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 light-upregulated 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.

INTRODUCTION

Light is an essential growth factor for sustaining autotrophic plant life. The quality, quantity, direction, and length of light exposure affect plant growth and development [1,2,3]. The natural light-dark cycle maintains the carbon and nitrogen metabolism of rice plants [4]. Light induces transcriptional reprogramming in various plant species [4–12] via an array of photoreceptors [13,14]. The photoreceptors phytochrome (red light) and cryptochrome (blue light) are involved in light signaling and photomorphogenesis [15,16]. Genes encoding phytochrome interacting factors (PIFs) promote skotomorphogenesis and development in the dark [17–19]. Light-mediated gene expression modulation is also triggered by translational enhancement of the preexisting mRNA pool instead of an enhanced transcription rate [20,21].

Intron splicing in multiexonic mRNA is a post-transcriptional regulatory process that often produces different mRNA isoforms transcribed from a single gene locus, thus contributing to proteome plasticity. Light-induced alternative splicing (AS) was observed in ~10% of the protein-coding genes of *Arabidopsis* and *Physcomitrella patens* [22,23]. A flash of light applied in the middle of the dark or nighttime is sufficient to induce splicing [24]. In contrast to animals, where the most common AS event is exon skipping (ES), intron retention (IR) is the most common AS event in rice [25], Arabidopsis [25,26], and Poplar [27]. It is now well established that transcriptome modulation via AS is vital for plant growth, development, and stress response [28–30].

Compared to the protein-coding genes, long non-coding RNAs (lncRNAs) are transcripts >200 bp in length that do not have protein-coding potential. lncRNAs are classified into categories: (i) sense lncRNAs, (ii) antisense lncRNAs, (iii) intergenic RNAs (lincRNAs), (iv) intronic RNAs, and (v) bidirectional lncRNAs [31]. In addition to the numerous studies coupling gene expression and AS studies on protein-coding genes, it is now known that lncRNAs also play a role in regulating gene expression through transcriptional, post-transcriptional, and chromatin remodeling mechanisms [31–34]. In rice, lncRNAs regulate biological processes, such as ovule development and female gametophyte abortion [35], sexual reproduction [36], and stress response [37,38]. However, the role of light in regulating lncRNAs is not well studied. *Arabidopsis* non-coding RNA *HID1* is a known positive regulator of photomorphogenesis in continuous red light [39].

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

64 MATERIALS & METHODS

65 Plant material, growth conditions, and treatment

66 Seeds of rice (*Oryza sativa spp. japonica* cv. Nipponbare) were grown and processed for the
67 experiment by following the growth conditions and sampling described previously [40]. Seeds were
68 sown in the dark and germinated on day 2. After sowing, these germinated seedlings grew in the
69 dark for 8 days (8DD). At the end of day 8, three biological replicates of the dark-grown
70 etiolated shoots were harvested. The remaining dark-grown seedlings were exposed to
71 continuous white light at 120 µmol/m2/sec (measured at the soil surface) for 48 hours or 2 days
72 (days 9 and 10 after sowing). The shoots of three biological replicates of light-treated green-
73 colored seedling samples (8DD-2LL) were harvested at the end of day 10. Harvested samples
74 were frozen using liquid nitrogen and stored at -80°C until further processing. Throughout this
75 report, 8DD-treated plants are called dark samples, and 8DD-2LL light-treated plants are called
76 light samples. Data analysis is described for light regulation.

77 Sample preparation and sequencing

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

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

89 **RNA-seq data analysis**

90 The generation of FASTQ files from the RNA-Seq sequences was performed by CASAVA
91 software v1.8.2 (Illumina Inc.). Sequence reads were filtered and trimmed for low quality at a
92 score of 20 using Sickle v1.33 [41]. Clean, high-quality reads from each sample and replicates were
93 aligned to the *Oryza sativa japonica* cv Nipponbare reference genome (IRGSP-1.0.31) using
94 TopHat v2.1.1 [42]. Mapped reads were assembled using Cufflinks, and the reference-guided
95 assembled transcripts from each replicate were merged using Cuffmerge [43]. Assembled
96 transcripts were compared to the reference genome annotation using Cuffcompare. The RSEM
97 software package estimated normalized baseline expression from the aligned sequence reads [44].
98 For differential gene expression analysis, read count data obtained from RSEM were used in
99 EBSeq [45]. Differentially expressed (DE) genes were filtered based on the false discovery rate
100 corrected P value ≤0.05.

101 **Functional annotation**

102 We carried out the Gene Ontology (GO) enrichment analysis tool provided by the GO
103 consortium [46] to determine the biological roles played by the enriched gene set. Plant pathway
104 enrichment analysis was done by mapping the DE genes using the Plant Reactome analysis tool
105 [47,48,49] (http://plantreactome.gramene.org/PathwayBrowser/#TOOL=AT).

106

107 **Alternative splicing analysis**

108 Splicing events in the transcripts from the samples were identified by the SpliceGrapher v0.2.5
109 pipeline [50]. Sequence reads from each sample, and replicates were aligned to the reference rice
110 genome. Splice site-specific classifiers were built using build_classifiers.py script using
111 canonical (GT) and noncanonical (GC) donor sites and acceptor site (AG) for *Oryza sativa*
112 genome annotation version 31 (Oryza_sativa.IRGSP-1.0.31). Read alignments in SAM format
113 from each replicate were used as input for SpliceGrapher's sam_filter.py script to filter out false-
114 positive sites. SpliceGrapher Python scripts were used for the generation of depth files

115 (sam_to_depths.py), splice graph prediction (predict_graphs.py), generating statistics
116 (splicegraph_statistics.py) from a set of splice graphs, gene-by-gene summary
117 (genewise_statistics.py) of splicing events and splice graph visualization (plotter.py). The
118 Realignment pipeline was used to construct putative transcripts from unresolved exons with
119 sufficient coverage from the alignments [50]. In the following steps, IsoLasso [51], an extension of
120 the SpliceGrapher workflow, was used to predict novel splicing events.

121 **Prediction of long non-coding RNA (lncRNA)**

122 All transcripts annotated as intergenic transcripts, intron transcripts, antisense exon transcripts
123 overlapping the reference exons, and antisense intron transcripts overlapping the reference
124 introns were considered potential lncRNA candidates. Transcript sequences of length <=200
125 nucleotides were filtered out, and the gffread function of Cufflinks was used to extract fasta
126 sequences of potential lncRNA transcripts from the gtf file. CPC2 [52] was used to predict the
127 coding potential of transcripts. Predicted lncRNAs were scanned by InterProScan [53] to ensure the
128 absence of protein-coding domains. To identify novel lncRNAs, a BLASTn [54] search was
129 performed against a custom BLAST database generated using rice lncRNAs downloaded from
130 the CANTATAdb2.0 (http://cantata.amu.edu.pl), PNRD [55], GreeNC [56], and RiceLNCPedia [57]
131 databases. Secondary structures for the lncRNA molecules were predicted by the RNAfold
132 software [58,59].
133

