCYGKVMVAEQAAWETA

Lfil 1 -----VDVKDAALAHV  
Ljap 79 KEKGVDLVVVNPVLVIGPLLOPTINASTIHLKYLTGSAKTYVNATQAYVDVKDAALAHV  
Gmax 177 KERGVVDLVVVNPVLVIGPLLOPTINASTIHLKYLTGSAKTYVNATQAYVHVVDVALAHI  
Mtru 181 KENQVDLVVVNPVVLIGPLLOPTINASTIHLKYLNAAKTYVNATOSYYVHVKDVALAHL  
Atha 177 KEKGVDLVVVNPVLVIGPLLOPTINASLYHVLKYLTGSAKTYANLTQAYVDVFDVALAHV

Lfil 12 LVYETPSASGRYICAESSLHRGELVEILAKYFPEYPIPTKCSDEKNPRVKPYIIFSNQKLK  
Ljap 139 LVYETPSASGRYICAESSLHRGELVEILAKYFPEYPIPT-----  
Gmax 237 LVYETPSASGRFICAESSLHRGELVEILAKYFPEYPIPTKCSDEKNPRVKPYIIFSNQKLK  
Mtru 241 LVYETNSASGRYICCTALHRGEVVEILAKYFPEYPIPTKCSDEKNPRVKPYKFSNQKLK  
Atha 237 LVYEAPSASGRYLAEASARHRGEVVEILAKLFPEYPIPTKCKDEKNPRAKPYKFTNQKIK

Lfil 72 DLGLEFTPV-----  
Ljap -----  
Gmax 297 DLGLEFTPVKQCLYDTVKNLQENGHLPPPKQKDS-----Y\*  
Mtru 301 DLGLEFTPVKQCLYDTVRSLQEKGHLPPIPMMQEDS-----A\*  
Atha 297 DLGLEFTSTKQSLYDTVKSLQEKGHAPPSSASQESVENGIKIGS\*

## Appendix 12

Supplementary Figures S41 – S47 Amino acid alignments for yellow-differentially expressed carotenoid genes in *Lotus sessilifolius*

Figure S41. Amino acid alignment for CRTISO

|      |     |   |
|------|-----|---|
| Lses | 1   | VKGARVLVLEKYVIPGGSSGFYQRDGTYFDVGSSVMFGSDKGNLNLTQALEAVGC <span style="background-color: black; color: black;">QMQ</span>   |
| Ljap | 1   | VKGARVLVLEKYVIPGGSSGFYQRDGTYFDVGSSVMFGSDKGNLNLTQALEAVGCK <span style="background-color: black; color: black;">MQ</span>   |
| Gmax | 1   | VKGARVLVLEKYVIPGGSSGFYQRDGTYFDVGSSVMFGSDKGNLNLTQALEAVGCR <span style="background-color: black; color: black;">MQ</span>   |
| Mtru | 1   | VKGAK <span style="background-color: black; color: black;">V</span> LVLEKYVIPGGSSGFYHR <span style="background-color: black; color: black;">D</span> GYTDVGSSVMFGSDKGNLNLTQALEAVGCR <span style="background-color: black; color: black;">MQ</span>  |
| Atha | 1   | VKE <span style="background-color: black; color: black;">A</span> RVLVLEKYL <span style="background-color: black; color: black;">I</span> PGGSSGFYERDGYT <span style="background-color: black; color: black;">D</span> VGSSVMFGSDKGNLNLTQALK <span style="background-color: black; color: black;">A</span> VGRK <span style="background-color: black; color: black;">ME</span>  |
| <br> |     |   |
| Lses | 61  | VVPDPTTVHFHLPNNLSVRVHREYDKFIEELTCYFPHEKEGILKFYGECKWIFNALNSLE  |
| Ljap | 61  | VVPDPTTVHFHLPNNLSVRVHREYDKFIEELTCYFPHEKEGILKFYGECKWIFNALNLLE  |
| Gmax | 61  | VVPDPTTVHFHLPNNLSVRVH <span style="background-color: black; color: black;">K</span> EYDKFIEELTSYFPHEKEGILKFYGECKWIFNALNSLE  |
| Mtru | 61  | VI <span style="background-color: black; color: black;">P</span> DPTTVHFHLPNNHLSVRVHREYDKFIEELTSYFPHEK <span style="background-color: black; color: black;">D</span> GILKFYGECKWIFNALNSLE   |
| Atha | 61  | VI <span style="background-color: black; color: black;">P</span> DPTTVHFHLPNNLSVR <span style="background-color: black; color: black;">T</span> HREYDD <span style="background-color: black; color: black;">F</span> IAELTSK <span style="background-color: black; color: black;">F</span> PHEKEGILGFYGD <span style="background-color: black; color: black;">C</span> WKIFNS <span style="background-color: black; color: black;">L</span> NSLE  |
| <br> |     |   |
| Lses | 121 | LKSLEEPYLFQFFFQKPLECLTLAYYL <span style="background-color: black; color: black;">P</span> QNAGAIARKYI <span style="background-color: black; color: black;">Q</span> DPQLLSFIDAECFIVSTVN   |
| Ljap | 121 | LKSLEEPYLFQFFFQKPLECLTLAYYL <span style="background-color: black; color: black;">P</span> QNAGAIARKYI <span style="background-color: black; color: black;">Q</span> DPQLLSFIDAECFIVSTVN   |
| Gmax | 121 | LKSLEEPYLFQFFF <span style="background-color: black; color: black;">R</span> QKPLECLTLAYYL <span style="background-color: black; color: black;">P</span> QNAGAIARKYI <span style="background-color: black; color: black;">Q</span> DPQLLSFIDAECFIVSTVN  |
| Mtru | 121 | LKSLEEPYLFQFFFQKPLECLTLAYYL <span style="background-color: black; color: black;">P</span> QNAGAIARKYI <span style="background-color: black; color: black;">Q</span> DPQLLSFIDAECFIVSTVN   |
| Atha | 121 | LKSLEEPY <span style="background-color: black; color: black;">I</span> YLFQFFFQKPLECLTLAYYL <span style="background-color: black; color: black;">P</span> QNAGAIARKYI <span style="background-color: black; color: black;">Q</span> DPQLLSFIDAECFIVSTVN   |
| <br> |     |   |
| Lses | 181 | ALQTPMINAAMVLCDRHFGGINYPLGGVGGIAKSLAKGL <span style="background-color: black; color: black;">I</span> DQGSEILYKANVTS <span style="background-color: black; color: black;">V</span> IEQG   |
| Ljap | 181 | ALQTPMINAAMVLCDRHFGGINYPLGGVGGIAKSLAKGL <span style="background-color: black; color: black;">I</span> DQGSEILYKANVTS <span style="background-color: black; color: black;">V</span> IEQG   |
| Gmax | 181 | ALQTPMINAAMVLCDRHFGGINYPLGGVGGIAKSLAKGL <span style="background-color: black; color: black;">V</span> DQGSEI <span style="background-color: black; color: black;">V</span> KANVTS <span style="background-color: black; color: black;">V</span> IEQG  |
| Mtru | 181 | ALQTPMINAS <span style="background-color: black; color: black;">M</span> VLCDRHFGGINYPLGGVGGIAKSLAKGL <span style="background-color: black; color: black;">V</span> DQGS <span style="background-color: black; color: black;">Q</span> ILYKANVTS <span style="background-color: black; color: black;">I</span> IEQG   |
| Atha | 181 | ALQTPMINAS <span style="background-color: black; color: black;">M</span> VLCDRH <span style="background-color: black; color: black;">Y</span> GGINYP <span style="background-color: black; color: black;">V</span> GGVGGIAKSLAE <span style="background-color: black; color: black;">G</span> LVDQGSEI <span style="background-color: black; color: black;">Q</span> YKANV <span style="background-color: black; color: black;">K</span> SI <span style="background-color: black; color: black;">I</span> LDHG  |
| <br> |     |   |
| Lses | 241 | KAVGVRLSDGREFFAKTIISNATRWDT-----  |
| Ljap | 241 | KAVGVRLSDGREFFAKTIISNATRWDTFG-----  |
| Gmax | 241 | KAVGVRLS <span style="background-color: black; color: black;">N</span> GREFFAKTIISNATRWDTFGKLL <span style="background-color: black; color: black;">K</span> GVP <span style="background-color: black; color: black;">L</span> PKEEENFQKVYVKAPSFLSIHMG  |
| Mtru | 241 | KAVGVRLSDGREFFAKTIISNATRWDTFGKLV <span style="background-color: black; color: black;">K</span> GESLPKEEENFQKVYVKAPSFLSIHMG  |
| Atha | 241 | KAVGVRLADGREFFAKTIISNATRWDTFGKLL <span style="background-color: black; color: black;">K</span> GEKLPKEEENFQKVYVKAPSFLSIHMG  |
| <br> |     |   |
| Lses | 270 | -----   |
| Ljap | 270 | -----   |
| Gmax | 301 | VKAEVLPPTDCHHFVLE <span style="background-color: black; color: black;">S</span> N <span style="background-color: black; color: black;">W</span> K <span style="background-color: black; color: black;">L</span> EEPYGSIFLSIPT <span style="background-color: black; color: black;">V</span> LDSSLAP <span style="background-color: black; color: black;">E</span> GRHILHIFTSS <span style="background-color: black; color: black;">M</span> E   |
| Mtru | 301 | VKAEVLPPTDCHHFVLE <span style="background-color: black; color: black;">N</span> WTS <span style="background-color: black; color: black;">L</span> EEPYGSIFLSIPT <span style="background-color: black; color: black;">I</span> LDSSLAP <span style="background-color: black; color: black;">E</span> GRHILHIFTSSMD   |
| Atha | 301 | VKAEVLPPTDCHHFVLED <span style="background-color: black; color: black;">D</span> WKN <span style="background-color: black; color: black;">L</span> EEPYGSIFLSIPT <span style="background-color: black; color: black;">I</span> LDSSLAP <span style="background-color: black; color: black;">E</span> GRHILHIFTSSIE  |
| <br> |     |   |
| Lses | 361 | -----   |
| Ljap | 361 | -----   |
| Gmax | 361 | DWE <span style="background-color: black; color: black;">G</span> LSRV <span style="background-color: black; color: black;">E</span> YEAKK <span style="background-color: black; color: black;">Q</span> LV <span style="background-color: black; color: black;">A</span> DE <span style="background-color: black; color: black;">I</span> TS <span style="background-color: black; color: black;">R</span> LEN <span style="background-color: black; color: black;">K</span> LFP <span style="background-color: black; color: black;">G</span> LR <span style="background-color: black; color: black;">S</span> SI <span style="background-color: black; color: black;">D</span> F <span style="background-color: black; color: black;">M</span> EV <span style="background-color: black; color: black;">G</span> TPK <span style="background-color: black; color: black;">T</span> HR <span style="background-color: black; color: black;">R</span> Y <span style="background-color: black; color: black;">Y</span> LAR <span style="background-color: black; color: black;">D</span> EGTYG |
| Mtru | 361 | DW <span style="background-color: black; color: black;">Q</span> GLSRID <span style="background-color: black; color: black;">Y</span> YEAKK <span style="background-color: black; color: black;">Q</span> V <span style="background-color: black; color: black;">A</span> DE <span style="background-color: black; color: black;">I</span> IS <span style="background-color: black; color: black;">R</span> LEN <span style="background-color: black; color: black;">K</span> LFP <span style="background-color: black; color: black;">G</span> LR <span style="background-color: black; color: black;">S</span> SI <span style="background-color: black; color: black;">D</span> I <span style="background-color: black; color: black;">E</span> VG <span style="background-color: black; color: black;">G</span> TPK <span style="background-color: black; color: black;">T</span> HR <span style="background-color: black; color: black;">R</span> Y <span style="background-color: black; color: black;">Y</span> LAR <span style="background-color: black; color: black;">D</span> EGTYG |

Atha 361 DWEGLPPK**EYEAKKEDVAARI**IQRLEK**KLFPGLSSSITFKEVGTPFTHRRFLARDKGTYG**

Lses -----  
Ljap 270 -----  
Gmax 421 PMPRRIPKGLLGMPFNTTGIDGLYCVGDSCFPQGVIAVAFSGVMCAHRVAADIGLEKKS  
Mtru 421 PMPRSIPKGLLGMPFNTTSIDGLYCVGDSCFPQGVIAVAFSGVMCAHRVAADIGLEKKS  
Atha 421 PMPRGTPKGLLGMPFNTTAIDGLYCVGDSCFPQGVIAVAFSGVMCAHRVAADIGLEKKS

Lses -----  
Ljap 270 -----WFR  
Gmax 481 PVLD**SMILLRLLGWLR**  
Mtru 481 PVLD**GMLLGLLGWLR**  
Atha 481 RVLD**VGLLGLLGWLR**

