# Transcriptional modulation during photomorphogenesis in rice seedlings

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## ABSTRACT

Light is one of the most important factors regulating plant gene expression patterns, metabolism, physiology, growth, and development. To explore how light may induce or alter transcript splicing, we conducted RNA-Seq-based transcriptome analyses by comparing the samples harvested as etiolated seedlings grown under continuous dark conditions vs. the light-treated green seedlings. We identified 14,766 differentially expressed genes, of which 4369 genes showed alternative splicing. We observed that genes mapped to the plastid-localized methyl-erythritol-phosphate (MEP) pathway were upregulated in light compared to the cytosolic mevalonate (MVA) pathway genes. Many of these genes also undergo splicing. These pathways provide crucial metabolite precursors for the biosynthesis of secondary metabolic compounds needed for chloroplast biogenesis, the establishment of successful photosynthetic apparatus, and photomorphogenesis. In the chromosome-wide survey of the light-induced transcriptome, we observed intron retention as the most predominant splicing event. In addition, we identified 1709 novel lncRNA transcripts in our transcriptome data.

31 **INTRODUCTION**

32 Light is an essential growth factor for sustaining autotrophic plant life. The quality, quantity,
33 direction, and length of light exposure affect plant growth and development [1,2]. The natural light-
34 dark cycle maintains the carbon and nitrogen metabolism of rice plants [3]. Light induces
35 transcriptional reprogramming in various plant species [3–10] via an array of photoreceptors [11,12].
36 The photoreceptors phytochrome (red light) and cryptochrome (blue light) are involved in light
37 signaling and photomorphogenesis [13,14]. Genes encoding phytochrome interacting factors (PIFs)
38 promote skotomorphogenesis and development in the dark [15–17]. Light-mediated gene expression
39 modulation is also triggered by translational enhancement of preexisting mRNA pool instead of
40 an enhanced rate of transcription [18,19].

41 Intron splicing in multiexonic mRNA is a post-transcriptional regulatory process that often
42 produces different mRNA isoforms transcribed from a single gene locus, thus contributing to
43 proteome plasticity. Light-induced alternative splicing (AS) was observed in ~10% of the
44 protein-coding genes of *Arabidopsis* and *Physcomitrella patens* [20,21]. A flash of light applied in
45 the middle of the dark or nighttime is sufficient to induce splicing [22]. In contrast to animals,
46 where the most common AS event is exon skipping (ES), intron retention (IR) is the most
47 common AS event in rice [23], Arabidopsis [23,24], and poplar [25]. It is now well established that
48 transcriptome modulation via AS is vital for plant growth, development, and response to stress
49 [26–28].

50 Compared to the protein-coding genes, long noncoding RNAs (lncRNAs) are transcripts >200 bp
51 in length that do not have protein-coding potential. lncRNAs are classified into categories: (i)
52 sense lncRNAs, (ii) antisense lncRNAs, (iii) intergenic RNAs (lincRNAs), (iv) intronic RNAs,
53 and (v) bidirectional lncRNAs [29]. In addition to the numerous studies coupling gene expression
54 and AS studies on protein-coding genes, it is now known that lncRNAs also play a role in
55 regulating gene expression through transcriptional, post-transcriptional, and chromatin
56 remodeling mechanisms [29–32]. In rice, lncRNAs regulate biological processes, such as ovule
57 development and female gametophyte abortion [33], sexual reproduction [34], and stress response
58 [35,36]. However, the role of light in regulating lncRNAs is not well studied. *Arabidopsis*
59 noncoding RNA *HID1* is a known positive regulator of photomorphogenesis in continuous red
60 light [37].

Rice is a global staple crop and is a model for studying crop genomics. The transition from skotomorphogenesis under dark conditions to photomorphogenesis under light exposure is critical for seedling survival and requires precise control of gene expression by different regulatory mechanisms. Results from our rice study show that exposure to light alters the expression and splicing of a wide array of protein-coding genes but not so much for the noncoding lncRNA genes.

## MATERIALS & METHODS

### Plant material, growth conditions, and treatment

Seeds of rice (*Oryza sativa spp. japonica* cv. Nipponbare) were grown and processed for the experiment by following the growth conditions and sampling described previously [38]. Seeds were sown in the dark and germinated on day 2. After sowing, these germinated seedlings grew in the dark for 8 days (8DD). At the end of day 8, three biological replicates of the dark-grown etiolated shoots were harvested. The remaining dark-grown seedlings were exposed to continuous white light at 120 µmol/m2/sec (measured at the soil surface) for 48 hours or 2 days (days 9 and 10 after sowing). The shoots of three biological replicates of light-treated green-colored seedling samples (8DD-2LL) were harvested at the end of day 10. Harvested samples were frozen using liquid nitrogen and stored at -80°C until further processing. Throughout this report, 8DD-treated plants are called dark samples, and 8DD-2LL light-treated plants are called light samples. Data analysis is described for light regulation.

### Sample preparation and sequencing

Total RNA from frozen tissue samples was extracted using RNA Plant reagent (Invitrogen Inc., USA) and RNeasy kits (Qiagen Inc., USA) and treated with RNase-free DNase (Life Technologies Inc., USA) according to the manufacturer's protocol. The total RNA quality and concentration were determined using an ND-1000 spectrophotometer (Thermo Fisher Scientific Inc., USA) and Bioanalyzer 2100 (Agilent Technologies Inc., USA). PolyA-enriched mRNA libraries were prepared from three biological replicates of dark and light samples using the TruSeqTM RNA Sample Preparation Kits (v2) and sequenced as 51-bp single-end reads using the Illumina HiSeq 2000 instrument (Illumina Inc., USA) according to the manufacturer's protocol at the Center for Genomic Research and Biocomputing, Oregon State University. The

90 strand-specific sequencing reads and metadata were deposited at EMBL-EBI ArrayExpress
91 (accession number E-MTAB-5689).

92 **RNA-seq data analysis**

93 The generation of FASTQ files from the RNA-Seq sequences was performed by CASAVA
94 software v1.8.2 (Illumina Inc.). Sequence reads were filtered and trimmed for low quality at a
95 score of 20 using Sickle v1.33 [39]. Clean, high-quality reads from each sample and replicates were
96 aligned to the *Oryza sativa japonica* cv Nipponbare reference genome (IRGSP-1.0.31) using
97 TopHat v2.1.1 [40]. Mapped reads were assembled using Cufflinks, and the reference-guided
98 assembled transcripts from each replicate were merged using Cuffmerge [41]. Assembled
99 transcripts were compared to the reference genome annotation using Cuffcompare. The RSEM
100 software package estimated normalized baseline expression from the aligned sequence reads [42].
101 For differential gene expression analysis, read count data obtained from RSEM were used in
102 EBSeq [43]. Differentially expressed (DE) genes were filtered based on the false discovery rate
103 corrected P value ≤0.05.

104 **Functional annotation**

105 We carried out the Gene Ontology (GO) enrichment analysis tool provided by the GO
106 consortium [44] to determine the biological roles played by the enriched gene set. Plant pathway
107 enrichment analysis was done by mapping the DE genes using the Plant Reactome analysis tool
108 [46] (http://plantreactome.gramene.org/PathwayBrowser/#TOOL=AT).

109 **Alternative splicing analysis**

110 Splicing events in the transcripts from the samples were identified by the SpliceGrapher v0.2.5
111 pipeline [47]. Sequence reads from each sample, and replicates were aligned to the reference rice
112 genome. Splice site-specific classifiers were built using build_classifiers.py script using
113 canonical (GT) and noncanonical (GC) donor sites and acceptor site (AG) for *Oryza sativa*
114 genome annotation version 31 (Oryza_sativa.IRGSP-1.0.31). Read alignments in SAM format
115 from each replicate were used as input for SpliceGrapher's sam_filter.py script to filter out false-
116 positive sites. SpliceGrapher Python scripts were used for the generation of depth files
117 (sam_to_depths.py), splice graph prediction (predict_graphs.py), generating statistics
118 (splicegraph_statistics.py) from a set of splice graphs, gene-by-gene summary

119 (genewise_statistics.py) of splicing events and splice graph visualization (plotter.py). The
120 Realignment pipeline was used to construct putative transcripts from unresolved exons with
121 sufficient coverage from the alignments [47]. In the following steps, IsoLasso [48], an extension of
122 the SpliceGrapher workflow, was used to predict novel splicing events.