134

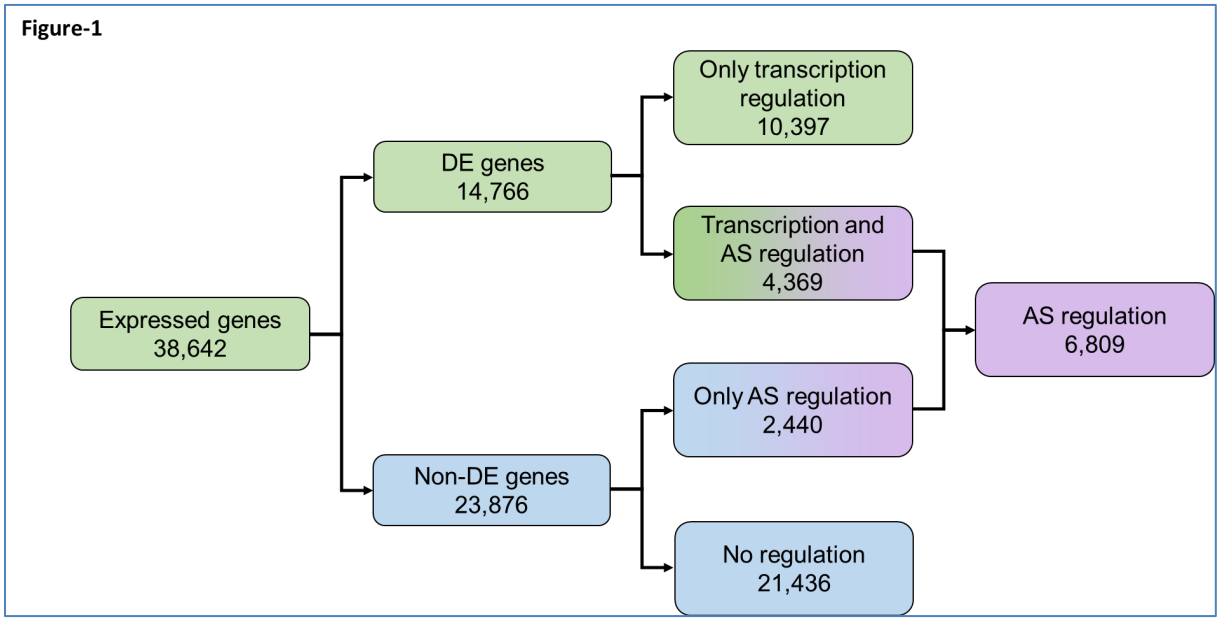
135 **RESULTS**

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

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

144 respectively. Differential expression analysis identified 14,766 light-regulated genes (Figure 1,
145 Supplementary Figure S1, Supplementary Table S2).

146



147

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

150

151 **Gene function and pathway enrichment analyses**

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

159 Pathway enrichment analysis using the Plant Reactome pathway analysis tool [60] mapped 475
160 light-upregulated genes to 243 pathways (Supplementary Table S3). A total of 164 pathways

161 overlapped between light-up and light-downregulated genes. Most pathways showed higher
162 mappings to light-upregulated genes, except hormone auxin and brassinosteroid signaling and
163 reproductive structure development (seed). Pathways unique to light-upregulated genes include
164 those for the biosynthesis of photosynthesis components chlorophyll, carotenoid, and
165 phylloquinone, hormones like gibberellin, auxin, abscisic acid, *etc*. The pathways with unique
166 mapping to light-downregulated genes include polar auxin transport, mevalonate (MVA)
167 pathway, circadian clock, salicylic acid metabolism and signaling, reproductive plant part
168 development, root-specific gene network of NAC10 transcription factor (Supplementary Figure
169 S3). To our surprise, we observed enrichment of the

170 **Identifying light-regulated transcription factors**

171 To identify the light-regulated transcription factors (TF), a list of rice TFs was downloaded from
172 the Plant Transcription Factor Database [61] and searched against the DE genes. We found 429
173 light-upregulated and 498 light-downregulated TFs (Supplementary Table S4). We found
174 WRKY, NAC, and orphans were the most abundantly expressed TFs in light, compared to many
175 light-downregulated bHLH, bZIP, and C3H gene family members (Table 1). Highly upregulated
176 (fold change ≥10) TFs belong to the MYB, AP2-EREBP, WRKY, Orphans, NAC, MADS, and
177 bHLH gene families; however, down upregulated TFs belong to the AP2-EREBP, C2C2-CO-
178 like, C3H, HB, and NAC gene families (Supplementary Table S4). To investigate whether TFs
179 targeted the MVA and MEP pathway genes, we surveyed the list of TFs and their potential
180 targets identified by the Plant Transcription Factor Database. We identified 23 TFs that
181 potentially bind to the promoter region of 6 MVA pathway genes. Two MVA pathway genes,
182 hydroxymethylglutaryl-CoA synthase (*HMGS*; Os08g0544900) and 3-hydroxy-3-methylglutaryl
183 coenzyme A reductase (*HMGR*; Os08g0512700), were targeted by bZIP and AP2-EREBP
184 factors, respectively, whereas the mevalonate 5-diphosphate decarboxylase (*MDD*;
185 Os02g0109100) gene was a target of by 13 AP2-EREBPs, one C3H protein, and one C2H2
186 protein (Supplementary Table S5). None of the TFs we found bind to the promoter of MEP
187 pathway genes.

188

189 **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

190

191

192 **Transcript splicing during photomorphogenesis**

193 To study how light exposure modulates transcript splicing (AS) events in the rice seedling
194 shoots, we analyzed the transcriptome data by using SpliceGrapher [50]. We created highly
195 accurate splice site classifiers for rice with canonical (GT-AG) and semi-canonical (GC-AG)
196 splice sites to filter the splice junctions in the sequence reads aligned to the reference genome.
197 After removing the false positive splice sites, we predicted chromosome-wise splice graphs from
198 the two transcriptome sample datasets. In the dark-treated dataset, 63.9% were true positive
199 junctions, with 5.5% novel splice sites. In the light-treated dataset, 64.3% of splice junctions
200 were true positives, of which 5.8% were predicted as novel sites. We observed 6214 spliced
201 genes with 9685 splicing events in the dark samples compared to the 6809 spliced genes with
202 10432 splicing events in the light samples (Figure 1, Table 2). The highest number of spliced
203 genes and events were on chromosome 1, and the lowest was on chromosome 10 (Table 2).
204 Intron retention (IR) was the most prevalent type of splicing event in both samples, followed by
205 exon skipping (ES). Alternative 3' splicing (Alt.3') was the least common event. To resolve
206 ambiguous combinations of donor and acceptor splice sites, we realigned the sequenced reads
207 from the two samples to the putative transcripts and resolved the novel exons to generate splice
208 graphs. Light-induced splicing in 2162 unique genes compared to 1567 unique genes that
209 undergo splicing in the dark. Of the 4647 spliced genes shared between the two samples, 165
210 genes displayed differential splicing events.

211

212 **Table 2:** Summary of spliced genes and events under dark and light conditions detected by the
213 SpliceGrapher and the IsoLasso workflows. IR: Intron retention; ES: Exon Skipping; Alt5':
214 alternative 5′ splice site; Alt3': alternative 3′ splice site. Color scales: Red (Large counts) to Blue
215 (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

216

217 In our differentially expressed and spliced genes (DES), we found that approximately 30%
218 (4369) differentially expressed (DE) genes also undergo splicing (AS). Approximately 10%
219 (2440) of the non-DE genes undergo splicing (Figure 1). Light-upregulated DES genes show
220 enrichment for various molecular functions like oxidoreductase, hydrolase, isomerase activity,
221 and RNA binding, which play roles in photosynthesis, transmembrane transport, small molecule
222 metabolic process, and located in the cellular components, chloroplast, and membrane-bound
223 organelles. In contrast, the light-downregulated DES genes enriched for RNA processing,
224 response to stress, protein binding, and macromolecule metabolic process, and localized in
225 cellular components cytoplasm and nucleus (Supplementary Figure S4).