Figure S42. Amino acid alignment for CYB

|      |     |  |
|------|-----|--|
| Lses | 1   | RASSSALLELVPEFKKENLDFELPLYDSTKGAVVVDLAVVGGGPAGLAVAQQVSEAGLSVC  |
| Ljap | 1   | RASSSALLELVPEVKKENLDFELPLYDSMKGAVVVDLAVVGGGPAGLAVAQQVSEAGLSVC  |
| Gmax | 1   | RASSSALLELVPEFKKENLDFELPLYDSSKGAVVDLAVVGGGPAGLAVAQQVSEAGLSVC   |
| Mtru | 1   | RASSSALLELVPEFKKENLDFELPLYDSTKGTVVVLVVVGGGPAGLAVAQQVSEAGLSVC   |
| Atha | 1   | VSGSAALLLVPETKKENLDFELPLYDTSKSQVVDLATVGGGPAGLAVAQQVSEAGLSVC    |
| <br> |     |  |
| Lses | 61  | AIDPNPRLIWPNNYGVWVDFEAMDLLCCLDTWSGAVVYIDDKTKKYLDRPYGRVNRKL     |
| Ljap | 61  | AIDPNPRLIWPNNYGVWVDFEAMDLLCCLDTWSGAVVYIDDKTKKYLDRPYGRVNRKL     |
| Gmax | 61  | AIDPNPRLIWPNNYGVWVDFEAMDLLCCLDTWSGAVVYIDDKTKKDLDRPYGRVNRKL     |
| Mtru | 61  | AIDPNPRLIWPNNYGVWVDFEAMDLLCCLDTWSGAVVYIDDKTKKDLDRPYGRVNRKL     |
| Atha | 61  | SIDPSPKLIWPNNYGVWVDFEAMDLLCCLDTWSGAVVYDEGVKKDLSRPYGRVNRKL      |
| <br> |     |  |
| Lses | 121 | LKSMLQKCISNGVKFHQAQVIKVIHEESKSMICNDGVTIQATVVLGFSRSLVQYD        |
| Ljap | 121 | LKSMLQKCISNGVKFHQAQVIKVIHEESKSMICNDGVTIQATVVLGFSRSLVQYD        |
| Gmax | 121 | LKSMLQKCISNGVKFHQAQVIKVIHEEAKSLICNDGVTMATHVVLGFSRCLVQYD        |
| Mtru | 121 | LKSMLQKCITANGVKFHQAQVIKVIHEESKSMICNDGVTQATVVLGFSRCLVQYD        |
| Atha | 121 | LKSMLQKCITANGVKFHQSKVTVNHEANSTVVCSDGVKIQASVVLGFSRCLVQYD        |
| <br> |     |  |
| Lses | 181 | KPYNPQYQVAYGILAEVEEHPFDVKMLFMDWRDSHLDNDMELKERNNSIPTFLYAMPFS    |
| Ljap | 181 | KPYNPQYQVAYGILAEVEEHPFDVKMLFMDWRDSHLDNNMELKERNNSIPTFLYAMPFS    |
| Gmax | 181 | KPYNPQYQVAYGILAEVDEHPFDVKMLFMDWRDSHLDNDMELKQRNSIPTFLYAMPFS     |
| Mtru | 181 | KPYNPQYQVAYGILAEVEEHPFDVKMLFMDWRDSHLDNDLKLKERNNSIPTFLYAMPFS    |
| Atha | 181 | KPYNPQYQVAYGIVAEVDGHPFDVDMVFMDWRDKHLDSYPELKERNNSIPTFLYAMPFS    |
| <br> |     |  |
| Lses | 241 | STKIFLEETSLVARPGLRMDDIQDRMVARLKHLGIKVKSIEEDEHCVIPMGGPLPVLPQR   |
| Ljap | 241 | STKIFLEETSLVARPGLRMDDIQDRMVARLKHLGIKVKSIEEDEHCVIPMGGPLPVLPQR   |
| Gmax | 241 | STKIFLEETSLVARPGLRMDDIQERMAARLKHLGIKVKSIEEDEHCVIPMGGPLPVLPQR   |
| Mtru | 241 | STKIFLEETSLVARPGLRMDDIQDRMVARLKHLGINVKSIEDEQCVIPMGGPLPVLPQR    |
| Atha | 241 | SNRIFLEETSLVARPGLRMEDIQERMAARLKHLGINVKRIEEDERCVIPMGGPLPVLPQR   |
| <br> |     |  |
| Lses | 301 | VVGIGGTAGMVHPSTGYMVARTLAAAPIVANSIVQYLGSDRGISGDHLSAQVWKDLWPIE   |
| Ljap | 301 | VIGGGTAGMVHPSTGYMVARTLAAAPIVANAIVQYLGSDRGISGDGLSSLVWKDLWPIE    |
| Gmax | 301 | VVGIGGTAGMVHPSTGYMVARTLAAAPIVANSIVQCLGSDRGFSGDEISAQVWKDLWPIQ   |
| Mtru | 301 | VVGIGGTAGMVHPSTGYMVARTLAAAPIVANAIVQYLGSDRGILLGDEV SARVWKDLWPIE |
| Atha | 301 | VVGIGGTAGMVHPSTGYMVARTLAAAPIVANAIVRYLGSPNSLRGDQLSAEVWFDLWPIE   |
| <br> |     |  |
| Lses | 361 | RRRQREFFCFGMDILLKLDLAGT[KRFFDAFFDLEPRYWHGFSSRLFLPELLFFGSLSLFS  |
| Ljap | 361 | RRRQREFFCFGMDILLKLDLPGTRRRFFDAFFDLEPHYWHGFSSRLFLPELLVFGSLSLFS  |
| Gmax | 361 | RRRQREFFCFGMDILLKLDLPGT[KRFFDAFFDLEPHYWHGFSSRLFLHELLFFGSLSLFS  |
| Mtru | 361 | RRRQREFFCFGMDILLKLDLPGT[RFFDAFFDLEPHYWHGFSSRLFLYLPFETFGSLSLFS  |
| Atha | 361 | RRRQREFFCFGMDILLKLDLDA[TRRFFDAFFDLQPHYWHGFSSRLFLPELLVFGSLSLFS  |
| <br> |     |  |
| Lses | 421 | YASNTSR[EIMAKGTVP  |
| Ljap | 421 | YASNTSR[EIMAKGTVP  |
| Gmax | 421 | YASNTSRVEIMAKGTVP  |
| Mtru | 421 | YASNTSRLEIMAKGTVP  |
| Atha | 421 | HASNTSRLEIMTKGTVP  |

Figure S43. Amino acid alignment for E-CHY

Lses 1 -----  
Ljap 1 SLSLSPSLKSSINKTPSSWVSPDWLTSLRSRLTAGKNDDSGIPIASAKLDDVSDLLGGA  
Gmax 1 SLSLTPSLKSSINKTPSSWSPDWLTSLRSRLTAGNDVSGIPVASAKLDDVSDLLGGA  
Mtru 1 SLEPLSHLSKSSTNKTTSWVSPDWLTSLSKSLTTSKNDDSNIPIASAKLDDVSDLLGGA

Lses 1 -----  
Ljap 61 LFLPLFKWMKEYGPIYRLAAGPRNFVVVSDPAIAKHVLKNYGKYGKGLVAEVSEFLFGSG  
Gmax 60 LFLPLFKWMQDYGPIYRLAAGPRNFVVVSDPAIAKHVLNYGKYAKGLVAEVSEFLFGSG  
Mtru 61 LFLPLFKWMNEYGPIYRLAAGPRNFVVVSDPAIAKHVLKNYGKYGKGLVAEVSEFLFGDG

Lses 1 -----KLQPAALNGTAVNMEDKF  
Ljap 121 FAIAEGPLWTARRRAVVPSSLHKRYLSIVDRVFCRCAERLVEKLQPDALNGTAVNMEDKF  
Gmax 120 FAIAEGPLWTARRRAVVPSSLHKRYLSIVDRVFCRCAERLVEKLQPDALNGTAVNMEDKF  
Mtru 121 FAIAEGPLWTARRRAVVPSSLHKRYLSIMVDRVFCKCAERLVEKLQADAINGTAVNMEDKF

Lses 19 SQLTLDVIGLSVFNYDFDSLNAADSPVIGAVYTALKAEEARSTDLLPYWQGEFLRKINPRQ  
Ljap 181 SQLTLDVIGLSVFNYDFDSLNAADSPVIGAVYTALKAEEARSTDLLPYWQVEFLRKIIIPRQ  
Gmax 180 SQLTLDVIGLSVFNYNFDALNSDSDSPVIEAVYTALKAEEARSTDLLPYWKFKFLCKIIIPRQ  
Mtru 181 SQLTLDVIGLSVFNYNFDALNSDSDSPVIEAVYTALKAEEARSTDLLPYWKIDFLCKIIIPRQ

Lses 79 -----  
Ljap 241 IKAENAVTIIRKTVEDLIEKCKEIVESEGERIDGDEYVNDSDPSILRFILLASREEVSSDQ  
Gmax 240 IKAEAVSVIRKTVEDLIEKCREIVESEGERIDVEYVNDSDPSILRFILLASREEVSSVQ  
Mtru 241 IKAENAVTVIRKTVEDLIECKEIVESEGERIDADEVVNADPSILRFILLASREEVSSVQ

Lses 301 -----  
Ljap 301 LRDDLLSMLVAGHETTGSQLTWTLYLLSKDSSSLAKVQEEVDRVLQGRRTPTEDMKNLKF  
Gmax 300 LRDDLLSILVAGHETTGSQLTWTLYLLSKDSSSLAKAQEEVDRVLQGRRTTYEDIKDLKF  
Mtru 301 LRDDLLSMLVAGHETTGSQLTWTLYLLSKDSSSLAKAQEEVDRVLQGRRTYEDMKDLKF

Lses 361 -----  
Ljap 361 LTRCIITESLRLYPHPPVLIRRQVPDELPGAYKVNAGQDIMISVYNIHSSEVWDRAEEF  
Gmax 360 LTRCIIESLRLYPHPPVLIRRQVPDELPGGYKL DAGQDIMISVYNIHRSSEVWDRAEEF  
Mtru 361 LNRCIIESLRLYPHPPVLIRRQTPDELPGDYKIDAGQDIMISVYNIHSSKVWDRAEEF

Lses 421 -----  
Ljap 421 LPERFDLDGPMPNETNTDFRFIPFSGGPRKCVGDQFALLEATVSLAIFLQHMNFELVPDQ  
Gmax 420 VPERFDLDGPVPNETNTDFRFIPFSGGPRKCVGDQFALMEAVVALAIFLQHMNFELVPDQ  
Mtru 421 LPERFDLDGPVPNETNTDFRFIPFSGGPRKCVGDQFALLEATVALAVFLQHMNFELVPDQ

Lses 481 -----  
Ljap 481 NISMTTGATIHTTNGLYMKLSQRVK  
Gmax 480 NISMTTGATIHTTNGLYMKLSRRLK  
Mtru 481 NICMTTGATIHTTNGLYMKLSQRLK

Figure S44. Amino acid alignment for PSY

Lses 1 VLLWVNCGKGNSISLIGLGGRRGSRSQRRFRLCSGISFASFSSAVAEPSSRSSEERVYEVVL  
 Ljap 1 -----LGGRRGSRSQRRYRLCSGISFASFSSAVAEPSSRSSEERVYEVVL  
 Gmax 1 VLLWVSCGKENPISIVGLGGRRGRSQRFGLCNGISFASFSPAVAPSRSSEERVYEVVL  
 Mtru 1 VLLWVNCGKENEAIISLMGLVGRDVRQRRFRHCSGISFACFSSAVVEPIRSSEERVYEVVL  
 Atha 1 AVLWVATSNPDPMNNCGLV-RVLESSRLESPCQNQRLSLVAPSNGEIALSSEEKVYNVVL

Lses 61 KQAALVKEQKRNTKRDLNLDIEDDFTNKDLLNAAYDRCGEVCAEYAKTFYLGTQLMTEER  
 Ljap 44 KQAALVKEQKRDTKRLNLDIEDDFTNKDLLSGAYDRCGEVCAEYAKTFYLGTQLMTEER  
 Gmax 61 KQAALVKEQDKGTKRALNLDIEGDLTNQGDLLSDAYDRCGEVCAEYAKTFYLGTQLMTOER  
 Mtru 61 KQAALVKEQRKDIKRGGLNLDIEGDFTNQELLSSAYDRCGIVCAEYAKTFYLGTQLMTOER  
 Atha 60 KQAALVNKQIIRSSSYDIDVKQDVVLPSLSLLGEAYDRCGEVCAEYAKTFYLGTILMTPER

Lses 121 RKAIWAIYVWCRTDELVDGPNSPHITPKALDRWEQRLSDVFEGRPYDMYDAALSDTVTK  
 Ljap 104 RKAIWAIYVWCRTDELVDGPNSPHITPKALDRWEQRLSDVFEGRPYDMYDAALSDTVTK  
 Gmax 121 RKAIWAIYVWCRTDELVDGPNASHITPKALDRWEQRLSDVFEGRPYDMYDAALSDTVSK  
 Mtru 121 RKAIWAIYVWCRTDELVDGPNASHITPKALDRWEQRLTDVFEGRPYDMYDAALSDTVTK  
 Atha 120 RKAIWAIYVWCRTDELVDGPNASHITPMALDRWEARLEDIFRGRPFDMIDAIAADTVAR

Lses 181 YPVDIQPFKDMIEGMRLDLRKSRYDNFDELYLYCYYVAGTVGLMSVPVMGIAFESKASTE  
 Ljap 164 YPVDIQVNLMITG-----  
 Gmax 181 YPVDIQPFKDMIEGMRLDLRKSRYNSFDELYLYCYYVAGTVGLMSVPVMGIAFESKATTE  
 Mtru 181 YPVDIQPFKDMIEGMRLDLRKSRYNNFDELYLYCYYVAGTVGLMSVPVMGIEFQSKASTE  
 Atha 180 YPVDIQPFEDMIEGMRMDLKKSRYCNFDDLYLYCYYVAGTVGLMSVPVMGIDFQSKATTE

Lses 241 SVYNAALALGIANQLTNILRDVGEDARRGRVYLPQDELAKAGLSDEDIFSGRVTDKWRNF  
 Ljap -----  
 Gmax 241 SVYNAALALGIANQLTNILRDVGEDARRGRVYLPQDELAQAGLTDDDIFRGKVTDKWRNF  
 Mtru 241 STYNAALALGIANQLTNILRDVGEDARRGRVYLPQDELAQAGLSDDDIFRGRVTDKWRNF  
 Atha 240 SVYNAALALGIANQLTNILRDVGEDARRGRVYLPQDELAQAGLSDEDIFAGKVTDKWRNF

Lses 301 MKGQIIRRARMFFDEAEKGVSELSSASRWPVWASLLLRYQILDSIEANDYNNFTKRAYVGK  
 Ljap -----  
 Gmax 301 MKGQIQRARMFFDEAEKGVSELNSASRWPVWASLLLGYQILDSIEANDYNNFTKRAYVGK  
 Mtru 301 MKGQIKRARMFFDEAEKGVSELSSASRWPVWASLLLRYQILDSIEANDYNNFTKRAYVGK  
 Atha 300 MKMQLKRAMFFDEAEKGVTELSAASRWPVWASLLLRYRILDEIEANDYNNFTKRAYVGK

Lses 361 AKKLLSLPAA  
 Ljap -----  
 Gmax 361 VKKLLSLPAA  
 Mtru 361 AKKLLSLPVA  
 Atha 360 VKKIAALPLA

Figure S45. Amino acid alignment for ZEP-1

Lses 1 -----  
 Gmax 1 MATTLCYNSLNPSSTVFSRTHFSVPGCCTRKKQRKKVMHVKTPSKKQLRIILVAGGGIGGLV  
 Mtru 1 MVSTLSHKCLSPSMTTFSRTHFSNPGNRTTKQRRKLMQVKTPQKKQLKVLVAGGGIGGLV  
 Atha 1 MGSTPFCYSINPSKLDFTRTHVFSPGVSGFESRALLGVKTEKKKSRVLVAGGGIGGLV

Lses 1 -----IT  
 Gmax 61 FALAAKRKGFEMVFEKDLAIRGEQYRGPIQIQSNALAALEAIDSEVADEVMRVGCIT  
 Mtru 61 FALAAKRKGFVVVFKEKDLAIRGEQYRGPIQIQSNALAALEAIDMNVADEVMRVGCIT  
 Atha 61 FALAAKKKGFDVLFKEKDLAIRGEQYRGPIQIQSNALAALEAIDIEVAEQVMEAGCIT

Lses 3 GDRINGLVDGVSGSWYVKFDFTPAVERGLPVTRVISRMVLOGILARAVGDDIMNASNV  
 Gmax 121 GDRINGLVDGVSGSWYVKFDFTPAVERGLPVTRVISRMVLOEILARAVGEDIMNASNV  
 Mtru 121 GDRINGLVDGVSGSWYIKFDFTPAERGLPVTRVISRMALQEILARAVGDDVIMNGSNV  
 Atha 121 GDRINGLVDGTSGTWYVKFDFTPAASRGLPVTRVISRMTLQQILARAVGEDVIRNESNV