123 **Prediction of long noncoding RNA (lncRNA)**

124 All transcripts annotated as intergenic transcripts, intron transcripts, antisense exon transcripts
125 overlapping the reference exons, and antisense intron transcripts overlapping the reference
126 introns were considered potential lncRNA candidates. Transcript sequences of length <=200
127 nucleotides were filtered out, and the gffread function of Cufflinks was used to extract fasta
128 sequences of potential lncRNA transcripts from the gtf file. CPC2 [49] was used to predict the
129 coding potential of transcripts. Predicted lncRNAs were scanned by InterProScan [50] to ensure the
130 absence of protein-coding domains. To identify novel lncRNAs, a BLASTn [51] search was
131 performed against a custom BLAST database generated using rice lncRNAs downloaded from
132 the CANTATAdb2.0 (http://cantata.amu.edu.pl), PNRD [52], GreeNC [53], and RiceLNCPedia [54]
133 databases. Secondary structures for the lncRNA molecules were predicted by the RNAfold
134 software [55,56].
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136 **RESULTS**

137 **Light-mediated differential gene expression during photomorphogenesis**

138 To explore the transcriptome modulation in rice in response to light, we sequenced the strand-
139 specific poly-A enriched RNA fraction isolated from three biological replicates of rice plants
140 grown under dark and light exposure conditions (see methods). A total of ~38 million and ~42
141 million high-quality reads were generated from the dark and light-treated samples, respectively.
142 More than 92% of reads from each sample aligned to the rice reference genome (Supplementary
143 Table S1). We found 38,642 genes showed baseline normalized expression in the samples, of
144 which 33,943 genes expressed in the dark vs 35,772 genes that expressed under light,
145 respectively. Differential expression analysis identified 14,766 light-regulated genes (Figure 1,
146 Supplementary Figure S1, Supplementary Table S2).

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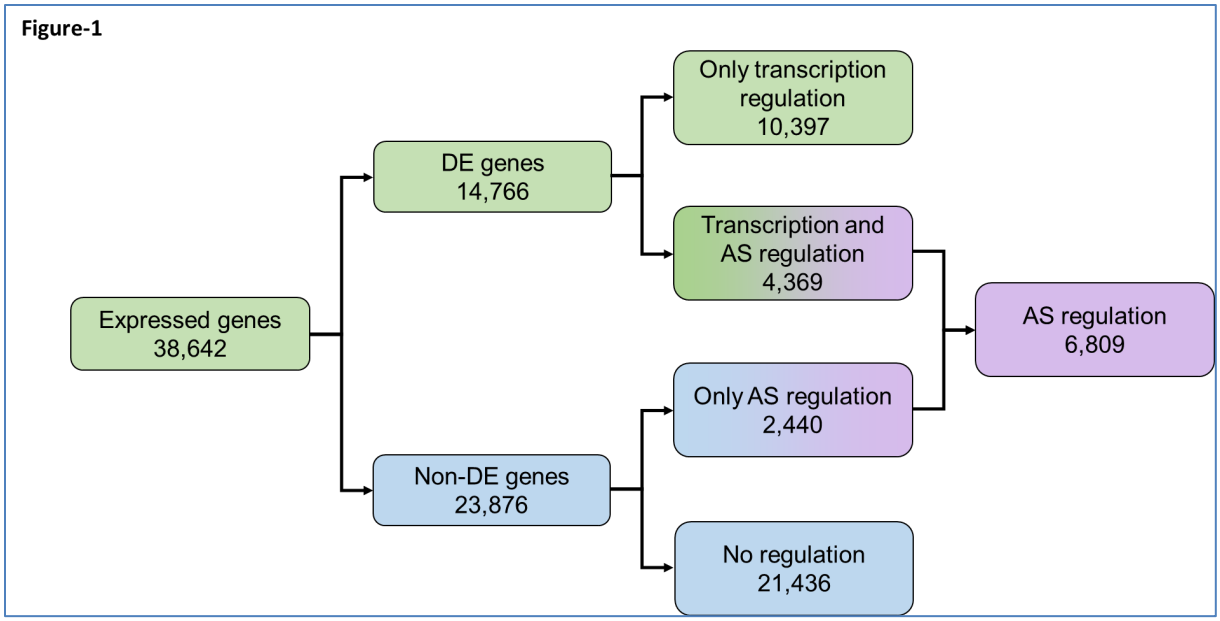


**Figure 1:** Summary of differential gene expression and transcript splicing observed in rice seedling shoots undergoing photomorphogenesis.

## Gene function and pathway enrichment analyses

Gene Ontology-based functional annotation analysis of the light-regulated differentially expressed gene set (Supplementary Figure S2) revealed enrichment for biological processes (BP), such as chlorophyll and cell wall biosynthesis, besides photosystem light reaction pathways and ribosome assembly. Dominant molecular functions (MF) were rRNA binding, cytoskeletal motor activity, oxidoreductase and metallopeptidase activities, translation elongation, and chaperone binding. As expected, chloroplast, cytoskeleton, ribosomes, peroxisomes, and nucleus were the major cellular component sites of activity.

Pathway enrichment analysis using the Plant Reactome pathway analysis tool [57] mapped 475 light-upregulated genes to 243 pathways (Supplementary Table S3). A total of 164 pathways overlapped between light-up and light-downregulated genes. Most pathways showed higher mappings to light-upregulated genes, except hormone auxin and brassinosteroid signaling and reproductive structure development (seed). Pathways unique to light-upregulated genes include those for the biosynthesis of photosynthesis components chlorophyll, carotenoid, and

166 phylloquinone, hormones like gibberellin, auxin, abscisic acid, *etc*. The pathways with unique
167 mapping to light-downregulated genes include polar auxin transport, mevalonate (MVA)
168 pathway, circadian clock, salicylic acid metabolism and signaling, reproductive plant part
169 development, root-specific gene network of NAC10 transcription factor (Supplementary Figure
170 S3).

171 **Identifying light-regulated transcription factors**

172 To identify the light-regulated transcription factors (TF), a list of rice TFs was downloaded from
173 the Plant Transcription Factor Database [58] and searched against the DE genes. We found 429
174 light-upregulated and 498 light-downregulated TFs (Supplementary Table S4). We found
175 WRKY, NAC, and orphans were the most abundantly expressed TFs in light, compared to many
176 light-downregulated bHLH, bZIP, and C3H gene family members (Table 1). Highly upregulated
177 (fold change ≥10) TFs belong to the MYB, AP2-EREBP, WRKY, Orphans, NAC, MADS, and
178 bHLH gene families; however, down upregulated TFs belong to the AP2-EREBP, C2C2-CO-
179 like, C3H, HB, and NAC gene families (Supplementary Table S4). To investigate whether TFs
180 targeted the MVA and MEP pathway genes, we surveyed the list of TFs and their potential
181 targets identified by the Plant Transcription Factor Database. We identified 23 TFs that
182 potentially bind to the promoter region of 6 MVA pathway genes. Two MVA pathway genes,
183 hydroxymethylglutaryl-CoA synthase (*HMGS*; Os08g0544900) and 3-hydroxy-3-methylglutaryl
184 coenzyme A reductase (*HMGR*; Os08g0512700), were targeted by bZIP and AP2-EREBP
185 factors, respectively, whereas the mevalonate 5-diphosphate decarboxylase (*MDD*;
186 Os02g0109100) gene was a target of by 13 AP2-EREBPs, one C3H protein, and one C2H2
187 protein (Supplementary Table S5). None of the TFs we found bind to the promoter of MEP
188 pathway genes.