226 We also surveyed the DES aspects of the 57 known spliced genes in rice [25,62] and observed
227 splicing in 41 genes (Table 3). Of these, seven spliced under dark conditions, three under light
228 conditions, and 31 genes spliced under both dark and light conditions. Of the known genes, we
229 also observed that 18 genes were light-upregulated and 24 downregulated. Only 32 genes
230 showed a DES profile. Three light-upregulated genes (Os02g0130600, Os05g0348100,
231 Os12g0567300) show complete splicing in light compared to intron retention in the dark. In
232 contrast, the two light-downregulated genes (Os02g0666200 and Os04g0656100) show intron
233 retention.

234

235 **Table 3:** Expression and splicing profile of genes in our data that are known to undergo splicing
236 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

237

238

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

240 ***Circadian clock pathway genes***

241 The circadian clock is entrained by light, and the diurnal photoperiod regulated clock genes [63,64].
242 We investigated the light-regulated DES profile of clock genes. Most of the circadian clock
243 genes spliced under either or both conditions. Four genes, *Casein kinase alpha subunit* (*CK2α-*
244 *1/Hd6*, Os03g0762000), *Casein kinase beta subunit* (*CK2β-2*, Os10g0564900), *Phytochrome B*
245 (*PhyB,* Os03g0309200;), and *Timing of CAB Expression 1* (*TOC1*, Os02g0618200), showed
246 differential splicing (only reference splicing events or complete splicing and no novel isoforms)
247 upon light treatment compared to the presence of unspliced intron in the absence of light
248 (Supplementary Figure S5). In contrast, only one gene, *Phytochrome Interacting Factor 3* (*PIF3*,
249 Os01g0286100), showed intron retention under light. All five genes were upregulated in light
250 (Supplementary Figure S5). Seven genes, *Casein kinase alpha subunit* (*CK2α-2*,
251 Os03g0763000), *Casein kinase beta subunit* (*CK2β-1*, Os07g0495100), blue light receptor
252 *Cryptochrome 2* (*CRY2*, Os02g0625000), *Gigantea* (*GI*, Os01g0182600), *Pseudo-Response*
253 *Regulator 37* or *Heading date 2* (*PRR37*, *Hd2*, Os07g0695100), and *Pseudo-Response Regulator*
254 *73* (*PRR73*, Os03g0284100), showed differential splicing under both conditions (Supplementary
255 Figure S5, S6). Exon skipping and alternative 5' (Alt.5') splicing events in the *CK2α-2*
256 (Os03g0763000) gene were induced in light. Both *CK2β-1* (Os07g0495100) and *GI*
257 (Os01g0182600) display incomplete splicing of intron (IR) in the dark. *PRR37* showed three
258 types of splicing events (Alt.3', ES, and IR) under light, whereas the second IR event observed
259 toward the 3' end under dark was spliced in light. Conversely, *PRR73* showed three types of
260 events (Alt.3', ES, and IR) under dark conditions, whereas under light conditions, two new Alt.5'
261 events were identified. Expression of both PRR genes (*PRR37* and *PRR73*) was light
262 downregulated (Supplementary Figure S5). Light-upregulated *PRR95* shows an additional
263 unspliced intron in light.

264 ***MVA and MEP pathway genes***

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

regulated by light [65,66], 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 [67].

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.

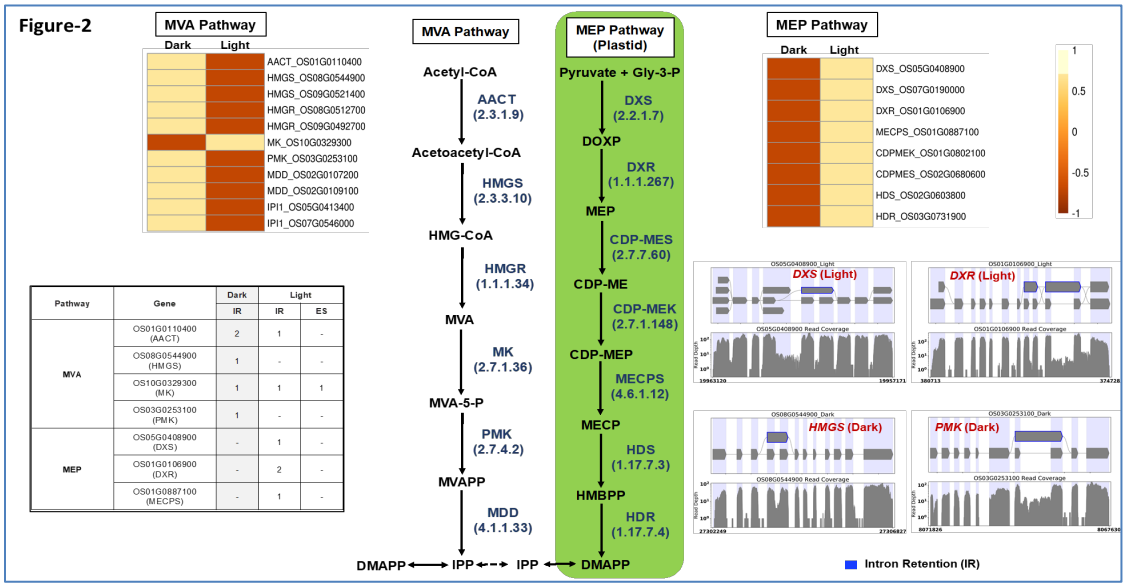


Figure 2: Gene expression and splicing of cytosolic mevalonate (MVA) and plastid-localized methyl erythritol 4-phosphate (MEP) pathway genes. *AACT*, acetyl-CoA acetyltransferase; *HMGS*, hydroxymethyl glutaryl CoA synthase; *HMGR*, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; *MK*, mevalonate kinase; *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-phosphate cytidylyl transferase; *CDPMEK*, 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase; *MECPS*, 2-C-methyl-D-erythritol2,4-cyclodiphosphate synthase; *HDS*, 4-hydroxy-3-methylbut-2-enyldiphosphate synthase; *HDR*, 4-hydroxy-3-methylbut-2-enyldiphosphate reductase. Blue: intron retention, Green: exon skipping, Orange: Alt3' splicing, Purple: Alt5' splicing.

290

291 **lncRNA discovery and splicing during photomorphogenesis**

292 lncRNAs are known to regulate diverse biological processes in plants, including
293 photomorphogenesis [68], and as described above, are categorized into four major types based on
294 their location in the gene space and on the DNA strand. We used our transcriptome data to
295 characterize light-induced rice lncRNAs (Supplementary Figure S7). We identified 1485 and
296 1407 lncRNA transcripts in the dark and light samples, of which 309 were common. Many
297 lncRNAs from intergenic and intronic regions were identified (Supplementary Figure S7). To
298 discover novel lncRNAs, the transcripts from our datasets were queried against the custom
299 BLAST database of rice lncRNAs. About 44% of the total lncRNA transcripts (2583) found a
300 match in the BLAST database (Supplementary Table S6). 173 lncRNAs spliced under dark and
301 142 spliced under light conditions, most located in the intergenic regions (Supplementary Figure
302 S7). Interestingly, all the spliced lncRNA genes from both datasets were from Chr1, Chr10,
303 Chr11, and Chr12. The maximum number of spliced lncRNA genes was from Chr1, followed by
304 Chr11, 12, and 10. Among all the splicing events, IR was predominant under both conditions.