Lses 63 VNFVDDGNKVTVELENGKYEGLLLVGADGIWSKVRKQLFGLTEAVYSGYTC-----  
 Gmax 181 VNFVDDGNKVTVELENGQKYEGDVLVGADGIWSKVRKQLFGLTEAVYSGYTCYTGIADFV  
 Mtru 181 VDFIDHETKVTVVLDNGQKYDGDLLVGADGIWSKVRKLFGSTEATYSGYTCYTGIADFV  
 Atha 181 VDEEDSGDKVTVVLENGQRYEGDLLVGADGIWSKVRNNLFGRSEATYSGYTCYTGIADFV

Lses 241 PADIESTVGYRVFLGHKQYFVSSDVAGKMQWYAFHKEPPGGVDEPNGKERLLRIFEGWC  
 Gmax 241 PEDIESVGYRVFLGHKQYFVSSDVAGKMQWYAFHQEPAGGVDTPNGKERLLKIFEGWC  
 Mtru 241 PADIESVGYRVFLGHKQYFVSSDVGGGKMQWYAFHEEPAGGADAPNGMKKRLFEIFDGWC  
 Atha 241 PADIESVGYRVFLGHKQYFVSSDVGGKMQWYAFHEEPAGGADAPNGMKKRLFEIFDGWC

Lses 301 DNAIDLILATEEEEAILRRDIYDRPTLTWKGRTVLLGDSVHAMQPNMGQGGCMAIEDSY  
 Gmax 301 DNAIDLIVATEEEEAILRRDIYDRPTLTWKGRTVLLGDSVHAMQPNMGQGGCMAIEDGY  
 Mtru 301 DNVIDLHATEEEEAILRRDIYDRSPGFTWGKGRTVLLGDSIHAMQPNMGQGGCMAIEDSF  
 Atha 301 DNVIDLHATEEEEAILRRDIYDRSPGFTWGKGRTVLLGDSIHAMQPNMGQGGCMAIEDSF

Lses 361 QLAELENAWEQSIKSGSPIDIDSSLRSYERERRLRVAIIHGMARMAALMASTYKAYLG  
 Gmax 361 QLAFIELDNAWQQSAKSGSTIDIASSLKSYERERRLRVTFVHGMARMAALMASTYKAYLG  
 Mtru 361 QLAELDEAWKQSVETTIPVDVVSLLKRYEESRRLRVAIIHAMARMAAIMASTYKAYLG  
 Atha 361 QLAELDEAWKQSVETTIPVDVVSLLKRYEESRRLRVAIIHAMARMAAIMASTYKAYLG

Lses 421 GLGPLEFLTKFRIPHGRVGGGRFFVDIMMPSMLSWVLGGNSDKLEGRPLSCRLTDK  
 Gmax 421 GLGPFEFLTKFRIPHGRVGGGRFTQKSMPLMLNWVLGGNSSKLEGRPLCCRLISDK  
 Mtru 421 GLGPLSFLTKFRVPHGRVGGGRFFVDIAMPSMLDWVLGGNSEKQGRPPSCRLTDK  
 Atha 421 GLGPLSFLTKFRVPHGRVGGGRFFVDIAMPSMLDWVLGGNSEKQGRPPSCRLTDK

Figure S46. Amino acid alignment for ZEP-2

|      |     |   |
|------|-----|---|
| Lses | 1   | CRLSDKANDQLQRWFEDDDALERAVNGE WILL PCGDEAGLSKPICLSQDEM KPCMIGQED   |
| Ljap | 1   | -----QED  |
| Gmax | 1   | CRLSDKANDQLHRWFEDNDALERAINGE WILL PCGDEAGPITKPICLIQDEM KPCIIGQKD  |
| Mtru | 1   | CRLSDKASDQLHTWFEDDDALETITINGE WILL PCGDVPGHVKPISLNQDDTKPYIIGQED   |
| <br> |     |   |
| Lses | 61  | YPGCSITIPLPQVSQM HARINYKDGAFFLTDIQSQHGTWITDNGGRRYRVPPNDPARVRP     |
| Ljap | 4   | YPGSSITITLPQVSQMHAQINYKDGAFFLTDIQSQHGTWITDNEGRRNRVPPNC PARVRP     |
| Gmax | 61  | HPGSSIITIPLPQVSQM HARINYKDGAFFLTDI RS L HGTWITDNEGRRYRVPPNYPARVRP |
| Mtru | 61  | YPGSITIPLPQVSQI HARINFKDGAFFLTDI RS QHGTWITDNEGRRYMVSPNYPARIRP    |
| <br> |     |   |
| Lses | 121 | SDLIEFGSNKASYRVKVTR   |
| Ljap | 64  | SDLIEFGSNKVIDDP-----  |
| Gmax | 121 | SDVVEFGSDKASYRVKVTR   |
| Mtru | 121 | SHVIEFGCNOASYRVKVTR   |

Figure S47. Amino acid alignment for ZEP-3

|      |     |   |
|------|-----|---|
| Lses | 1   | KKKLRILVAGGGIGGLVFALAAKRKGFEVVVFEKDSAIRGEKYRGPIQIQSNALAALE  |
| Ljap | 1   | -----LTTAG-----   |
| Gmax | 1   | KKKLRLVLVAGGGIGGLVFALAAKRKGFEVVVFEKDSAIRGEKYRGPIQIQSNALAALE   |
| Mtru | 1   | KKQLKVLVAGGGIGGLVFALAAKRKGFEVVVFEKDSAIRGEKYRGPIQIQSNALAALE  |
| Atha | 1   | KKKSRLVLVAGGGIGGLVFALAAKKKGFDVLVFEKDSAIRGEKYRGPIQIQSNALAALE   |
| <br> |     |   |
| Lses | 61  | AID <span style="background-color: black; color: white;">KVADEVMRI</span> GCITGDRINGLVDGISGSWYIKFDTFTPAAERGLPVTRVISRMALQE   |
| Ljap | 6   | -----   |
| Gmax | 61  | AID <span style="background-color: black; color: white;">LEVAAEVILRV</span> GCITGDRINGLVDGISGSWYIKFDTFTPAAERGLPVTRVISRMALQE   |
| Mtru | 61  | AID <span style="background-color: black; color: white;">MVADEVMRVL</span> GCITGDRINGLVDGVSGSWYIKFDTFTPAAERGLPVTRVISRMALQE  |
| Atha | 61  | AID <span style="background-color: black; color: white;">LEVAEQVMEAGC</span> ITGDRINGLVDGIS <span style="background-color: black; color: white;">TWYV</span> KFDTFTPAAASRGLPVTRVISRMTLQQ  |
| <br> |     |   |
| Lses | 121 | ILACAVGEDVIL <span style="background-color: black; color: white;">NSNVVDFIDHGN</span> KVTVELENGQKYDGDLVGADGIWSKVRKKLFGES  |
| Ljap | 6   | -----   |
| Gmax | 121 | ILA <span style="background-color: black; color: white;">HAVGEDVIMND</span> NSNVVDFIDHGD <span style="background-color: black; color: white;">KVTVELENGQKYDGDLVGADGIWSKVRKKLFQ</span> T   |
| Mtru | 121 | ILARAVG <span style="background-color: black; color: white;">DDVIMN</span> GSNVVDFIDHET <span style="background-color: black; color: white;">KVTVVL</span> ENGQKYDGDLVGADGIWSKVRTKLFGST   |
| Atha | 121 | ILARAVGEDVIRNE <span style="background-color: black; color: white;">NSNVVDFEDSGD</span> KVTVVLENGQR <span style="background-color: black; color: white;">YEGD</span> LLVGADGIWSKVRNNLFGRS   |
| <br> |     |   |
| Lses | 181 | EAT <span style="background-color: black; color: white;">YSGYTCYTGIADFVP</span> P <span style="background-color: black; color: white;">DIESVGYRVFLGHKQYFVSSDVGAGKM</span> QWYAFHQEP <span style="background-color: black; color: white;">PGGVD</span>   |
| Ljap | 6   | -----VSCK-----  |
| Gmax | 181 | EAT <span style="background-color: black; color: white;">YSGYTCYTGIADFVP</span> P <span style="background-color: black; color: white;">ADIESVGYRVFLGHKQYFVSSDVGAGKM</span> QWY <span style="background-color: black; color: white;">CF</span> HQEPAGGAD   |
| Mtru | 181 | EAT <span style="background-color: black; color: white;">YSGYTCYTGIADFVP</span> P <span style="background-color: black; color: white;">DIESVGYRVFLGHKQYFVSSDVGAGKM</span> QWYAFHQEPAGGVD  |
| Atha | 181 | EAT <span style="background-color: black; color: white;">YSGYTCYTGIADF</span> P <span style="background-color: black; color: white;">ADIESVGYRVFLGHKQYFVSSDVG</span> GGKMQWYAFH <span style="background-color: black; color: white;">HE</span> EPAGGAD  |
| <br> |     |   |
| Lses | 241 | IPNGRK <span style="background-color: black; color: white;">RVLKIFKGWC</span> DNVTELIVATEEEAILRRDIYDRTPTLTWGKGRVTLLGDSVHA   |
| Ljap | 10  | -----   |
| Gmax | 241 | IPNGKKERLLKIFKGWC <span style="background-color: black; color: white;">DNV</span> IDL <span style="background-color: black; color: white;">I</span> HATEEEAILRRDIYDRTPTFTWGKG <span style="background-color: black; color: white;">H</span> VTL <span style="background-color: black; color: white;">LGDS</span> IHA  |
| Mtru | 241 | TPNGKKERLLKIFEGWC <span style="background-color: black; color: white;">DNA</span> IDL <span style="background-color: black; color: white;">I</span> HATEEEAILRRDIYDRTPTLTWGKGRVTLLGDSVHA  |
| Atha | 241 | APNGMK <span style="background-color: black; color: white;">KRLFEI</span> D <span style="background-color: black; color: white;">GWCDNV</span> LD <span style="background-color: black; color: white;">I</span> HATEEEAILRRDIYDRSPGFTWGKGRVTLLGDSIHA  |
| <br> |     |   |
| Lses | 301 | MQPNL <span style="background-color: black; color: white;">GQGGCMAI</span> EDG <span style="background-color: black; color: white;">FQLALELD</span> TAWQQS <span style="background-color: black; color: white;">M</span> KSGSS <span style="background-color: black; color: white;">IDIDSSL</span> KSYERERRLRVA <span style="background-color: black; color: white;">VIHAM</span>   |
| Ljap | 10  | -----   |
| Gmax | 301 | MQPNMGQGGCMAI <span style="background-color: black; color: white;">EDSY</span> QLALELDNAWQQS <span style="background-color: black; color: white;">I</span> KSGSP <span style="background-color: black; color: white;">IDIDSSL</span> KSYERERRLRVA <span style="background-color: black; color: white;">IVHGM</span>   |
| Mtru | 301 | MQPNMGQGGCMAI <span style="background-color: black; color: white;">EDGY</span> QLAFELDNAWQQS <span style="background-color: black; color: white;">AKSG</span> ST <span style="background-color: black; color: white;">IDIAS</span> SSLKSYERERRLRV <span style="background-color: black; color: white;">TFVHGM</span>  |
| Atha | 301 | MQPNMGQGGCMAI <span style="background-color: black; color: white;">EDSF</span> QLALELDE <span style="background-color: black; color: white;">AWKQS</span> VE <span style="background-color: black; color: white;">TT</span> TPV <span style="background-color: black; color: white;">VSSLKRYE</span> ESRRLRVA <span style="background-color: black; color: white;">IIHAM</span>   |
| <br> |     |   |
| Lses | 361 | ARMAAL <span style="background-color: black; color: white;">MASTYKAYLGVGLGP</span> LEFLTMFRV <span style="background-color: black; color: white;">PHPGRVGG</span> Y <span style="background-color: black; color: white;">FIDKMMPLMLN</span> WVL <span style="background-color: black; color: white;">GGN</span> SK  |
| Ljap | 10  | -----   |
| Gmax | 361 | ARMAAM <span style="background-color: black; color: white;">MASTYKAYLGVGLGP</span> LEFLTKFRI <span style="background-color: black; color: white;">PHPGRVGG</span> RFF <span style="background-color: black; color: white;">IDKMMPLMLN</span> WVL <span style="background-color: black; color: white;">GGN</span> SK   |
| Mtru | 361 | ARMAAL <span style="background-color: black; color: white;">MASTYKAYLGVGLGP</span> LS <span style="background-color: black; color: white;">FLTKFRV</span> PHPGRVGG <span style="background-color: black; color: white;">FV</span> DIAMP <span style="background-color: black; color: white;">SMLD</span> WVL <span style="background-color: black; color: white;">GGN</span> SEK  |
| Atha | 361 | ARMAAI <span style="background-color: black; color: white;">MASTYKAYLGVGLGP</span> LS <span style="background-color: black; color: white;">FLTKFRT</span> PHPGRVGG <span style="background-color: black; color: white;">FV</span> DIAMP <span style="background-color: black; color: white;">SMLD</span> WVL <span style="background-color: black; color: white;">GGN</span> SEK  |
| <br> |     |   |
| Lses | 421 | LEGRPL <span style="background-color: black; color: white;">CCRLSDKANDQ</span> LR <span style="background-color: black; color: white;">WFEDDA</span> LES <span style="background-color: black; color: white;">AINGEW</span> T <span style="background-color: black; color: white;">LLPCGDE</span> T <span style="background-color: black; color: white;">GH</span> <span style="background-color: black; color: white;">SE</span> <span style="background-color: black; color: white;">PIRIS</span> <span style="background-color: black; color: white;">ONEM</span> KP         |
| Ljap | 10  | -----   |
| Gmax | 421 | LEGRPV <span style="background-color: black; color: white;">CCRLSDKANDQ</span> LR <span style="background-color: black; color: white;">HRWFED</span> ND <span style="background-color: black; color: white;">ALER</span> A <span style="background-color: black; color: white;">INGEW</span> ILL <span style="background-color: black; color: white;">LLPCGDE</span> A <span style="background-color: black; color: white;">GPTK</span> P <span style="background-color: black; color: white;">ICL</span> T <span style="background-color: black; color: white;">QDEM</span> KP |
| Mtru | 421 | LEGRPL <span style="background-color: black; color: white;">CCRLSDKASQ</span> L <span style="background-color: black; color: white;">HTWFEDDA</span> LER <span style="background-color: black; color: white;">TINGEW</span> ILL <span style="background-color: black; color: white;">LLPCGDV</span> PGHV <span style="background-color: black; color: white;">VKPI</span> SLN <span style="background-color: black; color: white;">QDDT</span> KP   |

Atha 421 LQGRPPS CRLTDKADDRLREWFEDDDALERTIKGEWYLIIPH GDDCCVSETLC LTKDEDQP

Lses 481 CIIGSGTTVTIPS PQVS PMHARINYKDGGFFLIDL RSE HGTWISDIEG KRYRVPPNYPAR  
Ljap 10 ----- ALQLQYLC PRVSP MHARINYKDGGFFLIDL RSE HGTWISDIEG KRYRVPPNYPAR  
Gmax 481 CIIGSGSSIIIPPL PQVS QMHARINYKDGAFFLTDLRSI HGTWITDNEGR RYRVPPNYPAR  
Mtru 481 YIIGN-----  
Atha 481 CIVGSGMRTIVIPSSQVYKL YACSCDLQRRSFLLD-----