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195 **Table 1:** Light-regulated transcription factor gene families and their members.

| Gene family | Upregulated | Downregulated | Gene family | Upregulated | Downregulated |
|---|---|---|---|---|---|
| ABI3VP1 | 6 | 4 | GRF | 1 | 2 |
| Alfin-like | 1 | 4 | HB | 21 | 24 |
| AP2-EREBP | 23 | 32 | HSF | 3 | 10 |
| ARF | 8 | 8 | LIM | 2 | 1 |
| ARR-B | 1 | 4 | LOB | 2 | 1 |
| BBR-BPC | 0 | 1 | MADS | 6 | 5 |
| BES1 | 0 | 3 | mTERF | 9 | 4 |
| bHLH | 19 | 27 | MYB | 28 | 14 |
| BSD | 1 | 6 | MYB-related | 16 | 25 |
| bZIP | 10 | 27 | NAC | 21 | 19 |
| C2C2-CO-like | 4 | 7 | OFP | 2 | 1 |
| C2C2-Dof | 5 | 4 | Orphans | 20 | 10 |
| C2C2-GATA | 7 | 4 | PBF-2-like | 1 | 0 |
| C2C2-YABBY | 3 | 1 | PLATZ | 1 | 4 |
| C2H2 | 14 | 19 | RWP-RK | 4 | 2 |
| C3H | 16 | 24 | S1Fa-like | 1 | 1 |
| CAMTA | 0 | 3 | SBP | 3 | 5 |
| CCAAT | 9 | 11 | Sigma 70-like | 4 | 0 |
| CPP | 1 | 4 | SRS | 1 | 0 |
| CSD | 0 | 1 | TAZ | 1 | 1 |
| DBP | 2 | 3 | TCP | 4 | 6 |
| E2F/DP | 2 | 1 | Tify | 6 | 2 |
| EIL | 1 | 1 | Trihelix | 3 | 9 |
| FAR1 | 5 | 10 | TUB | 2 | 5 |
| FHA | 7 | 6 | ULT | 1 | 0 |
| G2-like | 9 | 5 | VOZ | 0 | 2 |
| GeBP | 0 | 3 | WRKY | 22 | 10 |
| GRAS | 8 | 7 | zf-HD | 5 | 3 |
| **Other transcriptional regulators** | | | | | |
| **Gene family** | **Upregulated** | **Downregulated** | **Gene family** | **Upregulated** | **Downregulated** |
| ARID | 2 | 1 | PHD | 12 | 22 |
| AUX/IAA | 3 | 19 | Pseudo ARR-B | 2 | 1 |
| Coactivator p15 | 0 | 1 | RB | 0 | 1 |
| DDT | 3 | 0 | Rcd1-like | 0 | 2 |
| GNAT | 12 | 8 | SET | 11 | 6 |
| HMG | 4 | 5 | SNF2 | 11 | 9 |
| Jumonji | 4 | 5 | SOH1 | 0 | 2 |
| LUG | 0 | 2 | SWI/SNF-BAF60b | 4 | 4 |
| MBF1 | 0 | 1 | SWI/SNF-SWI3 | 2 | 1 |
| MED6 | 0 | 1 | TRAF | 7 | 11 |

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198 **Transcript splicing during photomorphogenesis**

199 To study how light exposure modulates transcript splicing (AS) events in the rice seedling
200 shoots, we analyzed the transcriptome data by using SpliceGrapher [47]. We created highly
201 accurate splice site classifiers for rice with canonical (GT-AG) and semi-canonical (GC-AG)
202 splice sites to filter the splice junctions in the sequence reads aligned to the reference genome.
203 After removing the false positive splice sites, we predicted chromosome-wise splice graphs from
204 the two transcriptome sample datasets. In the dark-treated dataset, 63.9% were true positive
205 junctions, with 5.5% novel splice sites. In the light-treated dataset, 64.3% of splice junctions
206 were true positives, of which 5.8% were predicted as novel sites. We observed 6214 spliced
207 genes with 9685 splicing events in the dark samples compared to the 6809 spliced genes with
208 10432 splicing events in the light samples (Figure 1, Table 2). The highest number of spliced
209 genes and events were on chromosome 1, and the lowest was on chromosome 10 (Table 2).
210 Intron retention (IR) was the most prevalent type of splicing event in both samples, followed by
211 exon skipping (ES). Alternative 3' splicing (Alt.3') was the least common event. To resolve
212 ambiguous combinations of donor and acceptor splice sites, we realigned the sequenced reads
213 from the two samples to the putative transcripts and resolved the novel exons to generate splice
214 graphs. Light-induced splicing in 2162 unique genes compared to 1567 unique genes that
215 undergo splicing in the dark. Of the 4647 spliced genes shared between the two samples, 165
216 genes displayed differential splicing events.

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225 **Table 2:** Summary of spliced genes and events under dark and light conditions detected by the
226 SpliceGrapher and the IsoLasso workflows. IR: Intron retention; ES: Exon Skipping; Alt5': 
227 alternative 5′ splice site; Alt3': alternative 3′ splice site. Color scales: Red (Large counts) to Blue
228 (Lower counts).

| Chromosome # | Treatment | SpliceGrapher Prediction | | | | | IsoLasso | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Genes | IR | ES | Alt5' | Alt3' | Genes | IR | ES | Alt5' | Alt3' |
| 1 | Dark | 924 | 897 | 214 | 163 | 97 | 939 | 925 | 214 | 181 | 99 |
| | Light | 1028 | 994 | 264 | 184 | 109 | 1048 | 1021 | 264 | 191 | 112 |
| 2 | Dark | 747 | 728 | 224 | 167 | 109 | 771 | 781 | 227 | 189 | 117 |
| | Light | 802 | 777 | 219 | 153 | 99 | 822 | 812 | 222 | 165 | 103 |
| 3 | Dark | 825 | 797 | 216 | 136 | 94 | 858 | 868 | 216 | 162 | 120 |
| | Light | 945 | 893 | 219 | 143 | 98 | 960 | 920 | 219 | 153 | 118 |
| 4 | Dark | 565 | 551 | 182 | 112 | 64 | 581 | 576 | 183 | 117 | 67 |
| | Light | 596 | 572 | 193 | 104 | 61 | 611 | 592 | 194 | 109 | 66 |
| 5 | Dark | 519 | 503 | 139 | 139 | 70 | 538 | 534 | 140 | 114 | 75 |
| | Light | 552 | 537 | 144 | 103 | 80 | 562 | 550 | 144 | 109 | 92 |
| 6 | Dark | 509 | 459 | 186 | 128 | 62 | 527 | 493 | 186 | 135 | 64 |
| | Light | 531 | 501 | 169 | 103 | 63 | 554 | 535 | 169 | 109 | 67 |
| 7 | Dark | 466 | 432 | 166 | 77 | 48 | 482 | 468 | 166 | 85 | 51 |
| | Light | 481 | 473 | 129 | 72 | 52 | 498 | 498 | 129 | 82 | 56 |
| 8 | Dark | 400 | 390 | 97 | 63 | 62 | 415 | 416 | 98 | 68 | 64 |
| | Light | 463 | 433 | 134 | 62 | 84 | 475 | 457 | 134 | 67 | 87 |
| 9 | Dark | 341 | 342 | 79 | 53 | 32 | 349 | 359 | 79 | 64 | 37 |
| | Light | 375 | 361 | 118 | 49 | 38 | 382 | 377 | 118 | 53 | 39 |
| 10 | Dark | 290 | 275 | 94 | 43 | 29 | 295 | 288 | 94 | 49 | 36 |
| | Light | 308 | 295 | 102 | 61 | 32 | 314 | 307 | 102 | 65 | 35 |
| 11 | Dark | 304 | 266 | 113 | 49 | 35 | 313 | 278 | 113 | 53 | 36 |
| | Light | 363 | 306 | 150 | 51 | 35 | 374 | 325 | 150 | 57 | 37 |
| 12 | Dark | 324 | 310 | 117 | 69 | 45 | 329 | 315 | 117 | 74 | 46 |
| | Light | 365 | 347 | 150 | 63 | 53 | 372 | 359 | 155 | 68 | 61 |

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231 In our differentially expressed and spliced genes (DES), we found that approximately 30%
232 (4369) differentially expressed (DE) genes also undergo splicing (AS). Approximately 10%
233 (2440) of the non-DE genes undergo splicing (Figure 1). Light-upregulated DES genes show
234 enrichment for various molecular functions like oxidoreductase, hydrolase, isomerase activity,
235 and RNA binding, which play roles in photosynthesis, transmembrane transport, small molecule
236 metabolic process, and located in the cellular components, chloroplast, and membrane-bound
237 organelles. In contrast, the light-downregulated DES genes enriched for RNA processing,
238 response to stress, protein binding, and macromolecule metabolic process, and localized in
239 cellular components cytoplasm and nucleus (Supplementary Figure S4).

