



# Alternative Splicing Regulation During Light-Induced Germination of *Arabidopsis thaliana* Seeds

Rocío Soledad Tognacca<sup>1,2</sup>, Lucas Servi<sup>2</sup>, Carlos Esteban Hernando<sup>3</sup>, Maite Saura-Sanchez<sup>1</sup>, Marcelo Javier Yanovsky<sup>3</sup>, Ezequiel Petrillo<sup>2\*</sup> and Javier Francisco Botto<sup>1\*</sup>

<sup>1</sup> Consejo Nacional de Investigaciones Científicas y Técnicas, Instituto de Investigaciones Fisiológicas y Ecológicas Vinculadas a la Agricultura (IFEVA), Facultad de Agronomía, Universidad de Buenos Aires, Buenos Aires, Argentina, <sup>2</sup> Facultad de Ciencias Exactas y Naturales, Departamento de Fisiología, Biología Molecular y Celular and CONICET-UBA, Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE), Universidad de Buenos Aires (UBA), Buenos Aires, Argentina, <sup>3</sup> Fundación Instituto Leloir, IIBBA-CONICET, Buenos Aires, Argentina

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### \*Correspondence:

Ezequiel Petrillo  
petry@fbmc.fcen.uba.ar  
Javier Francisco Botto  
botto@agro.uba.ar

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Seed dormancy and germination are relevant processes for a successful seedling establishment in the field. Light is one of the most important environmental factors involved in the relief of dormancy to promote seed germination. In *Arabidopsis thaliana* seeds, phytochrome photoreceptors tightly regulate gene expression at different levels. The contribution of alternative splicing (AS) regulation in the photocontrol of seed germination is still unknown. The aim of this work is to study gene expression modulated by light during germination of *A. thaliana* seeds, with focus on AS changes. Hence, we evaluated transcriptome-wide changes in stratified seeds irradiated with a pulse of red (Rp) or far-red (FRp) by RNA sequencing (RNA-seq). Our results show that the Rp changes the expression of ~20% of the transcriptome and modifies the AS pattern of 226 genes associated with mRNA processing, RNA splicing, and mRNA metabolic processes. We further confirmed these effects for some of the affected AS events. Interestingly, the reverse transcriptase–polymerase chain reaction (RT–PCR) analyses show that the Rp modulates the AS of splicing-related factors (*At-SR30*, *At-RS31a*, *At-RS31*, and *At-U2AF65A*), a light-signaling component (*At-PIF6*), and a dormancy-related gene (*At-DRM1*). Furthermore, while the phytochrome B (phyB) is responsible for the AS pattern changes of *At-U2AF65A* and *At-PIF6*, the regulation of the other AS events is independent of this photoreceptor. We conclude that (i) Rp triggers AS changes in some splicing factors, light-signaling components, and dormancy/germination regulators; (ii) phyB modulates only some of these AS events; and (iii) AS events are regulated by R and FR light, but this regulation is not directly associated with the intensity of germination response. These data will help in boosting research in the splicing field and our understanding about the role of this mechanism during the photocontrol of seed germination.

**Keywords:** dormancy, germination, light, phytochrome B (phyB), alternative splicing (AS), *Arabidopsis*

## INTRODUCTION

Seed dormancy is a developmental checkpoint that allows plants to regulate when and where they grow. Temperature, light, and nitrates are the most relevant environmental factors regulating the relief of seed dormancy to promote seed germination (Benesch-Arnold et al., 2000). These cues can trigger molecular responses including hormone signaling, mainly those of abscisic acid (ABA) and gibberellin (GA). The balance between the contents and sensitivity of these hormones is key for the regulation of the dormancy status of the seeds. ABA promotes primary dormancy induction and later maintenance, whereas GA promotes seed germination. Environmental signals regulate this balance by modifying the expression of metabolic enzymes as well as those of positive and negative regulators of both hormones, many of which are feedback regulated (Finkelstein et al., 2008).

Light has been one of the most characterized factors regulating the relief of dormancy. Phytochromes are the best-known photoreceptors perceiving red (R) and far-red (FR) light. They are synthesized in its inactive form, Pr (with maximum absorption in R), and are photo-converted into their active form, Pfr (with maximum absorption in FR). The *Arabidopsis* genome encodes five phytochromes, named phyA to phyE. Among them, phytochrome B (phyB) has a prominent role as the main photoreceptor regulating the R/FR reversible response (Shinomura et al., 1994; Botto et al., 1995), and phyD and phyE can contribute to this regulation in *phyA/phyB* double mutant seeds (Hennig et al., 2002; Arana et al., 2014), suggesting some redundancy in phytochrome functions in the R-light-mediated seed germination. In the soils, weed seeds change their light sensitivity according with after-ripening and burial conditions (Scopel et al., 1991; Botto et al., 1998a; Botto et al., 1998b; Botto et al., 2000), being phyA responsible for the detection of very brief light stimulus-promoting seed germination (Botto et al., 1996; Shinomura et al., 1996).