Lses 541 IHP PSDV LEFG S KK  
Ljap 65 IHP PSDV LEFG SRK  
Gmax 541 VRF PSDV VEFG SDK  
Mtru -----  
Atha 515 ----- GSSK

## Appendix 13

Supplementary Figures S48 – S54 Amino acid alignments for red-differentially expressed flavonoid genes in *Lotus sessilifolius*

Figure S48. Amino acid alignment for ANS

|      |     |   |
|------|-----|---|
| Lses | 1   | M A P T V V E R V E S I S T S G I Q S I P K E Y V R P K E E L E N I G N V F E E E K K - D G P Q V P T I D L K E I D S P   |
| Ljap | 1   | M A P T V V E R V E S I S G S I Q S I P K E Y V R P K E E L A N I G D V F E E E K K - V G P Q V P T I D L K D I D S P     |
| Gmax | 1   | M G - T V A P R V E S I A S S G I K C I P K E Y V R P K E E L K S I G N V F E E E K K - E G P E V P T I D L K R E I D S E |
| Mtru | 1   | -----K E Y V R P K E E L A N I G N I F D E E K K - E G P Q V P T I D L K E I N S S  |
| Atha | 1   | M V -- A V E R V E S I A K S G I I S I P K E Y I R P K E E L E S I N D V F L E E K K E D G P Q V P T I D L K N I E S D    |
| <br> |     |   |
| Lses | 60  | D E F V R A N C R E K L R K A A E E W G V M H L V N H G I P D E L L I N Q L K T A G A E F F S L P V E E K E K Y A N D Q T |
| Ljap | 60  | D E F V R A K C R E K L R K A A E E W G V M H L V N H G I P D E L L I N Q L K S A G A E F F S L P V E E K E K Y A N D Q A |
| Gmax | 59  | D E V V R G K C R Q K L K K A A E E W G V M N L V N H G I Q D E L L I E R V K K A G E E F F G L A V E E K E K Y A N D Q E |
| Mtru | 39  | D E I V R G K C R E K L K K A A E E W G V M H L V N H G I S D D L I N R L K K A G E T F F E L P V E E K E K Y A N D Q S   |
| Atha | 59  | D E K I R E N C I E E L K K A S L D W G V M H L I N H G I P A D L M E R V K K A G E E F F S I S V E E K E K Y A N D Q A   |
| <br> |     |   |
| Lses | 120 | T G N V Q G Y G S K L A N N A S G Q L E W E D Y F F H L I F P E D K R D L S I W P K T P S Y Y T E V T S D Y A R R L R V   |
| Ljap | 120 | A G N V Q G Y G S K L A N N A S G Q L E W E D Y F F H L I F P E D K R D L S I W P K T P S Y Y T E V T S D Y A R R L R V   |
| Gmax | 119 | S G K I Q G Y G S K L A N N A S G Q L E W E D Y F F H L V F P E D K R D L S I W P K P K P D D Y I E V T S E Y A K R L R G |
| Mtru | 99  | S G K I Q G Y G S K L A N N A S G Q L E W E D Y F F H C I F P E D K R D L S I W P K T P A D Y T K V T S E Y A K E L R V   |
| Atha | 119 | T G K I Q G Y G S K L A N N A S G Q L E W E D Y F F H I A Y P E E K R D L S I W P K T P S D Y I E A T S E Y A K C L R L   |
| <br> |     |   |
| Lses | 180 | L A S K V L E V L S L E L G L E E G R L E K E V G G M E E L L L Q M K I N Y Y P K C P Q P E L A L G V E A H T D V S S L   |
| Ljap | 180 | L A S K I L E V L S L E L G L E E G R L E K E V G G M E E L L L Q M K I N Y Y P K C P Q P E L A L G V E A H T D I S A L   |
| Gmax | 179 | L A T K M L E A L S I G L G L E G G R L E K E V G G M E E L L L Q I K I N Y Y P I C P Q P E L A L G V E A H T D V S S L   |
| Mtru | 159 | L A S K I M E V L S L E L G L E G G R L E K E A G G M E E L L L Q M K I N Y Y P I C P Q P E L A L G V E A H T D V S S L   |
| Atha | 179 | L A T K V F K A L S V G L G L E P D R L E K E V G G I E E L L L Q M K I N Y Y P K C P Q P E L A L G V E A H T D V S A L   |
| <br> |     |   |
| Lses | 240 | T F L V H N M V P G L Q L F Y Q D K W I T A K C V P D S I L M H I G D T I E I L S N G K F K S I L H R G L V N K E K V R   |
| Ljap | 240 | T F L L H N M V P G L Q L F Y E G K W V T A K C V P D S I L M H I G D T T E I L S N G K E F K S I L H R G L V N K E K V R |
| Gmax | 239 | T F L L H N M V P G L Q L F Y Q G Q W F T A K C V P N S I L M H I G D T I E I L S N G K Y K S I L H R G L V N K E K V R   |
| Mtru | 219 | T F L L H N M V P G L Q L F Y E G K W V T A K C V P D S I L M H I G D T I E I L S N G K Y K S I L H R G L V N K E K V R   |
| Atha | 239 | T F I L H N M V P G L Q L F Y E G K W V T A K C V P D S I V M H I G D T I E I L S N G K Y K S I L H R G L V N K E K V R   |
| <br> |     |   |
| Lses | 300 | I S W A V F C E P P K E K I I I L K P L P E L V T E T E P A I F P P P R T F A Q H I H H K L F R K D Q E A A A Q -- S K X  |
| Ljap | 300 | I S W A V F C E P P K E K I I I L K P L P E L V T E T E P A R F P P P R T F A Q H I H H K L F R K D Q E A S A Q -- S K X  |
| Gmax | 299 | I S W A M F C E P P K E K I I I L Q P L P E L V T E T E P A R F P P P R T F A Q H I H H K L F R K D Q E G L P N -- X      |
| Mtru | 279 | I S W A V F C E P P K E K I I I L K P L P E L V T E K E P A R F P P P R T F A Q H I H H K L F R K D E E E K K D D P K K X |
| Atha | 299 | I S W A V F C E P P K D K I V L K P L P E M V S V E S P A K F P P P R T F A Q H I E H K L F G K E Q E E L V S E K N D X   |

Figure S49. Amino acid alignment for CHS-1

|      |     |   |
|------|-----|---|
| Lses | 1   | -----TVEEIRNAQRSHGPATILAFGTATPSHCVMQADYPDYYFRITNSEHMTDLKEK      |
| Ljap | 1   | MV-----TVEEIRNAQRSSGPATILAFGTATPSHCVMQADYPDYYFRITNSEHMTDLKEK    |
| Gmax | 1   | MV-----TVEEIRNAQRSHGPATILAFGTATPSNCVSQADYPDYYFRITNSEHMTDLKEK    |
| Mtru | 1   | MV-----TVEEIRKAQRSGPATILAFGTATPSHCVTQADYPDYYFRITNSEHMTDLKEK     |
| Atha | 1   | MVMAGASSLDEIROQAQRADGPA[GILA]GTAN[ENHVIQAEYPDYYFRITNSEHMTDLKEK  |
| <br> |     |   |
| Lses | 54  | FKRMCEKSMIRKRYMHLTEEIILKENPNMCAYMAPSLDARQDLVVVEVPKLGKDAAKAIK    |
| Ljap | 56  | FKRMCEKSMIRKRYMHLTEEIILKENPAMCAYMAPSLDARQDLVVVEVPKLGKEAAAKAIK   |
| Gmax | 56  | FKRMCEKSMIKKRYMHLTEEFLKLENPNMC[EYMAPSLDVRQDVVMEVPKLGKQAAITKAIK  |
| Mtru | 56  | FKRMCEKSMIKKRYMHLTEEFLKLENPNMCAYMAPSLDARQDLVVVEVPKLGKDAAKKAI    |
| Atha | 61  | FKRMC[DST]IRKRHMHLTEEFLKLENPHMCAYMAPSLDTRQDTVVVEVPKLGKEAAVKAIK  |
| <br> |     |   |
| Lses | 114 | EWGQPDKSKITHLVFCTTSGVDMPGADYQLTKLLGLKPSVKRLMMYQQGCFAGGTVLRLAK   |
| Ljap | 116 | EWGQPDKSKITHLVFCTTSGVDMPGADYQLTKLLGLKPSVKRLMMYQQGCFAGGTVLRLAK   |
| Gmax | 116 | EWGQPDKSKITHLVFCTTSGVDMPGADYQLTKLLGLRPSVKRLMMYQQGCFAGGTVLRLAK   |
| Mtru | 116 | EWGQPDKSKITHLVFCTTSGVDMPGADYQLTKLLGLKPSVKRLMMYQQGCFAGGTVLRLAK   |
| Atha | 121 | EWGQPDKSKITHLVFCTTSGVDMPGADYQLTKLLGLRPSVKRLMMYQQGCFAGGTVLRLAK   |
| <br> |     |   |
| Lses | 174 | DLAENNKGARVLVVCSEITAVTFRGSDTHLDLSLVGQALFGDGAAAMIVGSDPD[LAV-ER]  |
| Ljap | 176 | DLAENNKGARVLVVCSEITAVTFRGSDTHLDLSLVGQALFGDGAAAIIVGADPD[LAV-ER]  |
| Gmax | 176 | DLAENNKGARVLVVCSEITAVTFRGSDTHLDLSLVGQALFGDGAAALIIGSDPDPAV-ER    |
| Mtru | 176 | DLAENNKNARVLVVCSEITAVTFRGSDTHLDLSLVGQALFGDGAAAMIIGADPDITV-ER    |
| Atha | 181 | DLAENN[R]GARVLVVCSEITAVTFRGSDTHLDLSLVGQALFSDGAAALIVGSDPDTSVGEK  |
| <br> |     |   |
| Lses | 233 | PIFELVSAAQTLIPDSGAI[DGH]REVGLTFHLLKDVP[G]IISKHIEKSLTEAFAPIGVSD  |
| Ljap | 235 | PIFOLVSAQTLIPDSGAI[DGH]REVGLTFHLLKDVP[G]IISKHIEKSLTEAFAPIGISD   |
| Gmax | 235 | PIFEMISAAQTLIPDSGAI[DGH]REVGLTFHLLKDVP[G]IISKNIEKSLVEAFEPIGISD  |
| Mtru | 235 | PIFEIVSAAQTLIPDSGAI[DGH]REVGLTFHLLKDVP[G]IISKNIEKSLVEAFAPIGISD  |
| Atha | 241 | PIFEMVSAAQTLIPDSGAI[DGH]REVGLTFHLLKDVP[G]IISKNIVKSLDEAFKPLGISD  |
| <br> |     |   |
| Lses | 293 | WNSLFWIAHPGGPAILDQVEVKLRLKEEKL[RSTRHV]LSEYGNMSSACVLFILDEVRRRSK  |
| Ljap | 295 | WNSLFWIAHPGGPAILDQVEAKLRLKEEKL[RSTRHV]LSEYGNMSSACVLFILDEVRRRSK  |
| Gmax | 295 | WNSIFWIAHPGGPAILDQVEKLRLKEEKL[RSTRHV]LSEYGNMSSACVLFILDEM[R]KKSK |
| Mtru | 295 | WNSIFWVAHPGGPAILDQVEKLRLKEEKL[RSTRHV]LSEYGNMSSACVLFILDEM[R]RSK  |
| Atha | 301 | WNSLFWIAHPGGPAILDQVEIKLGLKEEKM[RATR]HVLSEYGNMSSACVLFILDEM[R]RSK |
| <br> |     |   |
| Lses | 353 | EEGKETTGEGLEWGVLFGFGPGLTVETVVLHSVP----                          |
| Ljap | 355 | EEGKETTG[G]EGLEWGVLFGFGPGLTVETVVLHSVP[LEAX]                     |
| Gmax | 355 | EEGKSTTGEGLEWGVLFGFGPGLTVETVVLHSVP[LEGX]                        |
| Mtru | 355 | EEGKITTGEGLEWGVLFGFGPGLTVETVVLHSVP[QGX]                         |
| Atha | 361 | KDG[VATT]GEGLEWGVLFGFGPGLTVETVVLHSVP--X                         |

Figure S50. Amino acid alignment for CHS-6

Lses 1 MVSVAEIRKAQRAEGPATILAIGTANPPNCVDQSTYPDFYFRITNSEHMTTELKEFQRMC  
Ljap 1 MVSVAEIRKAQRAEGPATIFAI GTANPPNCVDQSTYPDFYFRITNSEHMTTELKEFQRMC  
Gmax 1 MVSVAEIRQAQRAEGPATILAIGTANPPNCVDQSTYPDYYFRITNSEHMTTELKEFQRMC  
Mtru 1 MVSVSEIRKAQRAEGPATILAIGTANPANCVEQSTYPDFYFKITNSEHKTTELKEFQRMC  
Atha 1 MVMA GASSLDEIRQAQRADGPAGILAIGTANPENHVLQAEYPDYYFRITNSEHMTDLKEK

Lses 61 DKSMIKKRYMHLTEDILKENPNMCA-----  
Ljap 61 DKSMIKKRYMHLTEDILKENPNMCAYMAPSLDARQDMVVVEVPRLGKEAAVKAIKEWGQP  
Gmax 61 DKSMIKKRYMYLNEEILKENPNMCAYMAPSLDARQDMVVVEVPRKLGKEAAVKAIKEWGQP  
Mtru 61 DKSMIKKRYMYLTEEILKKKNPSVCEYMAPSLDARQDMVVVEVPRLGKEAAVKAIKEWGQP  
Atha 61 FKRMCDCDKSTIRKRHMHLTEEFLK ENPHMCAYMAPSLDTRQDIVVVEVPKLGKEAAVKAIK

Lses -----  
Ljap 121 KSKITHLIFCTTSGVDM P GADYQLTKLLGLRPYVKRNKGALQVARCFVWPKTWIRRTKVL  
Gmax 121 KSKITHLIFCTTSGVDM P GADYQLTKQLGLRPYVKRYMMYQQGCFAGGT VRLAKDLAEN  
Mtru 121 KSKITHLIVCTTSGVDM P GADYQLTKLLGLRPYVKRYMMYQQGCFAGGT VRLAKDLAEN  
Atha 121 EWGQPKSKITHVVFC TTSGVDM P GADYQLTKLLGLRPSVKRLMMYQQGCFAGGT VRLIAK

Lses -----  
Ljap 181 VCXMFV LNSLQLPFVALVLTXTALLGKHCLEMEQLHSLLVPIQCLKLRNLCSSXYGLRK  
Gmax 181 NKCARV L VVCSEITAVTFRG PSDTHLDLSLVGQALFGDGA AAIVGSDPI PQVEKPL YELV  
Mtru 181 NKCARV L VVCSEITAVTFRG PSDTHLDLSLVGQALFGDGA ALIVGSDPI PEIERPI FEMV  
Atha 181 DLAENN R GARV L V CSEITAVTFRG PSDTHLDLSLVGQALFSDGA ALIVGSDPDT SVGEK