240 We also surveyed the DES aspects of the 57 known spliced genes in rice [23,59] and observed
241 splicing in 41 genes (Table 3). Of these, seven spliced under dark conditions, three under light
242 conditions, and 31 genes spliced under both dark and light conditions. Of the known genes, we
243 also observed that 18 genes were light-upregulated and 24 downregulated. Only 32 genes
244 showed a DES profile. Three light-upregulated genes (Os02g0130600, Os05g0348100,
245 Os12g0567300) show complete splicing in light compared to intron retention in the dark. In
246 contrast, the two light-downregulated genes (Os02g0666200 and Os04g0656100) show intron
247 retention.

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256 **Table 3:** Expression and splicing profile of genes in our data that are known to undergo splicing
257 from previous reports

| Known spliced genes | Spliced in | Regulation in light | Gene description |
|---|---|---|---|
| Os01g0619000 | Dark, Light | Down | RNA recognition motif domain-containing protein |
| Os01g0649900 | Dark, Light | - | GDSL esterase/lipase protein 20 |
| Os01g0764000 | Dark, Light | Up | PHI glutathione s-transferase 2 |
| Os01g0834400 | Dark | - | HAP3A subunit of CCAAT-box binding complex |
| Os02g0122800 | Dark, Light | Down | Similar to Arginine/serine-rich splicing factor |
| Os02g0130600 | Dark | Up | Conserved hypothetical protein |
| Os02g0161900 | Dark, Light | Down | Rice ubiquitin2 |
| Os02g0197900 | Dark, Light | Down | OsSTA53 |
| Os02g0274900 | Dark, Light | Up | Similar to metabolite transport protein CSBC |
| Os02g0577100 | Dark, Light | Down | Zinc finger, RING/PHD-type domain containing protein |
| Os02g0666200 | Light | Down | Aquaporin |
| Os02g0834000 | Dark, Light | Up | Rac-like GTP-binding protein 5 |
| Os02g0291000 | - | Down | Calcineurin B-like protein 7 |
| Os02g0291400 | - | - | Calcineurin B-like protein 8 |
| Os02g0823100 | - | Up | Plasma membrane intrinsic protein 1;3 |
| Os03g0265600 | Dark, Light | Down | Transformer-2-like protein |
| Os03g0314100 | Dark, Light | Down | DEAD-like helicase |
| Os03g0395900 | Dark, Light | Down | Splicing factor |
| Os03g0670700 | Dark, Light | Down | Glycine-rich rna-binding protein 3 |
| Os03g0698500 | Dark, Light | Down | Similar to Yippee-like protein 3 |
| Os03g0745000 | Dark, Light | - | Heat stress transcription factor A-2a |
| Os03g0717600 | - | Down | Zinc finger, C2H2-type |
| Os04g0115400 | Dark, Light | Down | D111/G-patch domain containing protein |
| Os04g0649100 | Dark, Light | - | Shattering abortion 1 |
| Os04g0656100 | Light | Down | $H^+$-ATPase |
| Os04g0665800 | Dark, Light | Up | Similar to H1005F08.12 protein |
| Os04g0402300 | - | - | Cysteine-type peptidase |
| Os04g0479200 | - | Up | Similar to NAD-dependent isocitrate dehydrogenase c;1 |

| | | | |
|---|---|---|---|
| Os05g0348100 | Dark | Up | Similar to CRR23 (chlororespiratory reduction 23) |
| Os05g0463800 | Dark, Light | Down | HAP3C subunit of CCAAT-box binding complex |
| Os05g0554400 | Dark, Light | Down | Phosphatidyl serine synthase family protein |
| Os05g0574700 | Dark, Light | - | Similar to cDNA clone:002-182-C01 |
| Os05g0534400 | - | Up | Calcineurin B-like protein 4 |
| Os05g0548900 | - | Up | Phosphoethanolamine N-Methyltransferase 2 |
| Os06g0172800 | Light | - | Similar to alkaline alpha galactosidase 2 |
| Os06g0651600 | Dark, Light | Down | Protein phosphatase 2C58 |
| Os06g0727200 | Dark | Down | Catalase B |
| Os06g0128500 | - | Up | Ribosomal protein L47, mitochondrial family protein |
| Os06g0133000 | - | - | Glutinous Endosperm, waxy |
| Os06g0506600 | - | - | Ubiquitin-conjugating enzyme 17 |
| Os07g0490400 | Dark, Light | Up | FK506 binding protein 20-2 |
| Os07g0574800 | - | Up | Tubulin alpha-1 chain |
| Os07g0613300 | - | - | Similar to PAUSED |
| Os08g0191600 | Dark, Light | Up | Autophagy associated gene 8C |
| Os08g0530400 | Dark, Light | Up | Moco containing protein, Similar to sulfite oxidase |
| Os08g0436200 | - | - | Zinc finger, RING/PHD-type domain containing protein |
| Os10g0115600 | Dark, Light | Down | U1 snRNP 70K |
| Os10g0535800 | Dark, Light | - | Cys-rich domain containing protein |
| Os10g0564900 | Dark | Down | Similar to protein kinase CK2 regulatory subunit CK2B2 |
| Os10g0567400 | Dark, Light | Up | Chlorophyll a oxygenase 1 |
| Os10g0577900 | Dark, Light | - | Glycerol-3-phosphate acyltransferase |
| Os10g0411500 | - | Down | Q calmodulin-binding region domain containing protein |
| Os11g0157100 | Dark, Light | Down | Cyclin-T1-4 |
| Os11g0700500 | Dark | - | MybAS1 |
| Os11g0600700 | - | Up | Zinc finger, RING domain protein |
| Os12g0567300 | Dark | Up | MybAS2, R2R3-Myb |
| Os12g0632000 | Dark, Light | Down | Glycine-rich Protein GRP162 |

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260 **Light-mediated shift in gene expression and splicing events**

261 ***Circadian clock pathway genes***

262 The circadian clock is entrained by light, and the diurnal photoperiod regulated clock genes [60,61].
263 We investigated the light-regulated DES profile of clock genes. Most of the circadian clock
264 genes spliced under either or both conditions. Four genes, *Casein kinase alpha subunit* (*CK2α-*
265 *1/Hd6*, Os03g0762000), *Casein kinase beta subunit* (*CK2β-2*, Os10g0564900), *Phytochrome B*
266 (*PhyB,* Os03g0309200;), and *Timing of CAB Expression 1* (*TOC1*, Os02g0618200), showed
267 differential splicing (only reference splicing events or complete splicing and no novel isoforms)
268 upon light treatment compared to the presence of unspliced intron in the absence of light
269 (Supplementary Figure S5). In contrast, only one gene, *Phytochrome Interacting Factor 3* (*PIF3*,
270 Os01g0286100), showed intron retention under light. All five genes were upregulated in light
271 (Supplementary Figure S5). Seven genes, *Casein kinase alpha subunit* (*CK2α-2*,
272 Os03g0763000), *Casein kinase beta subunit* (*CK2β-1*, Os07g0495100), blue light receptor
273 *Cryptochrome 2* (*CRY2*, Os02g0625000), *Gigantea* (*GI*, Os01g0182600), *Pseudo-Response*
274 *Regulator 37* or *Heading date 2* (*PRR37*, *Hd2*, Os07g0695100), and *Pseudo-Response Regulator*
275 *73* (*PRR73*, Os03g0284100), showed differential splicing under both conditions (Supplementary
276 Figure S5, S6). Exon skipping and alternative 5' (Alt.5') splicing events in the *CK2α-2*
277 (Os03g0763000) gene were induced in light. Both *CK2β-1* (Os07g0495100) and *GI*
278 (Os01g0182600) display incomplete splicing of intron (IR) in the dark. *PRR37* showed three
279 types of splicing events (Alt.3', ES, and IR) under light, whereas the second IR event observed
280 toward the 3' end under dark was spliced in light. Conversely, *PRR73* showed three types of
281 events (Alt.3', ES, and IR) under dark conditions, whereas under light conditions, two new Alt.5'
282 events were identified. Expression of both PRR genes (*PRR37* and *PRR73*) was light
283 downregulated (Supplementary Figure S5). Light-upregulated *PRR95* shows an additional
284 unspliced intron in light.