305 We found lncRNA transcripts from light (TCONS_00012152) and dark (TCONS_00011794)
306 datasets that were present on the antisense strand overlapping an exon region of the *MADS27*
307 (Os02g0579600) gene. The transcript from the light-treated sample appears longer and showed
308 two alternative 3' splice sites and one exon skipping event. The TCONS_00011794 may
309 compete with MADS27 to form a complex with miRNA osa-miR444a known to play multiple
310 roles in the nitrate-dependent development pathway [69]. Among the list of lncRNAs present on
311 the antisense strand overlapping annotated genes, we identified a light-induced lncRNA
312 (TCONS_00001515) overlapping *ERF99* transcription factor (Os01g0868000) exon that may
313 play a role in its silencing. *ERF99* plays a central role in mediating abiotic stress responses [70].
314 Similarly, lncRNA transcript (TCONS_00015825) is present exclusively in light is transcribed
315 from the intronic region of the antisense strand of a B-type response regulator gene
316 (Os03g0224200), known for its involvement in cytokinin signaling, meristem maintenance, and
317 stress response [71,72]. The lncRNA transcript (TCONS_00026617) present exclusively in the dark
318 overlaps the intronic region of a phytochrome-interacting factor gene *PIF14* (Os07g0143200).

319 *PIF14* is known to bind the active form of phytochrome B and plays a crucial role in cross-talk
320 between light and stress signaling [73].

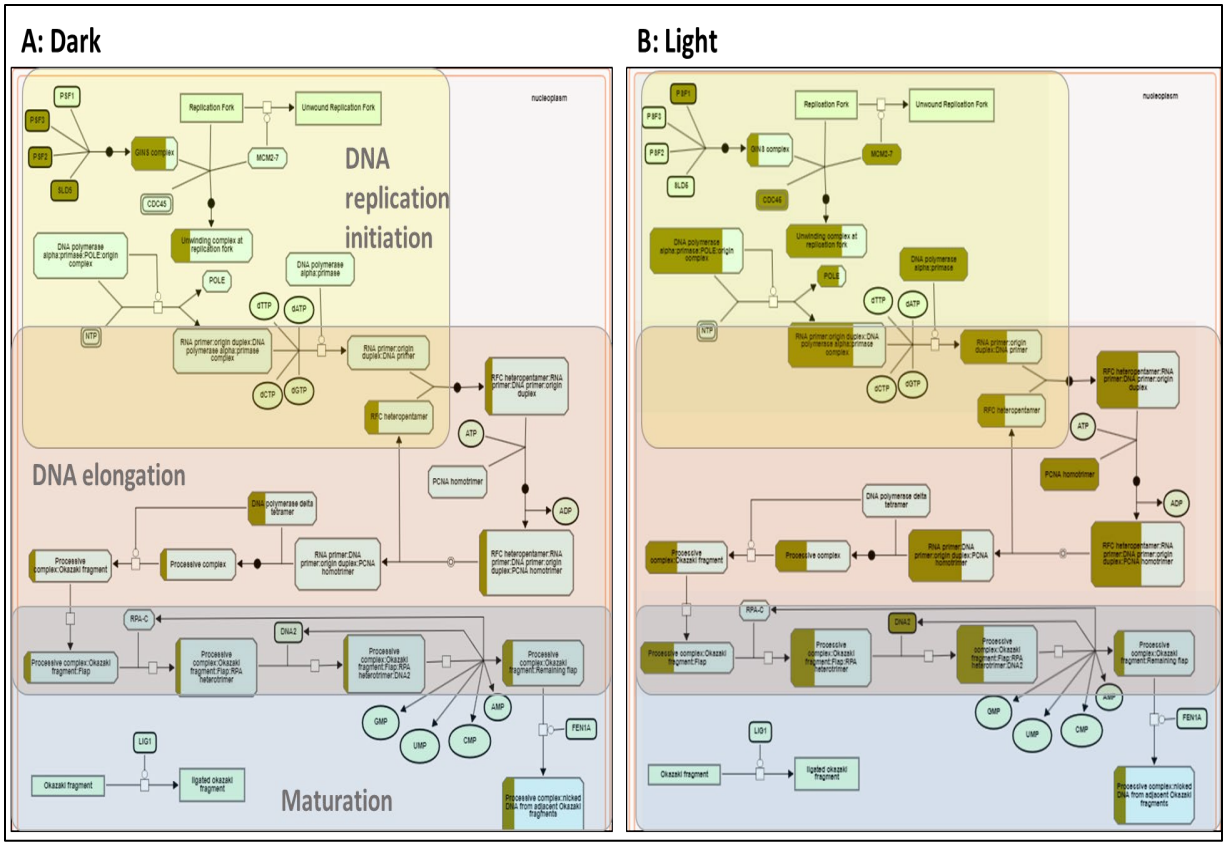
321 **Discussion**

322 Light is known to induce changes in the transcriptome, metabolome, and proteome of the plants
323 [74], which not only regulates the development, function, and physiology of the chloroplast but
324 also provides signals for modulating the plant's morphological, developmental, and
325 physiological adaptions in response to growth environment [8,40,75–77]. Though plants experience
326 constantly changing light conditions under the natural environment daily during their lifetime,
327 early development from germination to the seedling stage and acquiring the full autotrophic
328 capability is very much programmed by exposure to light. Therefore, we investigated
329 transcriptome modulation in rice seedlings during photomorphogenesis. Compared to earlier
330 studies in rice and *Arabidopsis*, where ~20% of the genes were reported differentially expressed
331 in dark-grown etiolated seedlings compared to light-exposed green seedlings [9,78,79], we observed
332 that the transition from skotomorphogenesis to photomorphogenesis alters differential expression
333 of ~38% of the rice genes.

334 Functional annotation of the differentially expressed genes enriched for roles in secondary
335 metabolism, chloroplast-related biosynthetic pathways, hormone biosynthesis and signaling,
336 amino acid biosynthesis, fatty acid metabolism, *etc*. (Supplementary Figure S3, Supplementary
337 Table S3). This result was expected based on the earlier reports on light regulation of such events
338 [24,80].