Lses -----  
Ljap 241 LLIQI VKEPLMVISVKLDXPFFISLKMFPGLFQRTL R KHXS RPSNRXAYLITTQY FGLHTQ  
Gmax 241 WTAQT IAPDSEGAI DGH LREV GLTFHLLKD VPGIVSKNIDKALFEAFNP LNISDM NSIFW  
Mtru 241 WTAQT IAPDSEGAI DGH LREAGLTFHLLKD VPGIVSKNITKALVEAFEP LGISDM NSIFW  
Atha 241 PIFEMVSAAQ TILPDSDGAI DGH LREVG LTFHLLKD VPGI SKNIVKS LDEAFKPLG ISD

Lses -----  
Ljap 301 AAQQFLIR SRTNSXSGQA-----  
Gmax 301 IAHPGGPA ILDQVEQKLGLKPEKMKA TRDV LSEYGNMSSACVL FILDEMRKSAENG LKT  
Mtru 301 IAHPGGPA ILDQVEQKLALKPEKMNA TREV LSEYGNMSSACVL FILDEM RKKSTQ NGLKT  
Atha 301 WNSLFWIAHPGGPA ILDQVEIKGLKEEKMRATR HVLSEYGNMSSACVL FILDEM RRKSA

Lses -----  
Ljap -----  
Gmax 361 TGEGLEWGVLFGF GPGLT IETVVLRSVAI-----X  
Mtru 361 TGEGLEWGVLFGF GPGLT IETVVLRSVAI-----X  
Atha 361 KDG VATTGEGLEW GVLFGFGPGLTVETVVLHSVPLX

Figure S51. Amino acid alignment for CHS-7

|      |     |   |
|------|-----|---|
| Lses | 1   | -----   |
| Ljap | 1   | MVSVAEIRKAQRAEGPATIFAI GTANPANCVDQSTYPDFYFRVTNSEHKTELKEKFQRMC   |
| Gmax | 1   | MVSVAEIRQAQRAEGPATILAIGTANPNRVDQSTYPDYYFRITNSDHTELKEKFQRMC      |
| Mtru | 1   | MVSVSEIRKAQRAEGPATIMAIGTANPANCVEQSTYPDFYFKITNSEHKVELKEKFQRMC    |
| <br> |     |   |
| Lses | 1   | -----   |
| Ljap | 61  | DKSMIKKRYMHLTEDILKENPNMCAYMAPSLDARQDMVVVEVPRLGKEAAVKAIKEWGQP    |
| Gmax | 61  | DKSMIKTRYMYLNNEEILKENPNMCAYMAPSLDARQDMVVVEVPKLGKEAAVKAIKEWGQP   |
| Mtru | 61  | DKSMIKKRRYMYLTLTEEILKENPSVCEYMAPSLDARQDMVVVEVPRLGKEAAVKAIKEWGQP |
| <br> |     |   |
| Lses | 1   | -----   |
| Ljap | 121 | KSKITHLIFCTTSGVDMMPGADYQLTKLLGLRPYVKRYMMYQQGCFAGGTVLRLAKDLAEN   |
| Gmax | 121 | KSKITHLIFCTTSGVDMMPGADYQLTKQLGLRPYVKRYMMYQQGCFAGGTVLRLAKDLAEN   |
| Mtru | 121 | KSKITHLIFCTTSGVDMMPGADYQLTKLLGLRPYVKRYMMYQQGCFAGGTVLRLAKDLAEN   |
| <br> |     |   |
| Lses | 1   | -----   |
| Ljap | 181 | NKGARVLVVCSEL TAVTFRGPDSDTHLDLSLVGQALFGDGAAALIVGSDPVPEIEKPLFELV |
| Gmax | 181 | NKGARVLVVCSEITAVTFRGPDSDTHLDLSLVGQALFGDGAAAVIVGSDPIPOVEKPLYELV  |
| Mtru | 181 | NKGARVLVVCSE TAVTFRGPDSDTHLDLSLVGQALFGDGAAALIVGSDPIPEIEKPIFEMV  |
| <br> |     |   |
| Lses | 1   | -----   |
| Ljap | 241 | WTAQTIAPDSEGAIDGHLREVGLTFHLLKDVPGIVSKNIEKALIEAFQPLGISDYN SIFW   |
| Gmax | 241 | WTAQTIAPDSEGAIDGHLREVGLTFHLLKDVPGIVSKNIDKALEFAFNPLNISDYN SIFW   |
| Mtru | 241 | WTAQTIAPDSEGAIDGHLREAGLTFHLLKDVPGIVSKNIDKALVEAFQPLNISDYN SIFW   |
| <br> |     |   |
| Lses | 36  | IAHPGGPAILDQVEQKLISLKPEKMRATREVLSEYGNMSSACVLFILDEMRKKSAQDGLKT   |
| Ljap | 301 | IAHPGGPAILDQVEQKLISLKPEKMRATREVLSEYGNMSSACVLFILDEMRKKSAQDGLKT   |
| Gmax | 301 | IAHPGGPAILDQVEQKLGLKPEKMRATRDVLSEYGNMSSACVLFILDEMRKKSAENGHKT    |
| Mtru | 301 | IAHPGGPAILDQVEEKLGKPEKMRATREVLSEYGNMSSACVLFILDEMRKKSAQEGLKT     |
| <br> |     |   |
| Lses | 96  | TGEGLEWGVLFGFGPGLTIETVVLR SVAIX                                 |
| Ljap | 361 | TGEGLEWGVLFGFGPGLTIETVVLR SVAIX                                 |
| Gmax | 361 | TGEGLEWGVLFGFGPGLTIETVVLSVAIX                                   |
| Mtru | 361 | TGEGLDWGVLFGFGPGLTIETVVLSVAIX                                   |

Figure S52. Amino acid alignment for DFR-2

|      |     |   |
|------|-----|---|
| Lses | 1   | -----ETVCVTGAAGFIGSWLVMRLIERGYTVRATVRDPANMKVKHLLELPNAKTKL   |
| Ljap | 1   | MSS--ESETVCVTGAAGFIGSWLVMRLIERGYTVRATVRDPANMKVKHLLELPDAKTKL   |
| Gmax | 1   | MGSSSASESVCVTGASGFIGSWLVMRLIERGYTVRATVRDPANMKVKHLVELPGAKTKL   |
| Mtru | 1   | MGS--VSETVCVTGASGFIGSWLVMRLIERGYTVRATVRDPDNMKVKHLLELPGANSKL   |
| Atha | 1   | MVS--QKETVCVTGASGFIGSWLVMRLIERGYFVRATVRDPGNLKKVQHLLDLPNAKTL   |
| <br> |     |   |
| Lses | 54  | TLWKADLAEEGSFDEAIKGCTGVFHATPMDFESKDPENEVIKPTINGLLDILKACEKAK   |
| Ljap | 59  | SLWKADLAEEGSFDEAI <del>G</del> GCTGVFHATPMDFESKDPENEVIKPTINGLLDILKACEKAK  |
| Gmax | 61  | SLWKADLAQEGSFDEAIKGCTGVFHATPMDF <del>D</del> SKDPENEVIKPTINGLLDIMKACVKAK  |
| Mtru | 59  | SLWKADLGEEGSFDEAIKGCTGVFHATPMDFESKDP <del>E</del> KEVINPTINGLLDIMKACKKAK  |
| Atha | 59  | TLWKADLSEEGSYDDAINGCDGVFHATPMDFESKDPENEVIKPTVNGMLGIMKACVKAK   |
| <br> |     |   |
| Lses | 114 | SVRRLVFTSSAGTVDVTEQQPKVIDETCWSDEFC <del>I</del> LRVKMTGWMYFVSKTRAEQEA <del>W</del> KYA  |
| Ljap | 119 | TVRRLVFTSSAGTVDVTEHPKPKVIDETCWSDEFC <del>I</del> LRVKMTGWMYFVSKTRAEQEA <del>W</del> KYA   |
| Gmax | 121 | TVRRLVFTSSAGTVDVTEHPNPVIDENCWSDVDFC <del>T</del> TRVKMTGWMYFVSKTLAEQEA <del>W</del> KYA   |
| Mtru | 119 | TVRRLVFTSSAGTLDVTEQQNSVIDETCWSDEFCRRVKMTGWMYFVSKTLAEQEA <del>W</del> KFS  |
| Atha | 119 | TVRRFVFTSSAGTVNV <del>E</del> HQKNVYDEN <del>D</del> WSDIEFI <del>M</del> SKKMTGWMYFVSKTLAEKA <del>W</del> DFA  |
| <br> |     |   |
| Lses | 174 | KEHNIDFVSVIPPLVVGPFLMPTMPPSLITALSLITGNEAHYSIIKQGQYVHLDDLCLAH  |
| Ljap | 179 | KEHNIDFVSVIPPLVVGPFLMPTMPPSLITALSLITGNEAHYSIIKQGQYVHLDDLCLAH  |
| Gmax | 181 | KEHNIDFISVIPPLVVGPFLMPTMPPSLITALSLITGNE <del>S</del> HYHI <del>I</del> KQGQFVHLDDLCLGH  |
| Mtru | 179 | KEHNIDFVSIIPPLVVGPFIIMP <del>S</del> MPPSLITALSLITGYEAHYSIIKQGQYI <del>I</del> HLDDLCLAH  |
| Atha | 179 | EEKG <del>L</del> D <del>F</del> ISIIP <del>T</del> LVVGPFITT <del>S</del> MPPSLITALSPITRNEAHYSII <del>R</del> QGQYVHLDDLCAH  |
| <br> |     |   |
| Lses | 234 | IFLFENPKAQGRYMC <del>S</del> AYEATIHEVARMINKKYPEFNVPTFKDIPD <del>E</del> LDIIKFSSKKIT   |
| Ljap | 239 | IFLFENPKAQGRYMC <del>S</del> AYEATIHEVARMINKKYPEFNVPTFKDIPD <del>E</del> LDIIKFSSKKIT   |
| Gmax | 241 | IFM <del>F</del> ENPKAEGRYICCSHEATIHDIAKLLNQ <del>K</del> YPEYNVLT <del>K</del> FKNIPD <del>E</del> LDIIKFSSKKIT  |
| Mtru | 239 | IFLFENPKAHGRYICCSHEATIHEVAKL <del>I</del> INKKYPEFNVPTFKDIPD <del>O</del> LEIIKFSSKKIT  |
| Atha | 239 | IFLYEQA <del>AA</del> AKGRYICSSH <del>A</del> TILT <del>I</del> SKFLRP <del>K</del> YPEYNVPST <del>F</del> EGV <del>D</del> N <del>L</del> K <del>S</del> IEFSSKKLT |
| <br> |     |   |
| Lses | 294 | DLGFKFKYSLEDMYTGAIETCREKGLLPKT <del>S</del> -----   |
| Ljap | 299 | DLGFKFKYSLEDMYTGAVETCREKGLLPKT <del>A</del> -----   |
| Gmax | 301 | DLGFKFKYSLEDMFTGAVETCREKGLLPKPE-----  |
| Mtru | 299 | DLGFI <del>F</del> FKYSLEDMFTGAIETCREKGLLPKVT-----  |
| Atha | 299 | DMG <del>F</del> NFKYSLEEMFIESIETCRQKGFLPV <del>S</del> LSYQSISEIKTKNENIDVKTGDGLTDGMKP  |
| <br> |     |   |
| Lses | 325 | ----ETPV <del>T</del> N-----GTTQKX  |
| Ljap | 330 | ----ETPATN-----GTTQKX   |
| Gmax | 332 | ----ETTVNNELLPKPAETTVNDTMOKX  |
| Mtru | 330 | ----ETPV <del>N</del> -----DTMKKX   |
| Atha | 359 | CNKTETG <del>G</del> ERTDAP---MLAQOMCAX   |

Figure S53. Amino acid alignment for F3H

|      |     |  |
|------|-----|--|
| Lses | 1   | -----LTTLAQONTLESSFVRDEDERPKVAYNNFSNEIPVISLAGIDEVDGRRSEIC        |
| Ljap | 1   | MASFKPKTLLAQONTLESSFVRDEDERPKVAYNNFSNEIPVISLAGIDEVDGRRSEIC       |
| Gmax | 1   | MAPT-AKTLTYLAQEKTLESSFVRDEDERPKVAYNEFSDEIPVISLAGIDEVDGRRREIC     |
| Mtru | 1   | MAP---AQTLTYLAQEKTLESSFVRDEDERPKVAYNNFSNEIPPIISLDGIDDAGGAAEIC    |
| Atha | 1   | MAP---GTLTELAGESKLNSKFVRDEDERPKVAYNVSDEIPVISLAGIDDDVGKRGAEIC     |
| <br> |     |  |
| Lses | 53  | NKIVEACENWGIFQVVNHGVDTTELVSHMTTLAKEFFALPPEEKLRFDMSGGGKKGGFIVSS   |
| Ljap | 61  | NKIVEACENWGIFQVVDHGVDTELVSHMTTLAKEFFALPPEEKLRFDMSGGGGGFIVSS      |
| Gmax | 60  | EKIVEACENWGIFQVVDHGVDQQQLVAEMTRLAKEFFALPPDEKLRFDMSGAKGGGFIVSS    |
| Mtru | 59  | NKIVEACENWGIFQVVDHGVDISKLISEMTRALEAKGFFDLPPEEKLRFDMSGGGKKGGFIVSS |
| Atha | 58  | RQIVEACENWGIFQVVDHGVDTNLVADMTRLARDFFALPPDKLRFDMSGGGKKGGFIVSS     |
| <br> |     |  |
| Lses | 113 | HLQGESVQDWREIVTYFSYPIRQRDYSRWPDTPAAGWKAATTEYSEKLMGLACKLLEVLS     |
| Ljap | 121 | HLQGESVQDWREIVTYFSYPIRNRDYSRWPDTPAAGWKAATTEYSEKLMGLACKLLEVLS     |
| Gmax | 120 | HLQGESVQDWREIVTYFSYPKRERDYSRWPDTPEGWRSVTEYSDKMGGLACKLMEVLSE      |
| Mtru | 119 | HLQGEAVKDWRRELVTYFSYPIRQRDYSRWPDKPEGWKEVTFQYSEKLMNLACKLLEVLS     |
| Atha | 118 | HLQGEAVQDWREIVTYFSYPVRNRDYSRWPDKPEGWVKVTEEYSERLMSLACKLLEVLS      |
| <br> |     |  |
| Lses | 173 | AMGLEKEALTKACVDMQKVNVYYPKCPQPDLTLGLKRHTDPGTITLLLQDQVGGQLQAT      |
| Ljap | 181 | AMGLEKEALTKACVDMQKVNVYYPKCPQPDLTLGLKRHTDPGTITLLLQDQVGGQLQAT      |
| Gmax | 180 | AMGLEKEGLSKACVDMQKVNVYYPKCPQPDLTLGLKRHTDPGTITLLLQDQVGGQLQAT      |
| Mtru | 179 | AMGLEKDALTKACVDMQKVVIINYYYPKCPQPDLTLGLKRHTDPGTITLLLQDQVGGQLQAT   |
| Atha | 178 | AMGLEKESLTNACVDMQKTIKVNVYYPKCPQPDLTLGLKRHTDPGTITLLLQDQVGGQLQAT   |
| <br> |     |  |
| Lses | 233 | RDNGKTWITVQPVEGAFVVNLGDGHYLSNGRFKNADHQAVVNSNSSRLSIATFQNPAPD      |
| Ljap | 241 | RDNGKTWITVQPVEGAFVVNLGDGHYLSNGRFKNADHQAVVNSNSSRLSIATFQNPAPD      |
| Gmax | 240 | RDNGKTWITVQPVEAFVVNLGDHAYHYLSNGRFKNADHQAVVNSNSRLSIATFQNPAPN      |
| Mtru | 239 | KDNGKTWITVQPVEGAFVVNLGDGHYLSNGRFKNADHQAVVNSNSRLSIATFQNPAPD       |
| Atha | 238 | RDNGKTWITVQPVEGAFVVNLGDGHFLSNGRFKNADHQAVVNSNSSRLSIATFQNPAPD      |
| <br> |     |  |
| Lses | 293 | ATVYPLKVREGEKSVLDEPITFAEMYRRKMSKDIELARMKKLAKEK-KLQDLE-----       |
| Ljap | 301 | ATVYPLKVREGEKSVMEEPITFAEMYRRKMSKDIELARMKKLAKEK-KLQDLE-----       |
| Gmax | 300 | ATVYPLKIREGEKPVMEEPITFAEMYRRKMSKDIETARMKKLAKEK-HLQDLENEKHLQE     |
| Mtru | 299 | ATVYPLKIREGEKSVMEEPITFAEMYRRKMSKDELIAARMKKLAKEEKELRDLE-----      |
| Atha | 298 | ATVYPLKVREGEKAILEEPITFAEMYRKMGKDLELARIKKLAKEE-RD-----            |
| <br> |     |  |
| Lses | 345 | ---KAKLEPKPMNEIF-----  |
| Ljap | 353 | ---KAKLEPKPMNEIFAX   |
| Gmax | 359 | LDQKAKLEAKPLKEILAX   |
| Mtru | 352 | ---KAKIEAKPLNEILAX   |
| Atha | 346 | ---HKEVDKPVDOIFAX  |