285 ***MVA and MEP pathway genes***

286 Light regulates the biosynthesis and accumulation of secondary plant products like isoprenoid-
287 derived metabolites required for plant growth and development. Isoprenoid precursors are
288 synthesized via cytosolic MVA and plastid-localized MEP pathways, and their fluxes are

regulated by light [62,63], and their products are essential for the successful development and function of chloroplast. We observed that MEP pathway genes were light-upregulated compared to the downregulated MVA pathway genes (Figure 2). Light positively regulates MEP pathway activity, whereas MVA pathway activity appears negatively regulated by light [64].

We investigated the light-induced differential splicing events in the MEP and MVA pathway genes. Four MVA pathway genes (*acetyl-CoA acetyltransferase: AACT, hydroxymethyl glutaryl CoA synthase: HMGS, mevalonate kinase: MK, phosphomevalonate kinase: PMK*) and 3 MEP pathway genes (*1-deoxy-D-xylulose-5-phosphate synthase: DXS, 1-deoxy-D-xylulose-5-phosphate reductoisomerase: DXR, 2-C-methyl-D-erythritol2,4-cyclodiphosphate synthase: MECPS*) showed intron retention events in dark (Figure 2). Gene *MK* showed a novel light-induced exon-skipping event.

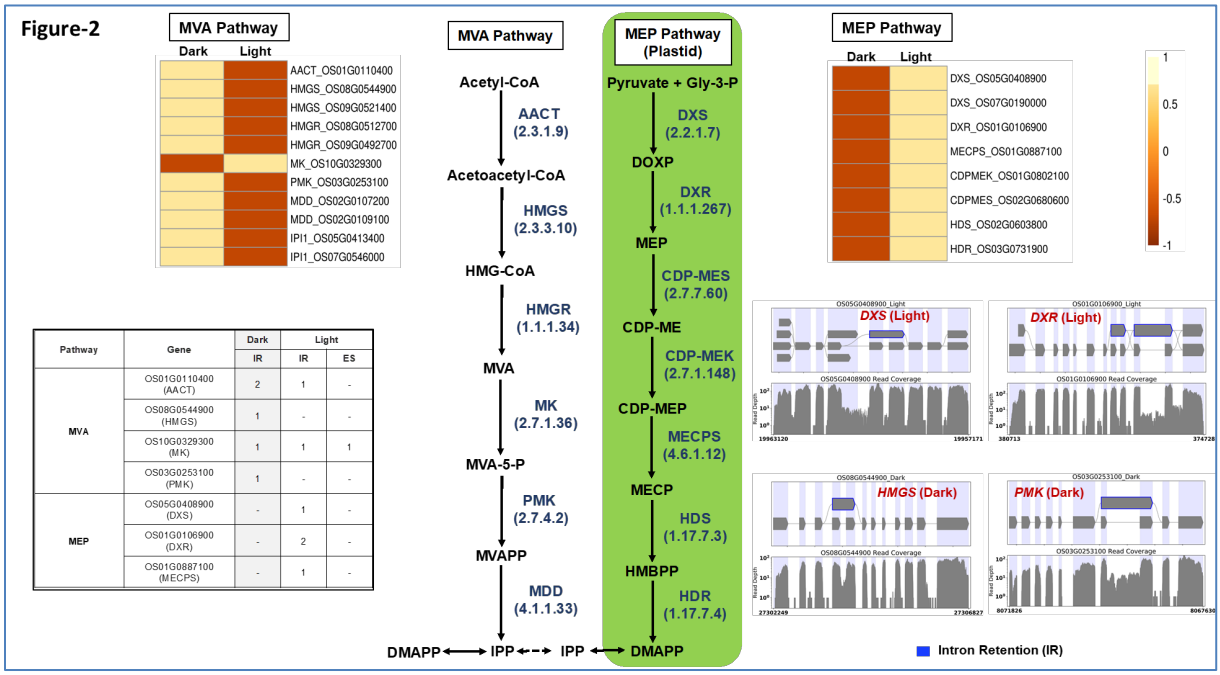


**Figure 2:** Gene expression and splicing of cytosolic mevalonate (MVA) and plastid-localized methyl erythritol 4-phosphate (MEP) pathway genes. *AACT*, acetyl-CoA acetyltransferase; *HMGS*, hydroxymethyl glutaryl CoA synthase; *HMGR*, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; *MK*, mevalonate kinase; *PMK*, phosphomevalonate kinase; *MDD*, mevalonate diphosphosphate decarboxylase; *DXS*, 1-deoxy-D-xylulose-5-phosphate synthase; *DXR*, 1-deoxy-D-xylulose-5-phosphate reductoisomerase; *CDPMES*, 2-C-methyl-D-erythritol4-

307 phosphate cytidylyl transferase; *CDPMEK*, 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase;
308 *MECPS*, 2-C-methyl-D-erythritol2,4-cyclodiphosphate synthase; *HDS*, 4-hydroxy-3-methylbut-
309 2-enyldiphosphate synthase; *HDR*, 4-hydroxy-3-methylbut-2-enyldiphosphate reductase. Blue:
310 intron retention, Green: exon skipping, Orange: Alt3' splicing, Purple: Alt5' splicing
311

312 **lncRNA discovery and splicing during photomorphogenesis**

313 lncRNAs are known to regulate diverse biological processes in plants, including
314 photomorphogenesis [65], and as described above, are categorized into four major types based on
315 their location in the gene space and on the DNA strand. We used our transcriptome data to
316 characterize light-induced rice lncRNAs (Supplementary Figure S7). We identified 1485 and
317 1407 lncRNA transcripts in the dark and light samples, of which 309 were common. Many
318 lncRNAs from intergenic and intronic regions were identified (Supplementary Figure S7). To
319 discover novel lncRNAs, the transcripts from our datasets were queried against the custom
320 BLAST database of rice lncRNAs. About 44% of the total lncRNA transcripts (2583) found a
321 match in the BLAST database (Supplementary Table S6). 173 lncRNAs spliced under dark and
322 142 spliced under light conditions, most located in the intergenic regions (Supplementary Figure
323 S7). Interestingly, all the spliced lncRNA genes from both datasets were from Chr1, Chr10,
324 Chr11, and Chr12. The maximum number of spliced lncRNA genes was from Chr1, followed by
325 Chr11, 12, and 10. Among all the splicing events, IR was predominant under both conditions.

326 We found lncRNA transcripts from light (TCONS_00012152) and dark (TCONS_00011794)
327 datasets that were present on the antisense strand overlapping an exon region of the *MADS27*
328 (Os02g0579600) gene. The transcript from the light-treated sample appears longer and showed
329 two alternative 3' splice sites and one exon skipping event. The TCONS_00011794 may
330 compete with MADS27 to form a complex with miRNA osa-miR444a known to play multiple
331 roles in the nitrate-dependent development pathway [66]. Among the list of lncRNAs present on
332 the antisense strand overlapping annotated genes, we identified a light-induced lncRNA
333 (TCONS_00001515) overlapping *ERF99* transcription factor (Os01g0868000) exon that may
334 play a role in its silencing. *ERF99* plays a central role in mediating abiotic stress responses [67].
335 Similarly, lncRNA transcript (TCONS_00015825) is present exclusively in light is transcribed
336 from the intronic region of the antisense strand of a B-type response regulator gene

337 (Os03g0224200), known for its involvement in cytokinin signaling, meristem maintenance, and
338 stress response [68,69]. The lncRNA transcript (TCONS_00026617) present exclusively in the dark
339 overlaps the intronic region of a phytochrome-interacting factor gene *PIF14* (Os07g0143200).
340 *PIF14* is known to bind the active form of phytochrome B and plays a crucial role in cross-talk
341 between light and stress signaling [70].