339 The plastids develop into chloroplasts by following an essential step of developing the thylakoid
340 membrane system and recruiting and assembling components of light and dark reactions to
341 establish a functional photosynthetic process. Many terpenoid compounds are essential to plants'
342 light-harvesting function and protect against damage from reactive oxygen species (ROS). The
343 basic isoprenoid units for terpenoid biosynthesis, such as tocopherols, plastoquinones,
344 carotenoids, chlorophylls, and precursors of the growth hormones gibberellins and abscisic acid,
345 are synthesized by the plastid-localized MEP pathway [81,82]. At the same time, isoprenoid units
346 synthesized by the MVA pathway contribute to the synthesis of triterpenes, phytosterols, and
347 phytohormones [83]. MEP and MVA pathways complement and contribute to the biosynthesis of

348 chlorophylls and carotenoids required for plastid development [84]. MEP pathway genes were
349 light-upregulated in our transcriptome data compared to the light-downregulated MVA pathway
350 genes (Figure 2). These results were consistent with earlier reports [85,86] and confirm that light
351 acts as a critical regulator in modulating the availability of isoprenoid precursors during
352 photomorphogenesis [87]. Arabidopsis circadian clock genes (*LHY*, *PRR9*, *CCA1*, *TOC1*) regulate
353 MVA and MEP pathways [87]. *AtTOC1* is also known to regulate the G1-to-S phase transition in
354 the mitotic cell cycle in early leaf development [88]. In our dataset, we did not observe any
355 significant change in the expression of *LHY/CCA1*(Os08g0157600), *PRR95* (Os09g0532400, a
356 homolog of *AtPRR9*), and *TOC1* (Os02g0618200). However, all three genes spliced differently.
357 Under the light, *TOC1* showed no splicing but retained two introns in the dark. We observed one
358 exon-skipping event in light of the *LHY/CCA1* gene, whereas *PRR95* showed one additional
359 intron retention (Supplementary Figure S5). Similar to *Arabidopsis* [88], the functional enrichment
360 analysis also identified the *TOC1*-regulated DNA biosynthesis pathway of the G1-to-S transition
361 of mitotic cell cycle (Figure 3) along with a greater coverage of reaction events in the light-
362 upregulated set. To our knowledge this is the first report of the light regulation of mitotic cell-
363 cycle pathway in rice.



364

365 **Figure 3:** Plant Reactome pathway enrichment showing light-regulated genes and reaction event
366 coverage for the DNA biosynthesis pathway of the G1-to-S phase mitotic cell cycle.

367 Previous transcriptome studies showed that ~42% of *Arabidopsis*, ~63% of *soybean*, and ~56%
368 of maize genes undergo alternative splicing events [89–91]. Studies on rice by Wang and Brendel [25]
369 reported IR for 69% of 6568 AS genes, and Campbell *et al.* [92] reported 44.7% of 8772 AS genes.
370 In our dataset, 4369 genes undergo differential expression and alternative splicing, called DES
371 genes (Figure 1A). About 80% (5395) of the spliced genes (6809) showed IR events, suggesting
372 that IR is the major splicing event. The more significant number of splicing events in the light-
373 treated samples suggests that light-mediated gene expression and post-transcriptional mRNA
374 splicing play an important role in photomorphogenesis. The splicing events in rice circadian
375 clock genes suggest that IR regulates the expression of PRR genes (Supplementary Figure S5).
376 Many of the light-regulated genes of the MEP and MVA pathways show IR events, suggesting
377 that light plays a positive role in completing the splicing and provides a hypothesis that exposure
378 to light is an adaptive feature and even if the gene is expressed in dark, it stays unspliced until it
379 encounters light. Our data showed the presence of all three states, partially spliced in the dark

380 (*GI*, Os01g0182600), fully spliced in light (*GI*, Os01g0182600), and both states in light (*CK2β-*
381 *1*, Os07g0495100) (Supplementary Figure S6).

382 lncRNAs play regulatory functions in essential biological processes such as vernalization,
383 photomorphogenesis, and stress regulation [39,93,94] and display splicing [95]. 6480 long intergenic
384 non-coding RNAs (lincRNAs) were identified from 200 *Arabidopsis* transcriptome datasets [96];
385 however, we identified 827 lincRNAs in the dark and 727 lincRNAs in the light datasets.
386 Corona-Gomez and coworkers [97] characterized the splicing conservation of lncRNAs in
387 *Brassicaceae*, which revealed that ~18% of lincRNAs display splicing conservation in at least
388 one exon. In our dataset, ~10% of lncRNA transcripts undergo splicing, and the majority were of
389 long intergenic non-coding RNA type (Supplementary Figure S7). Komiya and colleagues
390 reported [98] that phased small interfering RNAs (phasiRNAs) are generated from over 700
391 lincRNAs, and these phasiRNAs bind to rice argonaute protein MEL1. MEL1 has a specific
392 function in developing pre-meiotic germ cells and the progression of meiosis. In our dataset,
393 many lincRNAs transcribed from MEL1-phasiRNA clusters appeared.

394 Identification of light-induced lncRNAs antisense to *ERF99*, B-type response regulator genic
395 region may play a role in transcriptional regulation. *ERF99* is known to modulate root
396 architecture and downregulated in crown root primordia [99]. *MADS27* gene targeted by lncRNAs
397 present in both dark and light conditions. However, the dark lncRNA (TCONS_00011794) is
398 longer and, based on its predicted RNA fold structure and lower free energy, is likely more stable
399 than its transcript TCONS_00012152 in light. For the MADS27 gene, we also observed an
400 additional IR event in light. MADS27-miR444 complex is known to play a role in plant
401 development in a nitrate-dependent manner [100–102]. Therefore, we investigated the nitrogen
402 assimilation pathway genes. Nitrogen assimilation is necessary for sustaining plant growth and
403 development. Various nitrogen assimilatory enzymes are known to show isoform and cellular
404 component-specific responses under light and dark conditions [103,104]. We observed light
405 upregulation for the nitrate transporter, nitrate reductase, and nitrite reductase genes. We also
406 observed differential splicing patterns for these genes (Supplementary Figure S8). We also found
407 light regulation of the recently reported rice abiotic and biotic stress response genes [105,106].

408

409 **Conclusion**

410 This study suggests that light is a significant regulatory factor controlling genome-wide gene
411 expression through an alternative splicing mechanism in rice. All spliced genes did not
412 necessarily produce novel isoforms, which indicates the coupling of AS and nonsense-mediated
413 decay (NMD). NMD prevents the translation of mutant mRNAs harboring potential premature
414 stop codons by targeting them for degradation. In *Arabidopsis*, 77.2% of light-regulated AS
415 events exhibit NMD features within a splicing isoform [107]. We conclude that light induces a
416 significant number of splicing events in rice protein-coding and non-coding lncRNA genes. This
417 photomorphogenesis transcriptome study is a valuable resource for lncRNA research in rice and
418 provides insights into the portion of the genome regulated at the level of alternative splicing in
419 response to light. We expect the condition-dependent novel splice events discovered in this study
420 will help improve the annotation of the rice reference genome and pan-genomes [49,108]

421

422

423 **Conflict of interest**

424 The authors declare no conflict of interest.

425

426 **Availability of data**

427 The sequence reads and metadata were deposited at EMBL-EBI ArrayExpress (accession
428 number E-MTAB-5689).

429

430 **Figure legends**

431 **Figure 1:** Summary of differential gene expression and transcript splicing observed in rice
432 seedling shoots undergoing photomorphogenesis.
433
434 **Figure 2:** Gene expression and splicing of cytosolic mevalonate (MVA) and plastid-localized
435 methyl erythritol 4-phosphate (MEP) pathway genes. *AACT*, acetyl-CoA acetyltransferase;
436 *HMGS*, hydroxymethyl glutaryl CoA synthase; *HMGR*, 3-hydroxy-3-methylglutaryl-coenzyme
437 A reductase; *MK*, mevalonate kinase; *PMK*, phosphomevalonate kinase; *MDD*, mevalonate
438 diphosphosphate decarboxylase; *DXS*, 1-deoxy-D-xylulose-5-phosphate synthase; *DXR*, 1-
439 deoxy-D-xylulose-5-phosphate reductoisomerase; *CDPMES*, 2-C-methyl-D-erythritol4-
440 phosphate cytidylyl transferase; *CDPMEK*, 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase;
441 *MECPS*, 2-C-methyl-D-erythritol2,4-cyclodiphosphate synthase; *HDS*, 4-hydroxy-3-methylbut-
442 2-enyldiphosphate synthase; *HDR*, 4-hydroxy-3-methylbut-2-enyldiphosphate reductase. Blue:
443 intron retention, Green: exon skipping, Orange: Alt3' splicing, Purple: Alt5' splicing
444
445 **Figure 3:** Plant Reactome pathway enrichment showing light regulated genes and reaction event
446 coverage for the DNA biosynthesis pathway of the G1-to-S phase mitotic cell cycle.