Figure S54. Amino acid alignment for F3'H

|      |     |  |
|------|-----|--|
| Lses | 1   | MFOWM <small>I</small> IGFATITVIIIF <small>I</small> HRRVVKFAT-RP-SLPLPPGPKPWPIIGNLPHMGPVPHSLAA  |
| Ljap | 1   | MFPWM <small>I</small> IGFATITFLIF <small>I</small> HRRVVKEST-RP-SLPLPPGPKPWPIIGNFPHMGPVPHSLAA   |
| Gmax | 1   | MSPLI-VALATIAAAILIYRIIKFIT-RP-SLPLPPGPKPWPIVGNLPHMGPVPHSLAA  |
| Mtru | 1   | MSIWF-IIAIASF <small>T</small> LCILIYRFMKEAK-RSSSLPLPPGPKPWPIIGNMPHIGPAPHOSIAA   |
| Atha | 1   | MATLF-LTILLATVIFLIRIFSHRRN <small>R</small> SHNNRLPPGPNPWPIIGNLPHMGT <small>K</small> PHTLSA   |
| <br> |     |  |
| Lses | 59  | LAQTHGPLMHLKLGFDVVAASAIVAEQFLKVHDANFSSRPPNAGAKYIAYNYQDLVFA   |
| Ljap | 59  | LAFAHGPLMHLKLGFDVVAASAIVAEQFLKVHDANFSSRPPNAGAKYIAYNYQDLVFA   |
| Gmax | 58  | LARIHGPLMHLRLGFVDVVVAASASVAEQFLK <small>I</small> HD <small>S</small> NFSSRPPNAGAKYIAYNYQDLVFA   |
| Mtru | 59  | LAKIHGPLMHLKLGFDVTVAAASCVAEQFLKVHDANFSSRPPNTGAKYIAYNYQDLVFA  |
| Atha | 60  | MVTTYGP <small>I</small> ILHRLGFVDVVVAASKSVAEQFLK <small>I</small> HDANFASRPPNSGAKHMAYNQDLVFA  |
| <br> |     |  |
| Lses | 119 | PYGA <small>R</small> WRYLRKITSIHLFSGKALDNFKHRLQEEVSRLTRNI <span style="background-color: black; color: black;">SK</span> --SNSKVNLGQLLNVC   |
| Ljap | 119 | PYGA <small>R</small> WRYLRKIT <small>N</small> IHLFSGKALDNFKHRLQEEVSRLTRNI <span style="background-color: black; color: black;">SK</span> --SNSKVNLGQLLNVC                              |
| Gmax | 118 | PYGPRWRLLRKITSVHLFSGKAMNEFRHRLQEEVARLTCNLAS--SDTKAVNLGQLLNVC   |
| Mtru | 119 | PYGPRWRMLRKISVHLFSNKVMEFFKHLRQEEVARLTSNLASNYSDTKAVNLGQLLNVC  |
| Atha | 120 | PYGHWRWLLRKISVHLFSAKAEDFKHVRQEEVGTLTRELVR--VGTPVNGLGQLVNMC   |
| <br> |     |  |
| Lses | 177 | TTNALSRVMIGRRVFNDG <small>D</small> GGCDPRADEFKAMVVELMVLAGVF <small>N</small> IGDFIPSLEWLDLQGV   |
| Ljap | 177 | TTNALSRVMIGRRVFNDG <small>D</small> GGCDPRADEFKAMVVELMVLAGVF <small>N</small> IGDFIPSLEWLDLQGV   |
| Gmax | 176 | TTNALARAMIGRRVFNDGNGGCDPRADEFKAMVMEVMLLAGVF <small>N</small> IGDFIPSLEWLDLQGV  |
| Mtru | 179 | TTNALARVMIGRRVFNDGNGGCDPRADEFKEMVLELMVLAGVF <small>N</small> ISDFIPSLEWLDLQGV  |
| Atha | 178 | VVNALGREMIGRRLFGA---DADHKADEFRSMVT <span style="background-color: black; color: black;">EM</span> MALAGVF <small>N</small> IGDFVPSLDWLDLQGV  |
| <br> |     |  |
| Lses | 237 | QAKMKNLHNRFDEFLTSIIIEHDT-SSKSENHKDLLSTLLSLKEV-ADDDGNKLNDIEIK   |
| Ljap | 237 | QAKMKKLHNRFDEFLTSIIIEHNT-SSKSENHKDLLSTLLSLKDV-PDDDGNKLNDIEIK   |
| Gmax | 236 | QAKMKKLHKRFDALTSIIIEHHNNSSSKNENHKNFLSILLSLKDV-RDDHGNHLTDEIK  |
| Mtru | 239 | QAKMKKLHKKFDALTNIIDERENSFKSEKHKDLLSTLLLKEE-TDVGDNKLTYIEIK  |
| Atha | 235 | AGKMKRLHKRFDALSSILKEHEM-NGQDQKHTDMSTLISLKGTDLGDGGSLTDTEIK  |
| <br> |     |  |
| Lses | 295 | ALLLNMFATGTDTSASTTEWAIAELIRNP <span style="background-color: black; color: black;">K</span> ILAQVQQELDTVVGDRNVREDDLP <span style="background-color: black; color: black;">H</span> LPYL  |
| Ljap | 295 | ALLLNMFATGTDTSASTTEWIAELIRSPRILAQVQQELDTVVG <small>R</small> ERNVREDDLP <span style="background-color: black; color: black;">H</span> LPYL   |
| Gmax | 295 | ALLLNMFATGTDTSSTTEWAIAELIKNPQILAKI <span style="background-color: black; color: black;">L</span> QQELEDTVVGDRRSVKEEDIAHLPYL  |
| Mtru | 298 | ALLLNMFAGTDTSSTTEWAIAELIRNP <span style="background-color: black; color: black;">R</span> ILAQVQQELDNVVGRDRNVKEDDIPNL <span style="background-color: black; color: black;">P</span> LPYL |
| Atha | 294 | ALLLNMFATGTDTSASTV <span style="background-color: black; color: black;">D</span> WAIAELIRHPDIMVKAQEELDTIVVGRDRPVNESDIAQLPYL  |
| <br> |     |  |
| Lses | 355 | QAVKETFRLHPSTPLSLP <span style="background-color: black; color: black;">V</span> ASESCEVLGYHIPKGSTLLVNVWAIARDPKEADPLEFK  |
| Ljap | 355 | QAVKETFRLHPSTPLSLP <span style="background-color: black; color: black;">V</span> ASESCEVLGYHIPKGSTLLVNVWAIARDPKEADPLEFK  |
| Gmax | 355 | QAVIKETFRLHPSTPLSVP <span style="background-color: black; color: black;">R</span> AAAESCEIFGYHIPKGATLLVN <span style="background-color: black; color: black;">I</span> WAIARDPKEWNDPLEFR |
| Mtru | 358 | QAVIKETFRLHPSTPLSLPRIAESCEIFGYHIPKGSTLLVNVWAIARDPKEWVDPLEFK  |
| Atha | 354 | QAVIKNFRLHPPTPLSLPHIASESCEINGYHIPKGSTLLTN <span style="background-color: black; color: black;">I</span> WAIARDP <span style="background-color: black; color: black;">D</span> QWSDPLAFK  |
| <br> |     |  |
| Lses | 415 | PERFLQGGGDKVHV <span style="background-color: black; color: black;">D</span> VKGNDFEVIPFGAGRRICAGMSLGLRMVQLLTATLAHSFNWELEN   |
| Ljap | 415 | PERFLE--GDKV <span style="background-color: black; color: black;">D</span> VKGNDFKVIPFGAGRRICAGMSLGLRMVQLLTATLVH <span style="background-color: black; color: black;">S</span> FNWELEN   |
| Gmax | 415 | PERFLLG-GEKADVD <span style="background-color: black; color: black;">G</span> NDFEVIPFGAGRRICAGISLGLQMVQLLTATLAHSFDWELED   |
| Mtru | 418 | PERFLPG-GEKCDVD <span style="background-color: black; color: black;">G</span> NDFEVIPFGAGRRICPGMSLGLRMVQLLTATLAHSFDWELEN   |

Atha 414 PERFLPG-GEKSGVDVKGSDFELIPFGAGRRICAGLSLGLRTIQFLTATLVQGFDWELAG

Lses 475 GLNHEKLNMDAYGLTLQRAVPLSVYSRPRLSPHV-----  
Ljap 473 GLNHEKLNMDAYGLTLQRAVPLSVYSRPRLSPHVYAAS-HX  
Gmax 474 CMNPEKLNMDAYGLTLQRAVPLSVHPPRIAPHVYSMS-SX  
Mtru 477 GLNAGKMNMDEGYGLTLQRAVPLSVHPKPRLSPHVYSSC-FX  
Atha 473 GVTPEKLNMBE SYGLTLQRAVPLVVHPKPRLAPNVYGLGSGX

## Appendix 14

Supplementary Figures S55 Amino acid alignments for yellow-differentially expressed flavonoid genes in *Lotus sessilifolius*

Figure S55. Amino acid alignment for FLS

|      |     |  |
|------|-----|--|
| Lses | 1   | -EVLRVQTVAIQSKD-A--SIPSMFVRPKTEQPG TTVRGVLELEVPIIDLGSDEEKVLR     |
| Ljap | 1   | MEVLRVQSVAAQSKD-A--SIPAMFVRSETEQPGITTVRGVELEVPIIDLNGTDEVKVLS     |
| Gmax | 1   | MEVLRVQTIAKSQKD-A--AIPAMFVRAETEQPGITTVQGVNLELEVPIIDFSDPDEGKVVH   |
| Mtru | 1   | MEVLRVQTIAHQSKD-ATSTIPSMDRSETESPGTTVQGVKLEVPIIDFNNPDEGKIQN       |
| Atha | 1   | MEVERVQDISSSLLTE--AIPLEFIRSEKEQPAITTFRGPTPAIPVVVDLSDPDESVR       |
| Lses | 57  | EIVEASKEWGMFQVVNH I PVEVIAKLQSVGKHFELPQEKEVCGKIDGSDSVEGYGTK      |
| Ljap | 58  | EIVEASKEWGMFQVVNH I PSEVIAKLQAVGKEFFELPQEEKEVYGKIEGSDSIEGYGTK    |
| Gmax | 58  | EIEASRDWGMFQIVNHDI PSDVIRKLQSVGKMFELPQEEKELIAKPAGSDSIEGYGTK      |
| Mtru | 60  | EIMEASTKWGMFQIVNHDI PNEVIKKLQSVGKEFFELPQEEKEVIAKPIIGSDSIEGYGTK   |
| Atha | 59  | A VVKASEEWGLFQVVNHG I PTELIIRRQLQDVGRKFELPSSEKESVAKPEDSKDIEGYGTK |
| Lses | 117 | LQKEVNGKKGWVDHLFHI IWPTSAINYRFWPKNPPSYREVNEEYGKYLRSVGDKLFKSLS    |
| Ljap | 118 | LQKEVNGKKGWVDHLFHI IWPTSSINYRFWPKNPASYREVNEEYGKYLRSVADKLFKSMS    |
| Gmax | 118 | LQKEVNGKKGWVDHLFHI WPPSSINYFWFPQNPPSYREVNEEYCKHLRGVVDKLFKSMS     |
| Mtru | 120 | LEKEVNGKKGWVDHLFHI WPPSSINYRFWPNNPASYREVNEEYGKYLREVADKLFKSLS     |
| Atha | 119 | LQKDPEGKKAWVDHLFRIWPPSCVNYRFWPKNPEYREVNEEYAVHVKLSETILGILS        |
| Lses | 177 | IGLGLEENELKEAAGGDDMIHLKINYYPPCPCE LVLGVPPHTDMSFTILVPNEVQGL       |
| Ljap | 178 | IGLGLEENELKEAAGGDDMIHLKINYYPPCPCPDLVLGVPPHTDMSFTILVPNEVQGL       |
| Gmax | 178 | VGLGLEENELKEGANEDDMHYLLKINYYPPCPCPDLVLGVPPHTDMSFTILVPNEVQGL      |
| Mtru | 180 | IGLELEEHELKKAAGGDELIHMLKINYYPPCPVVDLVLGVPPHTDMSFTILVPNEVQGL      |
| Atha | 179 | DGLGLKRDALKEGLGGE MAEYMMKINYYPPCERPDALALGVPAHTDISGITLLVPNEVQGL   |
| Lses | 237 | QAFRDGHWYDVKYVPNALVIHGDQMEI VSNKGKYAVLHRTTVNKEETRMSWPVFIEPQG     |
| Ljap | 238 | QAFRDGHWYDVKYVPNALVIHGDQMEILSNGKYAVLHRTTVNKEETRMSWPVFIEPQG       |
| Gmax | 238 | QACRDGHWYDVKYVPNALVIHGDQMEILSNGKYKAVEHRTTVNKDETTRMSWPVFIEPQK     |
| Mtru | 240 | QASRDGQWYDVKYVPNALVIHGDQMEILSNGKYAVLHRTTVNKDETTRMSWPVFIEPQK      |
| Atha | 239 | QVFKDDHWFDAEYIPS AIVHGDQI LRLSNGRYKVNVLHRTVDKEKTRMSWPVFIEPQK     |
| Lses | 297 | HHEVGPHSKLVNHDNPPKYTKKYKDYAYCKLNKIPQ-X                           |
| Ljap | 298 | DHEVGPHSKLVNQDNPPKYTKKYKDYAYCKLNKIPQ-X                           |
| Gmax | 298 | EQEVGPHPKLVNQDNPPKYTKKYKDYAYCKLNKIPQ-X                           |
| Mtru | 300 | EHEIGPHPKLVNQENPPKYTKKFE DYLYCKLNKIPQ-X                          |
| Atha | 299 | EKIVGPLPELTGDDNPPKFKPFAFKDYSYRKLNKPLDX                           |