342 **Discussion**

343 Light is known to induce changes in the transcriptome, metabolome, and proteome of the plants
344 [71], which not only regulates the development, function, and physiology of the chloroplast but
345 also provides signals for modulating the plant's morphological, developmental, and
346 physiological adaptions in response to growth environment [7,38,72–74]. Though plants experience
347 constantly changing light conditions under the natural environment daily during their lifetime,
348 early development from germination to the seedling stage and acquiring the full autotrophic
349 capability is very much programmed by exposure to light. Therefore, we investigated
350 transcriptome modulation in rice seedlings during photomorphogenesis. Compared to earlier
351 studies in rice and *Arabidopsis*, where ~20% of the genes were reported differentially expressed
352 in dark-grown etiolated seedlings compared to light-exposed green seedlings [8,75,76], we observed
353 that the transition from skotomorphogenesis to photomorphogenesis alters differential expression
354 of ~38% of the rice genes.

355 Functional annotation of the differentially expressed genes enriched for roles in secondary
356 metabolism, chloroplast-related biosynthetic pathways, hormone biosynthesis and signaling,
357 amino acid biosynthesis, fatty acid metabolism, *etc*. (Supplementary Figure S3, Supplementary
358 Table S3). This result was expected based on the earlier reports on light regulation of such events
359 [22,77].

360 The plastids develop into chloroplasts by following an essential step of developing the thylakoid
361 membrane system and recruiting and assembling components of light and dark reactions to
362 establish a functional photosynthetic process. Many terpenoid compounds are essential
363 participants in plants' light-harvesting function and provide protection against damage from
364 reactive oxygen species (ROS). The basic isoprenoid units for terpenoid biosynthesis, such as
365 tocopherols, plastoquinones, carotenoids, chlorophylls, and precursors of the growth hormones

366 gibberellins and abscisic acid, are synthesized by the plastid-localized MEP pathway [78,79]. At the
367 same time, isoprenoid units synthesized by the MVA pathway contribute to the synthesis of
368 triterpenes, phytosterols, and phytohormones [80]. MEP and MVA pathways complement and
369 contribute to the formation of chlorophylls and carotenoids required for plastid development [81].
370 MEP pathway genes were light-upregulated in our transcriptome data compared to the light-
371 downregulated MVA pathway genes (Figure 2). These results were consistent with earlier
372 reports [82,83] and confirm that light acts as a critical regulator in modulating the availability of
373 isoprenoid precursors during photomorphogenesis [84]. Arabidopsis circadian clock genes (*LHY*,
374 *PRR9*, *CCA1*, *TOC1*) regulate MVA and MEP pathways [84]. In our dataset, we did not observe
375 any significant change in the expression of *LHY/CCA1*(Os08g0157600), *PRR95* (Os09g0532400,
376 a homolog of *AtPRR9*), and *TOC1* (Os02g0618200). However, all three genes spliced
377 differently. Under the light, *TOC1* showed no splicing but retained two introns in the dark. We
378 observed one exon-skipping event in light for the *LHY/CCA1* gene, whereas *PRR95* showed one
379 additional intron retention (Supplementary Figure S5).

380 Previous transcriptome studies showed that ~42% of *Arabidopsis*, ~63% of *soybean*, and ~56%
381 of maize genes undergo alternative splicing events [85–87]. Studies on rice by Wang and Brendel [23]
382 reported IR for 69% of 6568 AS genes, and Campbell *et al.* [88] reported 44.7% of 8772 AS genes.
383 In our dataset, 4369 genes undergo differential expression and alternative splicing, called DES
384 genes (Figure 1A). About 80% (5395) of the spliced genes (6809) showed IR events, suggesting
385 that IR is the major splicing event. The more significant number of splicing events in the light-
386 treated samples suggests that light-mediated gene expression and post-transcriptional mRNA
387 splicing play an important role in photomorphogenesis. The splicing events in rice circadian
388 clock genes suggest that IR regulates the expression of PRR genes (Supplementary Figure S5).
389 Many of the light-regulated genes of the MEP and MVA pathways show IR events, suggesting
390 that light plays a positive role in completing the splicing and provides a hypothesis that exposure
391 to light is an adaptive feature and even if the gene is expressed in dark, it stays unspliced until it
392 encounters light. Our data showed the presence of all three states, partially spliced in the dark
393 (*GI*, Os01g0182600), fully spliced in light (*GI*, Os01g0182600), and both states in light (*CK2β-*
394 *1*, Os07g0495100) (Supplementary Figure S6).

395 lncRNAs play regulatory functions in essential biological processes such as vernalization,
396 photomorphogenesis, and stress regulation [37,89,90] and display splicing [91]. 6480 long intergenic
397 noncoding RNAs (lincRNAs) were identified from 200 *Arabidopsis* transcriptome datasets [92];
398 however, we identified 827 lincRNAs in the dark and 727 lincRNAs in the light datasets.
399 Corona-Gomez and coworkers [93] characterized the splicing conservation of lncRNAs in
400 *Brassicaceae*, which revealed that ~18% of lincRNAs display splicing conservation in at least
401 one exon. In our dataset, ~10% of lncRNA transcripts undergo splicing, and the majority were of
402 long intergenic noncoding RNA type (Supplementary Figure S7). Komiya and colleagues
403 reported [94] that phased small interfering RNAs (phasiRNAs) are generated from over 700
404 lincRNAs, and these phasiRNAs bind to rice argonaute protein MEL1. MEL1 has a specific
405 function in developing pre-meiotic germ cells and the progression of meiosis. In our dataset,
406 many lincRNAs transcribed from MEL1-phasiRNA clusters appeared.

407 Identification of light-induced lncRNAs antisense to *ERF99*, B-type response regulator genic
408 region may play a role in its transcriptional regulation. *ERF99* is known to modulate root
409 architecture and downregulated in crown root primordia [95]. *MADS27* gene targeted by lncRNAs
410 present in both dark and light conditions. However, the dark lncRNA (TCONS_00011794) is
411 longer and, based on its predicted RNA fold structure and lower free energy, is likely more stable
412 than its transcript TCONS_00012152 in light. For the MADS27 gene, we also observed an
413 additional IR event in light. MADS27-miR444 complex is known to play a role in plant
414 development in a nitrate-dependent manner [96–98]. Therefore, we investigated the nitrogen
415 assimilation pathway genes. Nitrogen assimilation is necessary for sustaining plant growth and
416 development. Various nitrogen assimilatory enzymes are known to show isoform and cellular
417 component-specific responses under light and dark conditions [99,100]. We observed light
418 upregulation for the nitrate transporter, nitrate reductase, and nitrite reductase genes. We also
419 observed differential splicing patterns for these genes (Supplementary Figure S8).

420

421 **Conclusion**

422 This study suggests that light is a significant regulatory factor controlling genome-wide gene
423 expression through an alternative splicing mechanism in rice. All spliced genes did not
424 necessarily produce novel isoforms, which indicates the coupling of AS and nonsense-mediated

425 decay (NMD). NMD prevents the translation of mutant mRNAs harboring potential premature
426 stop codons by targeting them for degradation. In *Arabidopsis*, 77.2% of light-regulated AS
427 events exhibit NMD features within a splicing isoform [101]. We conclude that light induces a
428 significant number of splicing events in rice protein-coding and noncoding lncRNA genes. This
429 photomorphogenesis transcriptome study is a valuable resource for lncRNA research in rice and
430 provides insights into the portion of the genome regulated at the level of alternative splicing in
431 response to light. We expect the condition-dependent novel splice events discovered in this study
432 will help improve reference rice genome annotation.

433

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441

442 **Author's Contributions**

443 PJ conceptualized the project. PG performed the data analysis. PG and PJ wrote the manuscript.

444

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450

451 **Conflict of interest**

452 The authors declare no conflict of interest.

453

454 **Availability of data**

455 The sequence reads and metadata were deposited at EMBL-EBI ArrayExpress (accession
456 number E-MTAB-5689). https://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-5689/#.