447
448

449 **Table legends**

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

451

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

456

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

459

460 **Supplementary material**

461 **Supplementary Figure S1:**

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

463

464 **Supplementary Figure S2:**

465 Gene Ontology enrichment analysis of differentially expressed genes.

466

467 **Supplementary Figure S3:**

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

472

473 **Supplementary Figure S4**:

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

476

477 **Supplementary Figure S5**:

478 The summary of transcript splicing events and expression pattern of circadian clock genes during
479 morphogenesis.

480

481 **Supplementary Figure S6**:

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

484

485 **Supplementary Figure S7**:

486 Classification of lncRNAs identified in dark and light.

487

488 **Supplementary Figure S8:**

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

491

492 **Supplementary Table S1**:

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

494

495 **Supplementary Table S2:**

496 Differentially expressed genes

497

498 **Supplementary Table S3:**

499 Plant Reactome Pathway enrichment analysis for light-regulated genes

500

501 **Supplementary Table S4:**

502 Transcription factor genes regulated in light

503

504 **Supplementary Table S5:**

505 Transcription factors regulating MVA pathway genes

506

507 **Supplementary Table S6:**

508 lncRNA annotation using BLAST

509

510

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746
747

Figure-1

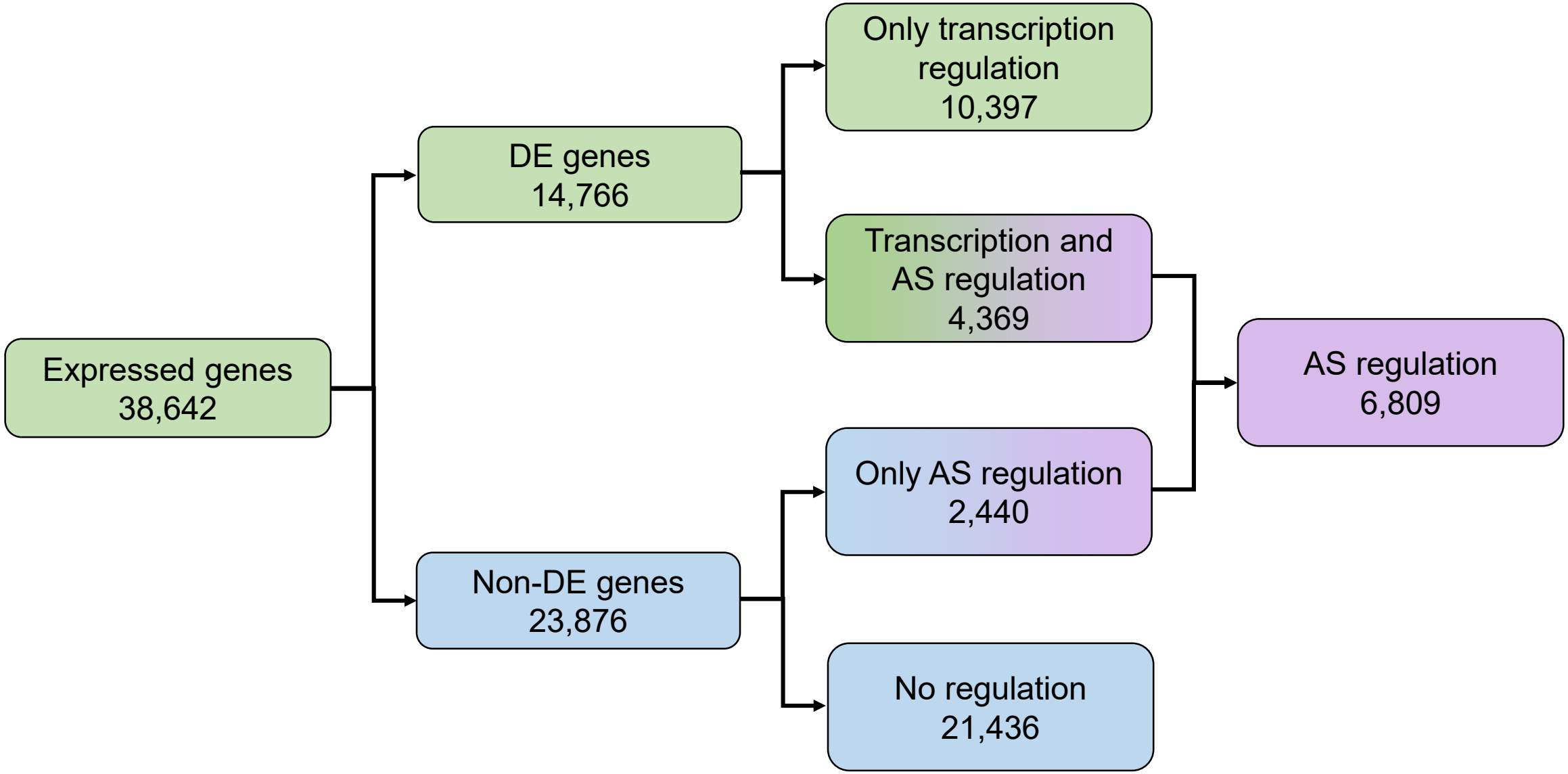
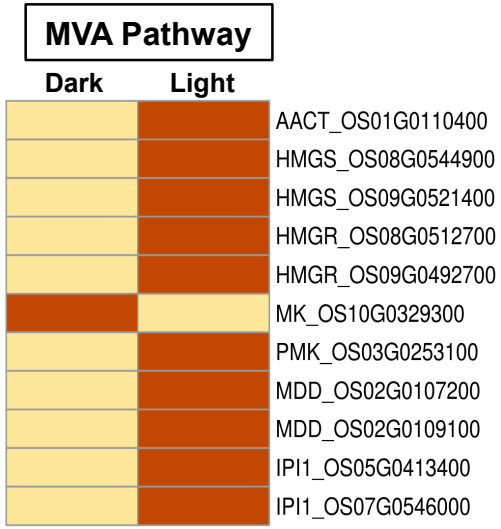
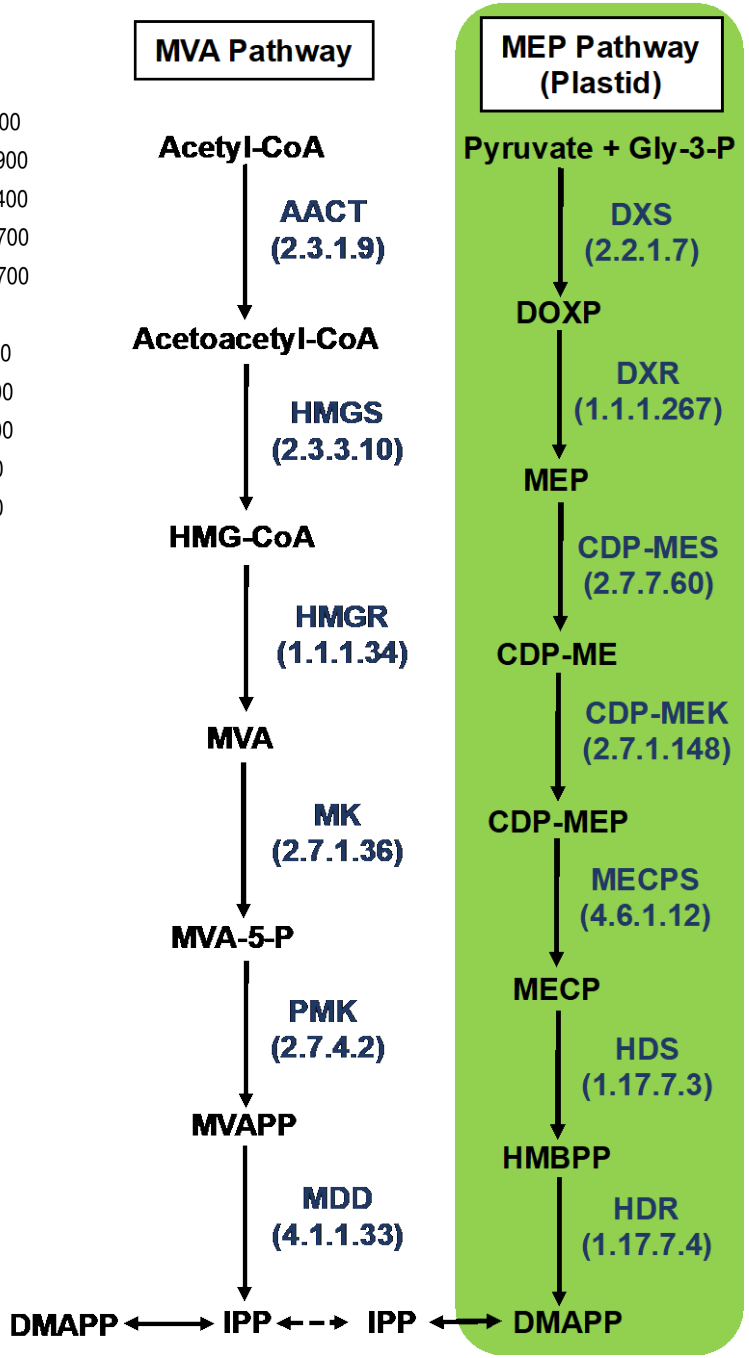
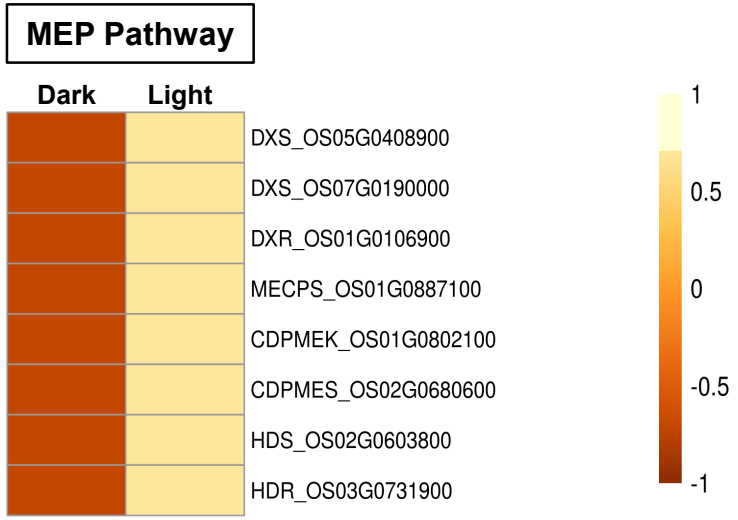