## Appendix 15

Supplementary Figures S56 – S57 Amino acid alignments for non-differentially expressed lignin genes in *Lotus sessilifolius*

Figure S56. Amino acid alignment for CAD

|      |     |   |
|------|-----|---|
| Lses | 1   | MGSLE-AERTTVGWAARDPSGILSPYTETLRNTGPDDVYIKVHYCGICHSDIHQVKNDLG    |
| Ljap | 1   | MGSLE-AERTTVGWAARDPSGILSPYTETLRNTGPDDVYIKVHYCGCHTDVHQVKNDLG     |
| Gmax | 1   | MGSLE-AERTTVGLAARDPSGILSPYTYNLRNTGPDDVYIKVHYCGICHSDLHQTKNDLG    |
| Mtru | 1   | MGSLEVAERTTVGLAARDPSGILTPYTTLRNTGPDDVYIKTHYCGVCHSDLHQTKNDLG     |
| Atha | 1   | MGIIME-AERKTTGWAARDPSGILSPYTTLRETGPEDVNIRIIICCGICHTDLHQTKNDLG   |
| <br> |     |   |
| Lses | 60  | MSNYPMVPGHEVVGEVLEVGSVDVTRFRAGEIVGAGLLVGCCCKNCTACQSDIEQYCSKKIW  |
| Ljap | 60  | MSNYPMVPGHEVVGEVLEVGSVDVTRFTVGEIVGAGLLVGCCCKNCTACQSDIEQYCKKKIW  |
| Gmax | 60  | MSNYPMVPGHEVVGEVLEVGSVDVSFRVGEIVGVGLLVGCCCKNCQPCQODIENYCSKKIW   |
| Mtru | 61  | MSNYPMVPGHEVVGEVLEVGSNVTRFKVGEIVGVGLLVGCCCKSCRACDSEIEQYCNKKIW   |
| Atha | 60  | MSNYPMVPGHEVVGEVLEVGSVDVSKFTVGDIVGVGCLVGCCGGCSPCERDLEQYCPKKIW   |
| <br> |     |   |
| Lses | 120 | NYNDVYVDGKPTQGGFAETTIVEQKFVVKIPEGMAPEQVAPLLCAGVTVYSPLAHFGLKE    |
| Ljap | 120 | NYNDVYVDGKPTQGGFAETTIVEQKFVVKIPEGMAPEQVAPLLCAAVTVYSPLSHFGLKE    |
| Gmax | 120 | SYNDVYVDGKPTQGGFAETMVEQKFVVKIPEGLAPEQVAPLLCAGVTVYSPLVHFGLKE     |
| Mtru | 121 | SYNDVYTDGKITQGGFAESTVVEQKFVVKIPEGLAPEQVAPLLCAGVTVYSPLSHFGLKT    |
| Atha | 120 | SYNDVYINGOPTQGGFAKATVHVQKFVVKIPEGMAVEQAAPLLCAGVTVYSPLSHFGLKQ    |
| <br> |     |   |
| Lses | 180 | SGLRGGILGLGGVGHMGVKIAKALGHHTVVISSSDRKKKKEAIEDLGVDAYLVSSDTTSMQ   |
| Ljap | 180 | SGLRGGILGLGGVGHMGVTLIAKAMGHHTVVISSSDRKKKKEAILEDLGADAYLVSSDTTSMQ |
| Gmax | 180 | SGLRGGILGLGGVGHMGVKIAKALGHHTVVISSSDRKKKKEALEDLGADQYLVSSDTVTAMQ  |
| Mtru | 181 | PGLRGGILGLGGVGHMGVKVAKAFGHHTVVISSSDRKKKKEALEDLGADSYLVSSDTVGMO   |
| Atha | 180 | PGLRGGILGLGGVGHMGVKIAKAMGHHTVVISSSNKKREREALQDLGADDYVIGSDQAKMS   |
| <br> |     |   |
| Lses | 240 | GAADSLDYIIDTPVPGHPLEPYLSLLKLDGKLILMGVINTPLOFIITPMVMLGRRSITGSF   |
| Ljap | 240 | EAADSLDYIIDTPVPGHPLEPYLSLLKLDGKLILMGVINTPLOFIITPMVMLGRRSITGSF   |
| Gmax | 240 | EAADSLDYIIDTPVPGHPLEPYLSLLKLDGKLILMGVINTPLOFVSPMVMLGRRSITGSF    |
| Mtru | 241 | EAADSLDYIIDTPVPGHPLEPYLSLLKIDGKLILMGVINTPLOFVTPMVMLGRKSITGSF    |
| Atha | 240 | EIADSLDYVIDTPVPHALEPYLSLLKLDGKLILMGVINNPLQFLTPLMLGEKVITGSF      |
| <br> |     |   |
| Lses | 300 | IGSIKETEEMLEFWKEKGSSMIEVVNMDYINKAFERLEKNDVRYRFVVVDVKGS-----     |
| Ljap | 300 | IGSIKETEEMLEFWKEKGLTSMIEVNMDYINKAFERLEKNDVRYRFVVVDVKGSKLIDQX    |
| Gmax | 300 | IGSMKETEEMLEFWKEKGSSMIEVVNMDYINKAFERLEKNDVRYRFVVVDVKGSKLVD-X    |
| Mtru | 301 | VGSVKETEEMLEFWKEKGSSMIEVTTMDYINKAFERLEKNDVRYRFVVVDVKGSKFED-X    |
| Atha | 300 | IGSMKETEEMLEFCKEKGSSIIIEVVKMDYVNTAFERLEKNDVRYRFVVVDVEGSNLDA-X   |

Figure S57. Amino acid alignment for CCR-3

Lses 1 -----KVCVTGAGGFVASWLVKLLL SKGYT VHGTVRQP--GSPKYEHLKLEKA  
Ljap 1 -----  
Gmax 1 MAA-----KVCVTGAGGFVASWLVKLLL SKGYT VHGTVRDPEP ATOKYEHLKLHGA  
Mtru 1 MEASGGVNNNKKVCVTGAGGFVASWLVKLLL SKGYF VHGTVRDP--GSPKYEHLKLEKA  
Atha 1 MAD----VHKGKVCVTGAGGF LGSWVV DLLLSKD YF VHGTVRDP--DNEKYAHLKLEKA

Lses 48 SENLTLLFKADLLNYESVHSAILGCTAVFHVASPV P STI SNPQVEVIEPAVKGTA NVLEA  
Ljap 1 -----NVFHVF-----  
Gmax 54 SENLTLLFKADLLNYES RSAISGCTAVFH IACPVPSISVPNPQVEMIEPAVKGTT NVLEA  
Mtru 59 SENLTLLFKAD LDYESVYSAIVGCSAVFHASPVPSTVVPNPEVEVIEPAVKGTA NVLEA  
Atha 55 GDKLKLKFADLLDYGSI QSAIAGC SGFHVACP VPPASVPNPEVELIAPAVDGTINVLKA

Lses 108 SLQAKVERVVFSSEAAICMNPNLPKDKLIDESY WSDKDYCKKTQ NWYCYSKTEAEEQAL  
Ljap 7 -----  
Gmax 114 SLEAKVQRLUVFVSSLAASNSPNLPDKVIDESY WSDKDYCKTTQ NWYCFSKTEAEEQAL  
Mtru 119 CILKANVERVVFSVSSVAATNPNLPKDKAIDESCWSKDKDYCKNTK NWYC YAKTEAEEQAL  
Atha 115 CIEANVKR VV VSSVAAA FMNP MWSKNQV IDEACWSDQ EYCKKTE NWYC CLAKTRA ESEAF

Lses 168 DFAKRTGL SVVSICPTTVLGPIQSVTNASSLILLKLLKAEGCDSMENKLNWIVDVRDL  
Ljap 7 -----GLVFLDCLKLKVLYSMENRLS WIVDVGDL  
Gmax 174 DFAKRTGLD VVSICPSLVLGPIQSTTVNASSLALLKLLK --GVNSMENKIRWIVDVRDV  
Mtru 179 HFAKRTGLN VV TICPTLVLGPIQSVTNASSLVLVKLLK --EGCD SVENKLRWIVDVRDV  
Atha 175 DFAKRTGLHIVSVCP TLVLGPIQNTVNASSLVLKLLK --EGFETRD NQERHIVDVRDV

Lses 227 VDAVLLAYEKLEAEGRYICTSHPIKKRDLVEKLKSIYPNYKYPTK FTEVDGYRRLSSEKL  
Ljap 36 VDAVLLAYEKLEAEGRYICTSHPIKKRDLVEKLKSIYPYKYPTK FTEVDGYRRLSSEKL  
Gmax 232 ADAVLLAYEKLEAEGRYICHSHTIKTRDMIEKLKSIYPNYKYPAKYTEVDDYISFSSEKL  
Mtru 237 VNAVLLAYENHEADGRYICTSHAI VTRDLVERLKGIYPNKYPTNYIE MDDYKMLSSEKL  
Atha 234 AQAVLLVYEKAEAEGRYICTSHTVKEEIVVEKLKSFYPHYNYPKKYIDAEDRV KVSS EKL

Lses 287 QRLGWKS RPLEETLIDSVESYREAGL LQSEX  
Ljap 96 QRLGWKS RPLEETLIDSVESYREAGL LQSE-  
Gmax 292 QRLGWK YRSLEETL VDSVESYREAGL LQSEX  
Mtru 297 QSLGWKLRPL EETLIDSVESYKEAGL LQSQX  
Atha 294 QKLGW TYRPLEETL VDSVESYRKAKLVD--X

## Appendix 16

Supplementary Figures S58 – S60 Amino acid alignments for yellow-differentially expressed lignin genes in *Lotus sessilifolius*

Figure S58. Amino acid alignment for CAD

|      |       |                                   |                                   |
|------|-------|-----------------------------------|-----------------------------------|
| Lses | 1     | RRKRMS-NNAGKLVCTGGSGYIASWIVKFLL   | HGYTVRATVRDPSNPKKVEHLLKLDGA       |
| Ljap | 1     | ----MS-NGAGKLVCTGGSGYIASWIVKFLL   | HGYTVRATVRDPNNPKVEHLLKLDGA        |
| Gmax | 1     | ----MSNNNAGKVVCTGASGGFIASWIVKFLL  | QRGYTVRATVRYPSNLKKVDHLVKLEGA      |
| Mtru | 1     | ----MS---GEGKVVCTGASGGFIASWIVKFLL | QRGYTVRATVRDPSNPKKVDHLLKLDGA      |
| <br> |       |                                   |                                   |
| Lses | 60    | KERIHLFKADLLEEGSFDSVV             | DGCDGVFHTASPVG                    |
| Ljap | 56    | KERIHLFKADLLEEGSFDSA              | I-----AELIDPAVKGTLNVLKSC          |
| Gmax | 57    | KERIQLFKADLLEEGSFDSV              | VEGCHGVFHTASPVRFVVNDPQAE          |
| Mtru | 55    | KERIQLFKADLLEEGSFDSV              | VEGCDGVFHTASPVRFVVNDPQ            |
| <br> |       |                                   |                                   |
| Lses | ----- | -----                             | -----                             |
| Ljap | 95    | AKSPSVRRVVLTSSVGAVQYNERPKTPEVL    | I                                 |
| Gmax | 117   | AKSPSVKRVVLTSISAVAFNRRPKTP        | QVVVDETWFSDPDVCRELELWYTLSKTLAEDAA |
| Mtru | 115   | AKSTSVKRVVLTSSNAAVSFNTRPKNPEVV    | VDETWFSNPDFCRESKLWYVLSKTLAEAAA    |
| <br> |       |                                   |                                   |
| Lses | ----- | -----                             | -----                             |
| Ljap | 155   | -----                             | I                                 |
| Gmax | 177   | WKFVNENSIDMISINPTMVAGPLLQPE       | I                                 |
| Mtru | 175   | WKFVNENNIDMISLNPTMVAGPLLQPE       | VNESVEPILNLNGIPFPNKAIIGWVNVKDVANA |
| <br> |       |                                   |                                   |
| Lses | ----- | -----                             | -----                             |
| Ljap | 157   | -----AHADGRCA                     | -----                             |
| Gmax | 237   | HIAYEIASASGRYCLVERVIHYSELATI      | LRLGLYPTLQIPDKCEVDEPYIPTYQ        |
| Mtru | 235   | HIAYEIASASGRCLLAERVVHYSELAM       | ILRDLYPTLPISDKQSTSSSKFVILAYTTL    |
| <br> |       |                                   |                                   |
| Lses | ----- | -----                             | -----                             |
| Ljap | 164   | -----                             | DSD                               |
| Gmax | 297   | KKDLGIEFTP                        | EVSLRETVESFREKKIVNF-NPX           |
| Mtru | 294   | FHQIGAEPTC                        | QGMQLNPLNPLIYSYYFGELPCX           |