457

458 **Figure legends**

459 **Figure 1:** Summary of differential gene expression and transcript splicing observed in rice
460 seedling shoots undergoing photomorphogenesis.
461
462 **Figure 2:** Gene expression and splicing of cytosolic mevalonate (MVA) and plastid-localized
463 methyl erythritol 4-phosphate (MEP) pathway genes. *AACT*, acetyl-CoA acetyltransferase;
464 *HMGS*, hydroxymethyl glutaryl CoA synthase; *HMGR*, 3-hydroxy-3-methylglutaryl-coenzyme
465 A reductase; *MK*, mevalonate kinase; *PMK*, phosphomevalonate kinase; *MDD*, mevalonate
466 diphosphosphate decarboxylase; *DXS*, 1-deoxy-D-xylulose-5-phosphate synthase; *DXR*, 1-
467 deoxy-D-xylulose-5-phosphate reductoisomerase; *CDPMES*, 2-C-methyl-D-erythritol4-
468 phosphate cytidylyl transferase; *CDPMEK*, 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase;
469 *MECPS*, 2-C-methyl-D-erythritol2,4-cyclodiphosphate synthase; *HDS*, 4-hydroxy-3-methylbut-
470 2-enyldiphosphate synthase; *HDR*, 4-hydroxy-3-methylbut-2-enyldiphosphate reductase. Blue:
471 intron retention, Green: exon skipping, Orange: Alt3' splicing, Purple: Alt5' splicing
472
473

474 **Table legends**

475 **Table 1:** Light-regulated transcription factor gene families and their members.

476

477 **Table 2:** Summary of spliced genes and events under dark and light conditions detected by the
478 SpliceGrapher and the IsoLasso workflows. IR: Intron retention; ES: Exon Skipping; Alt5':
479 alternative 5′ splice site; Alt3': alternative 3′ splice site. Color scales: Red (Large counts) to Blue
480 (Lower counts).

481

482 **Table 3:** Expression and splicing profile of genes in our data known to undergo splicing from
483 previous reports

484

485 **Supplementary material**

486 **Supplementary Figure S1:**

487 Expression pattern of differentially expressed genes (14,766) with target FDR controlled at 5%

488

489 **Supplementary Figure S2:**

490 Gene Ontology enrichment analysis of differentially expressed genes.

491

492 **Supplementary Figure S3:**

493 Pathway enrichment analysis using the Plant Reactome. **(A)** Plant Reactome pathway enrichment
494 analysis plots, **(B)** Unique and shared pathways enriched for the light upregulated and
495 downregulated gene sets; **(C)** Counts of genes mapped to some of the common Plant Reactome
496 pathways. Light upregulated (green) and downregulated (grey).

497

498 **Supplementary Figure S4**:

499 Bar plots of most significantly enriched GO terms for DES genes with -log10 transformed FDR
500 values. Light-upregulated (green), light-downregulated (grey).

501

502 **Supplementary Figure S5**:

503 The summary of transcript splicing events and expression pattern of circadian clock genes during
504 morphogenesis.

505

506 **Supplementary Figure S6**:

507 Transcript graphs showing splicing of *Casein kinase beta subunit:CK2β-1* (Os07g0495100),
508 *Gigantea:GI* (Os01g0182600) genes during photomorphogenesis.

509

510 **Supplementary Figure S7**:

511 Classification of lncRNAs identified in dark and light.

512

513 **Supplementary Figure S8:**

514 An overviw of splicing and expression pattern of nitrogen assimilation cycle genes during
515 photomorphogenesis. NRT: Nitrate transporter; NR: Nitrate reductase; NiR: Nitrate reductase.

516

517 **Supplementary Table S1**:

518 Summary of the RNA-Seq read mapping to the reference rice genome.

519

520 **Supplementary Table S2:**

521 Differentially expressed genes

522

523 **Supplementary Table S3:**

524 Plant Reactome Pathway enrichment analysis for light-regulated genes

525

526 **Supplementary Table S4:**

527 Transcription factor genes regulated in light

528

529 **Supplementary Table S5:**

530 Transcription factors regulating MVA pathway genes

531

532 **Supplementary Table S6:**

533 lncRNA annotation using BLAST

534

535

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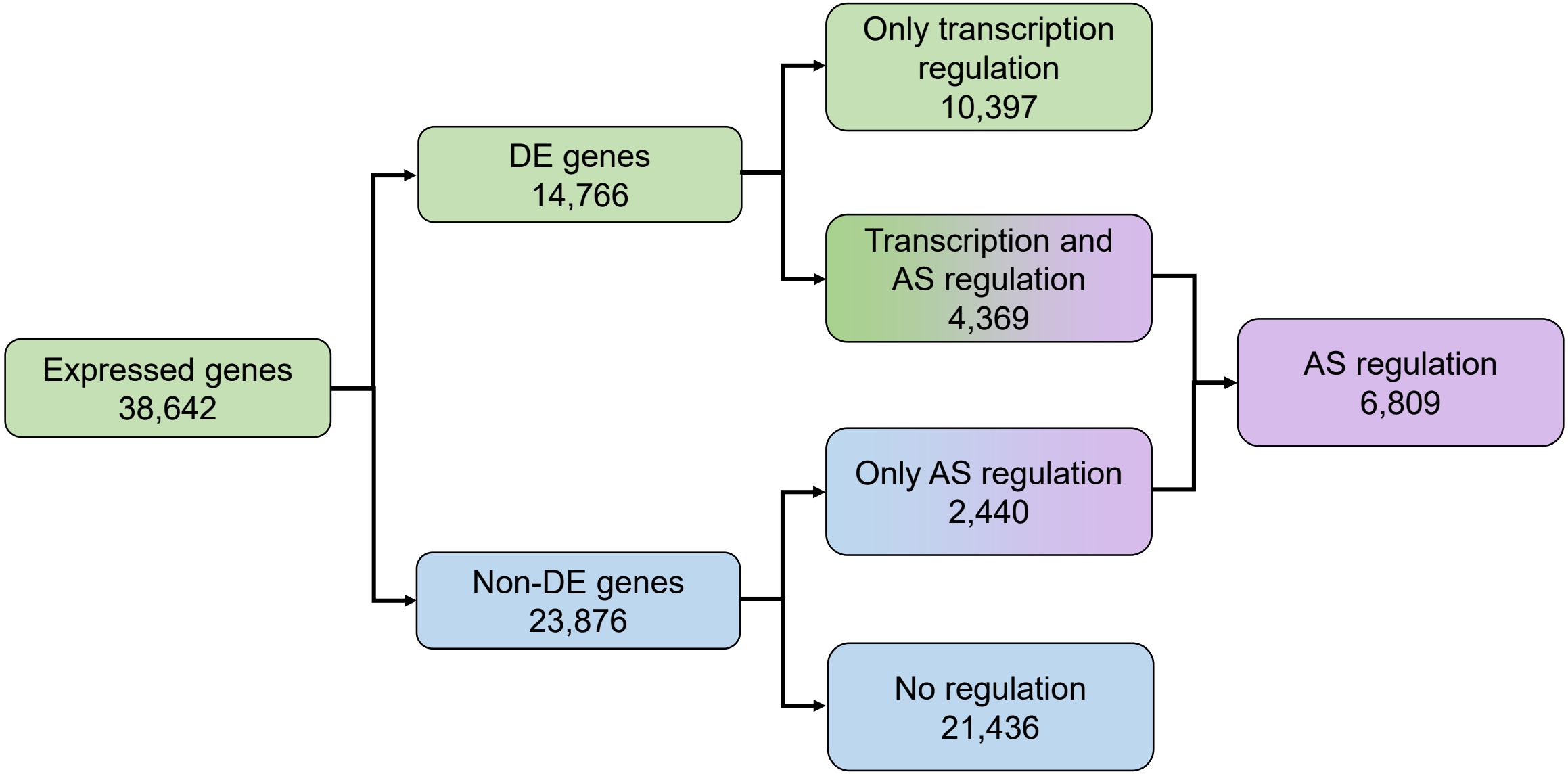
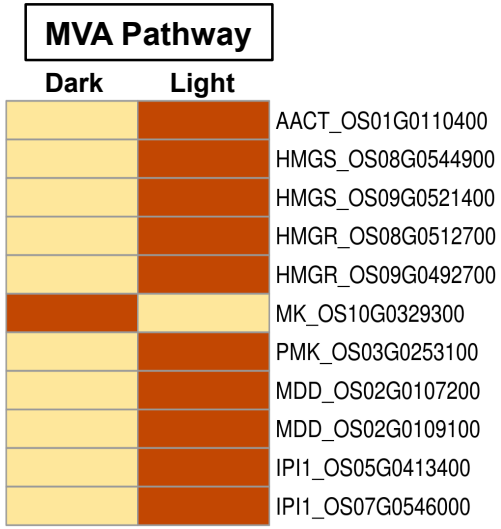
768