Expressed genes
38,642
DE genes
14,766
Non-DE genes
23,876
Only transcription regulation
10,397
Transcription and AS regulation
4,369
Only AS regulation
2,440
No regulation
21,436
AS regulation
6,809

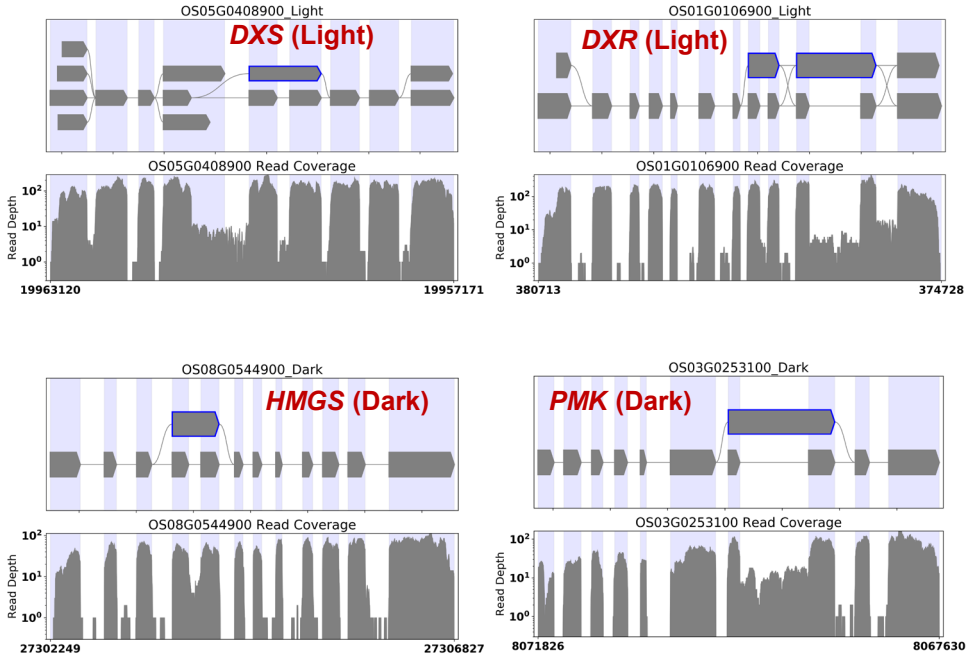
Figure-2







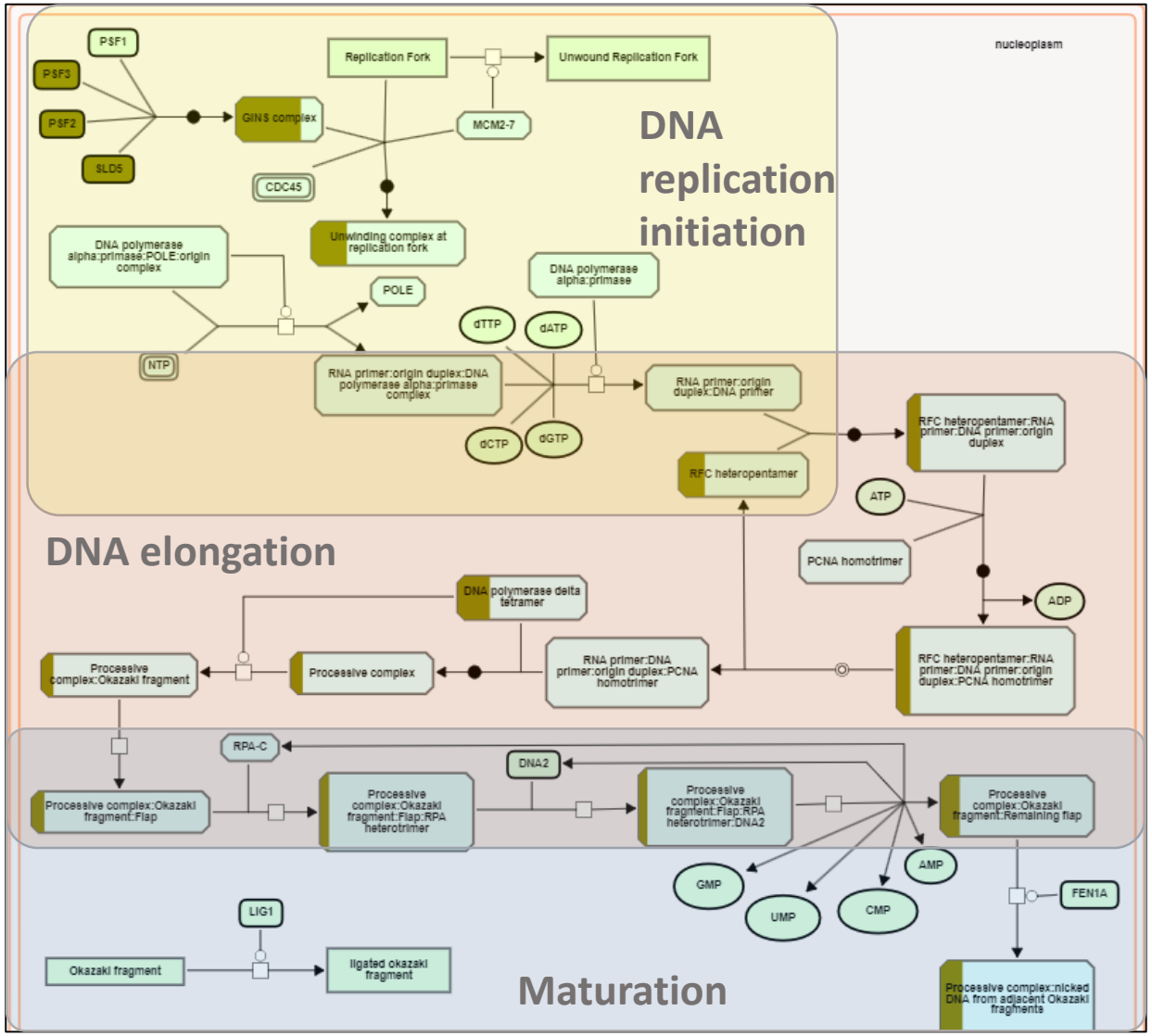