Figure S59. Amino acid alignment for CCoA-OMT

|      |     |   |
|------|-----|---|
| Lses | 1   | MATN-----EDQKOTEAGRHQEVGHKSLLQSDALYQYILETSVYPREHEAMKE           |
| Ljap | 1   | MATN-----EDQKOTEAGRHQEVGHKSLLQSDALYQYILETSVYPREHEAMKE           |
| Gmax | 1   | MAEQ-----NQQTTEAGRHQEVGHKSLLQSDALYQYILETSVYPREPESMKE            |
| Mtru | 1   | MATN-----EDQNQTESGRHQEVGHKSLLQSDALYQYILETSVFPREHEAMKE           |
| Atha | 1   | MATTTEATKTSSTNGEDQKQSQNLRHQEVGHKSLQLSDLYQYILETSVYPREPESMKE      |
| <br> |     |   |
| Lses | 49  | LRDLTAKHPWNIMTTSADEGQFLNMLLKLINAKNTMEIGVYTGYSLLATLAIPIPDGKIL    |
| Ljap | 49  | LRDLTAKHPWNIMTTSADEGQFLNMLLKLINAKNTMEIGVYTGYSLLATLAIPIPDGKIL    |
| Gmax | 49  | LRELTAHKPNIMTTSADEGQFLNMLLKLINAKNTMEIGVYTGYSLLATLAIPIPEDGKIL    |
| Mtru | 49  | LREVTAHKPNIMTTSADEGQFLSMLLKLINAKNTMEIGVYTGYSLLATLAIPIPEDGKIL    |
| Atha | 61  | LREVTAHKPNIMTTSADEGQFLNMLIILKVNNAKNTMEIGVYTGYSLLATLAIPIPEDGKIL  |
| <br> |     |   |
| Lses | 109 | AMDVNKENYELGLPVVIKKAGVAHKIDFREGPALPVLDEMVKDEKNHASYDFIFVDADKDN   |
| Ljap | 109 | AMDVNKENYELGLPVVIKKAGVAHKIDFREGPALPVLDEMVKDEKNHGSYDFIFVDADKDN   |
| Gmax | 109 | AMDINRENLYELGLPVVIKKAGVHDHKIDFREGPALPVLDEMVKDEKNHGSYDFIFVDADKDN |
| Mtru | 109 | AMDINKENYELGLPVVIKKAGVHDHKIDFREGPALPVLDEMVKDEKNHGSYDFIFVDADKDN  |
| Atha | 121 | AMDVNRENLYELGLPIIEKAGVAHKIDFREGPALPVLDEIVADEKNHGTYDFIFVDADKDN   |
| <br> |     |   |
| Lses | 169 | YLNYHKRLIELVKVGGVIGYDNTLWNGSVVAPPDAPLRKYVRYYRDFVLELNKALAADPR    |
| Ljap | 169 | YLNYHKRLIELVKVGGVIGYDNTLWNGSVVAPPDAPLRKYVRYYRDFVLELNKALAVDPR    |
| Gmax | 169 | YLNYHKRLIELVKVGGVIGYDNTLWNGSVVAPPDAPLRKYVRYYRDFVLELNKALAVDPR    |
| Mtru | 169 | YLNYHKRLIDLVKVGGVIGYDNTLWNGSVVAPPDAPLRKYVRYYRDFVLELNKALAVDPR    |
| Atha | 181 | YINYHKRLIDLVKIGGVIGYDNTLWNGSVVAPPDAPMRKYVRYYRDFVLELNKALAADPR    |
| <br> |     |   |
| Lses | 229 | IEICMLPVGDGITICRRRIKX   |
| Ljap | 229 | IEICMLPVGDGITICRRRIKX   |
| Gmax | 229 | IEICMLPVGDGITICRRRIKX   |
| Mtru | 229 | IEICMLPVGDGITICRRRIKX   |
| Atha | 241 | IEICMLPVGDGITICRRRIKSX  |

Figure S60. Amino acid alignment for OMT

|      |     |  |
|------|-----|--|
| Lses | 1   | MGSTGETQITPTHV <b>T</b> DEEANLFAMQLASASVLPVLKSA <b>I</b> ELDLLE <b>I</b> IAKAG <b>T</b> G <b>Q</b> <b>I</b> SPA  |
| Ljap | 1   | MGSTGETQITPTHVSDEEANLFAMQLASASVLPVLKSA <b>I</b> ELDLLE <b>F</b> IAKAG <b>T</b> G <b>Q</b> <b>I</b> SPA   |
| Gmax | 1   | MGSTGETQITPTHVSDEEANLFAMQLASASVLPVLKSA <b>E</b> LDLLE <b>I</b> IAKAG <b>P</b> G <b>V</b> H <b>L</b> SPS  |
| Mtru | 1   | MGSTGETQITPTH <b>S</b> DEEANLFAMQLASASVLPVLKSA <b>E</b> LDLLE <b>I</b> IAKAG <b>P</b> G <b>Q</b> <b>I</b> SPS  |
| Atha | 1   | MGSTA <b>E</b> TQ <b>I</b> TPV <b>Q</b> VT <b>D</b> DEA <b>A</b> LFAMQLASASVLP <b>M</b> ALKSA <b>E</b> LDL <b>I</b> MAKNG--SPMSPT  |
| <br> |     |  |
| Lses | 61  | EIASQLPTTNPDAPT <b>V</b> LDRILRLLACYNILTCS <b>V</b> QT <b>Q</b> QDGKV <b>Q</b> RLYGLAPVAKYLVKNED   |
| Ljap | 61  | EIASQLPTTNPDAPT <b>V</b> LDR <b>M</b> LRLLACYNILTCSVRTE <b>Q</b> D <b>G</b> KV <b>Q</b> RLYGLAPVAKYLVKNED  |
| Gmax | 61  | <b>D</b> IASR <b>L</b> PTHNPDA <b>P</b> VMLDRILRLLACYNIL <b>S</b> FL <b>R</b> TLPH <b>G</b> KVERLYGLAPVAKYLVRNED   |
| Mtru | 61  | EIASQLPTTN <b>P</b> EA <b>P</b> VMLDRILRLLACYNILTCSVR <b>T</b> Q <b>Q</b> D <b>G</b> KV <b>Q</b> RLYGLA <b>T</b> VAKYLVKNED  |
| Atha | 59  | EIA <b>S</b> KL <b>P</b> TK <b>N</b> PEA <b>P</b> VMLDRILRLL <b>T</b> SY <b>S</b> VL <b>T</b> CS <b>N</b> R <b>K</b> L <b>S</b> GD <b>G</b> VER <b>I</b> Y <b>G</b> LG <b>P</b> V <b>C</b> KY <b>I</b> T <b>K</b> NED                      |
| <br> |     |  |
| Lses | 121 | GVSISALNLMNQDKVLMESWYHLKDAVLEG <b>G</b> IP <b>F</b> FNKAYG <b>M</b> TAF <b>E</b> YHG <b>T</b> D <b>P</b> RFNKVF <b>N</b> KG <b>M</b>   |
| Ljap | 121 | GVSISALNLMNQDKVLMESWYHLKDAVLEG <b>G</b> IP <b>F</b> FNKAYG <b>M</b> TAF <b>E</b> YHG <b>T</b> D <b>P</b> RFNKVF <b>N</b> KG <b>M</b>   |
| Gmax | 121 | GVSIA <b>A</b> LNLMNQDK <b>I</b> LMESWY <b>Y</b> HLKDAVLEG <b>G</b> IP <b>F</b> FNKAYG <b>M</b> TAF <b>E</b> YHG <b>T</b> D <b>P</b> RFNKVF <b>N</b> KG <b>M</b>   |
| Mtru | 121 | GVSISALNLMNQDKVLMESWYHLKDAV <b>D</b> GG <b>G</b> IP <b>F</b> FNKAYG <b>M</b> TAF <b>E</b> YHG <b>T</b> D <b>P</b> RFNKVF <b>N</b> KG <b>M</b>  |
| Atha | 119 | GVSIA <b>A</b> AL <b>C</b> LMNQDKVLMESWYHLKDA <b>I</b> LD <b>D</b> GG <b>G</b> IP <b>F</b> FNKAYG <b>M</b> <b>S</b> A <b>E</b> YHG <b>T</b> D <b>P</b> RFNKVF <b>N</b> NG <b>M</b>   |
| <br> |     |  |
| Lses | 181 | SDHSTITMK <b>K</b> ILETYTG <b>F</b> EGL <b>K</b> SLVD <b>V</b> GGGTGAV <b>I</b> N <b>M</b> IVSKYPT <b>I</b> Q <b>G</b> INF <b>D</b> LPH <b>V</b> I <b>E</b> D <b>A</b> P   |
| Ljap | 181 | SDHSTITMK <b>K</b> ILETYTG <b>F</b> EGL <b>K</b> SLVD <b>V</b> GGGTGAV <b>I</b> N <b>M</b> IVSKYPT <b>I</b> K <b>G</b> INF <b>D</b> LPH <b>V</b> I <b>E</b> D <b>A</b> P   |
| Gmax | 181 | <b>A</b> DHSTITMK <b>K</b> ILETYTG <b>F</b> EGL <b>K</b> SLVD <b>V</b> GGGTGAV <b>V</b> N <b>M</b> IVSKYPT <b>I</b> K <b>G</b> INF <b>D</b> LPH <b>V</b> I <b>E</b> D <b>A</b> P   |
| Mtru | 181 | SDHSTITMK <b>K</b> ILETYTG <b>F</b> EGL <b>K</b> SLVD <b>V</b> GGGTGAV <b>I</b> N <b>T</b> IVSKYPT <b>I</b> K <b>G</b> INF <b>D</b> LPH <b>V</b> I <b>E</b> D <b>A</b> P   |
| Atha | 179 | <b>S</b> NHSTITMK <b>K</b> ILETY <b>K</b> FEGL <b>T</b> SLVD <b>V</b> GGG <b>I</b> GAT <b>L</b> K <b>M</b> IVSKY <b>P</b> N <b>I</b> K <b>G</b> INF <b>D</b> LPH <b>V</b> I <b>E</b> D <b>A</b> P  |
| <br> |     |  |
| Lses | 241 | SYPGVEHVGGDMFVSVPKADA <b>V</b> FM <b>K</b> W <b>I</b> CH <b>D</b> WS <b>D</b> E <b>H</b> CL <b>K</b> FL <b>K</b> NC <b>Y</b> EA <b>L</b> PD <b>N</b> KG <b>V</b> IV <b>A</b> EC <b>I</b> L   |
| Ljap | 241 | SYPGVEHVGGDMFVSVPKADA <b>V</b> FM <b>K</b> W <b>I</b> CH <b>D</b> WS <b>D</b> E <b>H</b> CL <b>K</b> FL <b>K</b> NC <b>Y</b> EA <b>L</b> PD <b>N</b> KG <b>V</b> IV <b>A</b> EC <b>I</b> L   |
| Gmax | 241 | SYPGVEHVGGDMFVSVPKADA <b>T</b> FM <b>K</b> W <b>I</b> CH <b>D</b> WS <b>D</b> E <b>H</b> CL <b>K</b> FL <b>K</b> NC <b>Y</b> EA <b>L</b> PD <b>N</b> KG <b>V</b> IV <b>A</b> EC <b>I</b> L   |
| Mtru | 241 | SYPGVEHVGGDMFV <b>S</b> TPKADA <b>V</b> FM <b>K</b> W <b>I</b> CH <b>D</b> WS <b>D</b> E <b>H</b> CL <b>K</b> FL <b>K</b> NC <b>Y</b> EA <b>L</b> PD <b>N</b> KG <b>V</b> IV <b>A</b> EC <b>I</b> L  |
| Atha | 239 | <b>S</b> HPG <b>T</b> EHVGGDMFVSVP <b>K</b> <b>C</b> DA <b>I</b> FM <b>K</b> W <b>I</b> CH <b>D</b> WS <b>D</b> E <b>H</b> C <b>V</b> K <b>F</b> LC <b>N</b> CY <b>E</b> SL <b>P</b> ED <b>G</b> K <b>V</b> I <b>A</b> EC <b>I</b> L       |
| <br> |     |  |
| Lses | 301 | PVAPDSSLAT <b>K</b> GV <b>V</b> H <b>I</b> D <b>V</b> IMLAHNPG <b>G</b> K <b>ER</b> TE <b>E</b> FE <b>A</b> LA <b>K</b> G <b>A</b> G <b>F</b> Q <b>G</b> FK <b>V</b> M <b>C</b> CAF <b>N</b> SY <b>V</b> ME                                |
| Ljap | 301 | PVAPDSSLAT <b>K</b> GV <b>V</b> H <b>I</b> D <b>V</b> IMLAHNPG <b>G</b> K <b>ER</b> TE <b>E</b> FE <b>A</b> LA <b>K</b> G <b>A</b> G <b>F</b> Q <b>G</b> F <b>Q</b> V <b>L</b> CCAF <b>N</b> SY <b>V</b> ME                                |
| Gmax | 301 | PVAPDSSLAT <b>K</b> GV <b>V</b> H <b>I</b> D <b>V</b> IMLAHNPG <b>G</b> K <b>ER</b> TE <b>E</b> FE <b>A</b> LA <b>K</b> G <b>S</b> G <b>F</b> Q <b>G</b> F <b>Q</b> V <b>L</b> CCAFNT <b>Y</b> V <b>ME</b>                                 |
| Mtru | 301 | PVAPDSSLAT <b>K</b> GV <b>V</b> H <b>I</b> D <b>A</b> IMLAHNPG <b>G</b> K <b>ER</b> T <b>Q</b> E <b>F</b> E <b>D</b> LA <b>K</b> G <b>A</b> G <b>F</b> Q <b>G</b> FK <b>V</b> H <b>C</b> NA <b>F</b> NT <b>Y</b> IME                       |
| Atha | 299 | <b>P</b> EPDSSL <b>T</b> K <b>Q</b> V <b>V</b> H <b>V</b> DC <b>I</b> MLAHNPG <b>G</b> K <b>ER</b> TE <b>E</b> FE <b>A</b> LA <b>K</b> A <b>S</b> G <b>F</b> K <b>G</b> I <b>K</b> V <b>V</b> C <b>D</b> A <b>F</b> GV <b>N</b> L <b>E</b> |
| <br> |     |  |
| Lses | 361 | <b>FL</b> KK <b>V</b> X  |
| Ljap | 361 | <b>FL</b> KK <b>V</b> X  |
| Gmax | 361 | <b>FL</b> KK <b>V</b> X  |
| Mtru | 361 | <b>FL</b> KK <b>V</b> X  |
| Atha | 359 | <b>L</b> LKK <b>L</b> X  |

## Appendix 17

**Figure S61.** Expanded phylogeny of *Lotus* spp. exhibiting PACC, including those studied in this thesis. Figure and caption are reproduced from Figure 2 in Ojeda et al. 2013:

Molecular tree based on one nuclear (ITS) and three plastid regions (*CYB6*, *trnH-psbA* and *matK*). The tree was randomly selected from a maximum parsimony (MP) analysis from Ojeda et al., 2012a and Ojeda et al., 2012b. Character mapping of the trait flower color change after anthesis in *Lotus* sections *Pedrosia* and the “*rhyncholotus* group”. Red branches show clades where this trait has evolved and the numbers on the tree the times this trait evolved within this groups (1–3). Arrows indicate the numbers of reversals, one of which occurred in three species of the “*rhyncholotus* group”. The species from the outgroup belong to the *Lotus* section are not endemic of the Macaronesian region. Species with (\*) are distributed in mainland Africa and/or in Europe (a) represents flower color at anthesis (pre-change) and (b) indicates flower color after change (post-change). (A) *L. jacobaeus*, (B) *L. purpureus*, (C) *L. eriosolen*, (D) *L. sessilifolius* subsp. *sessilifolius*, (E) *L. pyranthus*, (F) *L. emeroides*, (G) *L. argyrodes*, (H) *L. glaucus*, (I) *L. campylocladus*, and (J) *L. lancerottensis*.