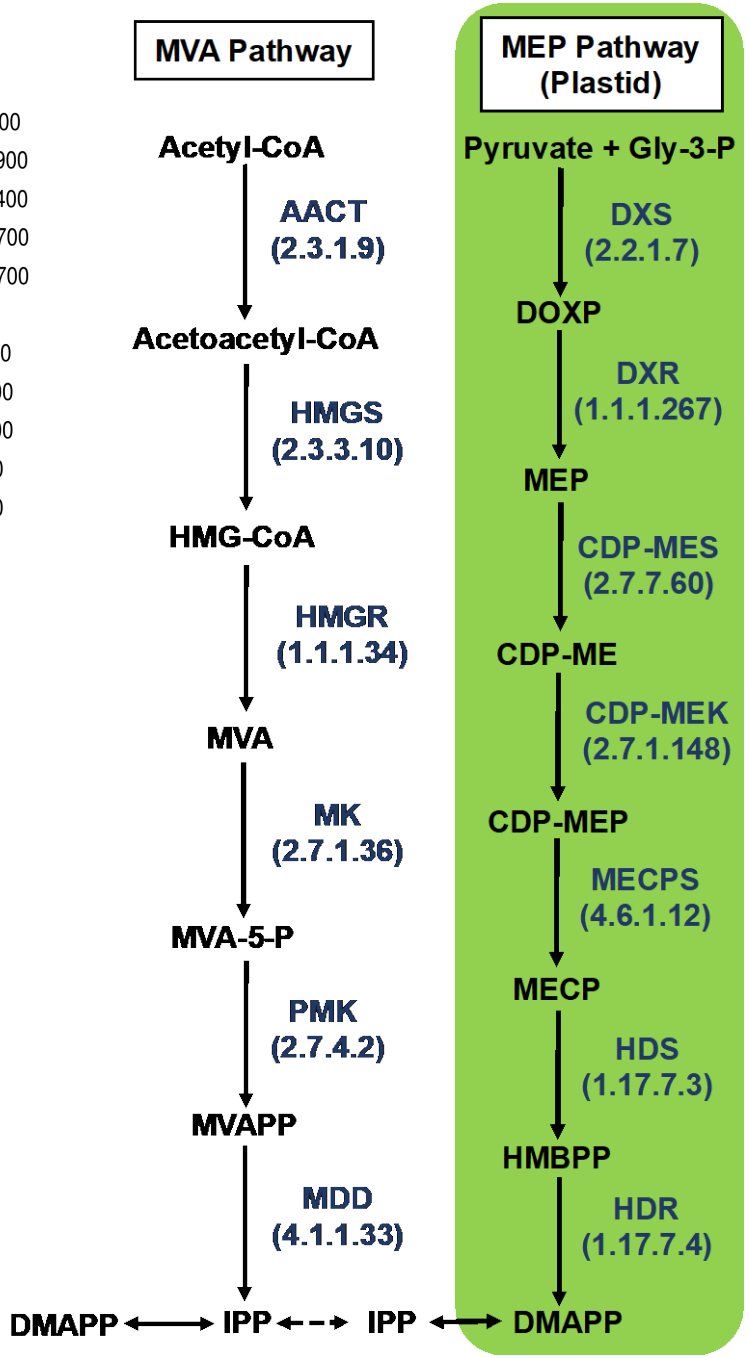
769

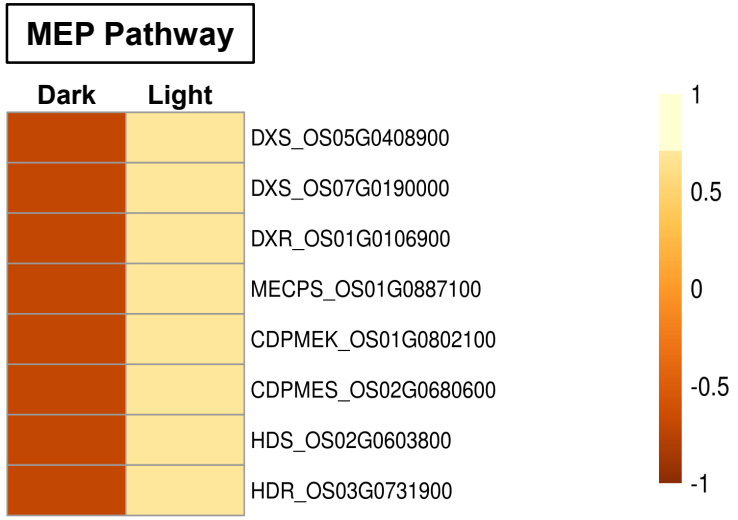
**Figure-1**


Expressed genes
38,642
DE genes
14,766
Non-DE genes
23,876
Only transcription regulation
10,397
Transcription and AS regulation
4,369
Only AS regulation
2,440
No regulation
21,436
AS regulation
6,809

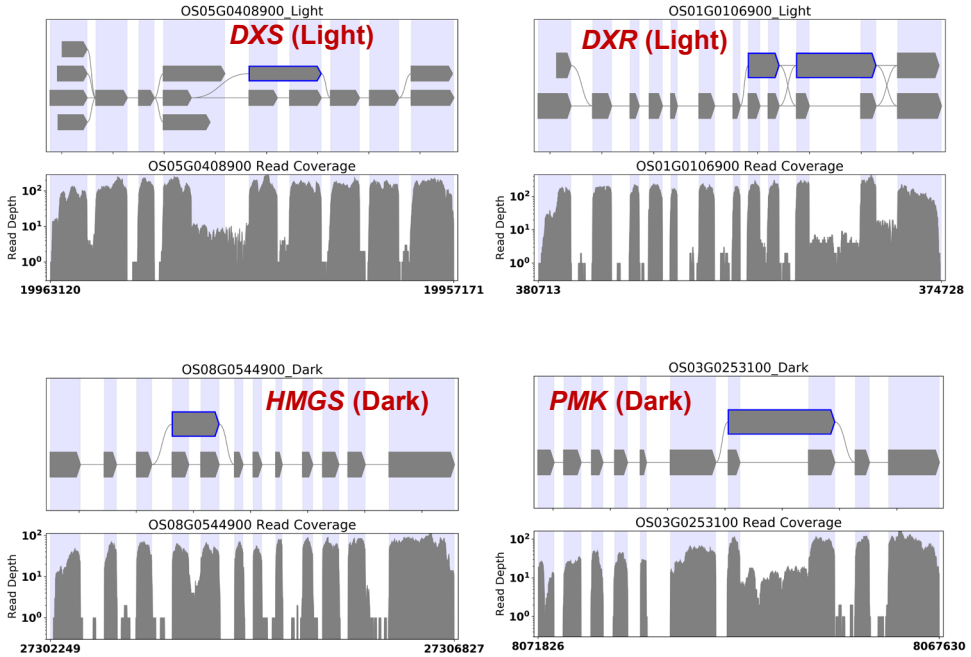
# Figure-2




| Pathway | Gene | Dark | Light | |
|---|---|---|---|---|
| | | IR | IR | ES |
| MVA | OS01G0110400 (AACT) | 2 | 1 | - |
| | OS08G0544900 (HMGS) | 1 | - | - |
| | OS10G0329300 (MK) | 1 | 1 | 1 |
| | OS03G0253100 (PMK) | 1 | - | - |
| MEP | OS05G0408900 (DXS) | - | 1 | - |
| | OS01G0106900 (DXR) | - | 2 | - |
| | OS01G0887100 (MECPS) | - | 1 | - |




Intron Retention (IR)