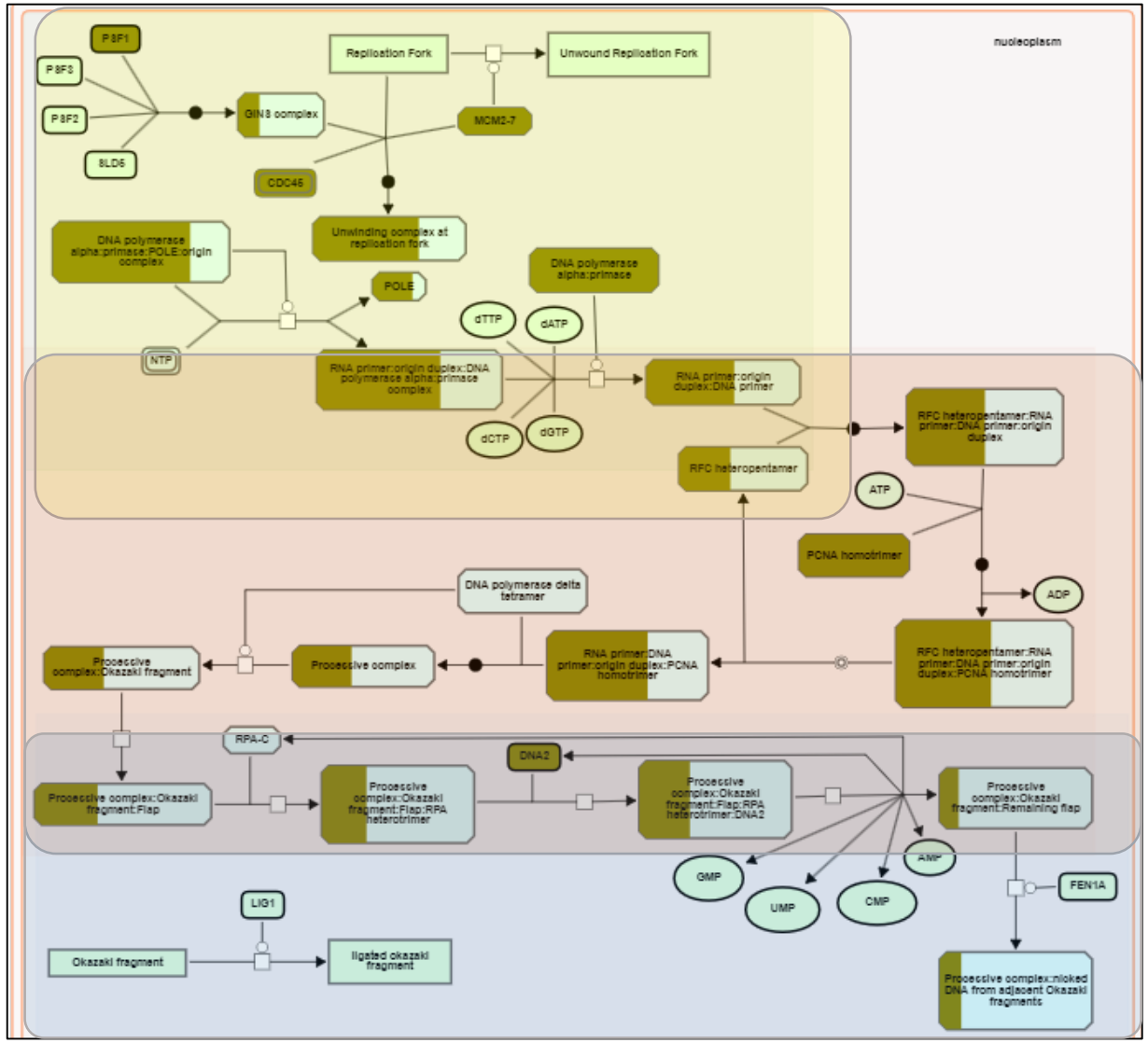
Pathway	Gene	Dark	Light	
		IR	IR	ES
MVA	OS01G0110400 (AACT)	2	1	-
	OS08G0544900 (HMGS)	1	-	-
	OS10G0329300 (MK)	1	1	1
	OS03G0253100 (PMK)	1	-	-
MEP	OS05G0408900 (DXS)	-	1	-
	OS01G0106900 (DXR)	-	2	-
	OS01G0887100 (MECPS)	-	1	-



Intron Retention (IR)

Figure-3

A: Dark



B: Light