Light tightly regulates the expression (transcript levels) of thousands of genes (Casal and Yanovsky, 2005; Galvao and Fankhauser, 2015) and also many other layers of gene expression such as mRNA splicing, translation, and stability (Mano et al., 1999; Yakir et al., 2007; Simpson et al., 2008; Jung et al., 2009; Juntawong and Bailey-Serres, 2012; Liu et al., 2012; Paik et al., 2012; Liu et al., 2013; Petrillo et al., 2014; Shikata et al., 2014; Tsai et al., 2014; Wang et al., 2014; Wu et al., 2014; Mancini et al., 2016). RNA splicing is a co-transcriptional molecular event that is carried out by a macromolecular complex called spliceosome. Alternative splicing (AS) is the process that generates multiple transcripts from a single gene by using different combinations of available splice sites. AS leads to different outcomes and produces transcripts encoding for proteins that may have altered or lost function. Several investigations have demonstrated the importance of AS in processes like photosynthesis, defense responses, circadian clock, hormone signaling, flowering time, and metabolism (Lightfoot et al., 2008; Matsumura et al., 2009; Sanchez et al., 2010; Martin-Trillo et al., 2011; James et al., 2012; Jones et al., 2012; Rosloski et al., 2013).

AS is also relevant during the early (Fouquet et al., 2011) and late stages of embryo development (Sugliani et al., 2010).

At the seed level, the work by Srinivasan et al. (2016) analyzed the AS during *Arabidopsis* seed development at a global level, both before and after seed desiccation. They identified 4,003 genes that are alternatively spliced, and 1,408 of those genes showing a differential pattern of splicing between both stages. Remarkably, most of these alternatively spliced transcripts had not been found in other tissues. More recently, another report highlights the relevance of AS in *Arabidopsis* seeds (Narsai et al., 2017). These authors analyzed the dynamics of gene expression over 10 developmental time points during seed germination and identified 620 genes undergoing AS. The regulation of these AS events during seed germination is time specific and/or tissue specific (Narsai et al., 2017). Interestingly, they also found complex variations in the relative abundance of *PIF6* (*PHYTOCHROME INTERACTING FACTOR 6*, *AT3G62090*) isoforms, as previously demonstrated (Penfield et al., 2010). *PIF6* transcription factor is expressed during seed development, and its expression is dramatically reduced during imbibition. It has four known AS isoforms (Narsai et al., 2017), one of them originating from an exon skipping (ES) event that creates a premature stop codon and encodes for a protein that lacks the DNA-binding domain (Penfield et al., 2010). As expected, the overexpression of this *PIF6* isoform reduces seed dormancy (Penfield et al., 2010). *DOG1* (*DELAY OF GERMINATION1*, *AT5G45830*), another main regulator of seed germination, is also affected at the AS level. *DOG1* protein accumulates during seed maturation, and its abundance in freshly harvested seeds determines the dormancy status of the seed (Graeber et al., 2014). *DOG1* transcripts are extensively alternatively spliced, giving place to five AS isoforms that are all functional but unstable if not expressed in combination (Bentsink et al., 2006; Nakabayashi et al., 2012; Nakabayashi et al., 2015). These findings clearly show the importance of AS control in the regulation of seed germination.

Photosensory-protein pathways are not the only way to sense light and regulate gene expression accordingly. Petrillo et al. (2014) have shown that *At-RS31* (*ARGININE/SERINE-RICH SPLICING FACTOR 31*, *AT3G61860*), *At-SR30* (*ARGININE/SERINE-RICH SPLICING FACTOR 30*, *AT1G09140*), and *At-U2AF65A* (*U2 SNRNP AUXILIARY FACTOR*, *AT4G36690*) AS patterns are modulated by different light conditions through retrograde signals arising from chloroplasts. They demonstrated that the chloroplast, which is able to sense and to communicate light signals to the nucleus, is the main actor triggering the AS changes in response to light (Petrillo et al., 2014). All these experimental evidences suggest that AS is a relevant mechanism even though there is still no information available about how light-promoting seed germination affects this process in *Arabidopsis thaliana* seeds. Here, we showed that (i) red pulse (Rp) triggers AS changes in some splicing factors, light-signaling components, and dormancy/germination regulators; and (ii) phyB is involved in the regulation of only some of these AS events. Furthermore, (iii) AS events are regulated by R and FR light, but this regulation is not directly associated with the intensity of germination response. We conclude that AS is a source of gene expression diversity, and a proper regulation of this process might be of key relevance for seed germination modulation under different light conditions.

## MATERIALS AND METHODS

### Plant Material and Growth Conditions

*A. thaliana* plants were grown under long day conditions [16-h L/8-h D, photosynthetically active radiation (PAR) =  $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ] with an average temperature of  $21 \pm 2^\circ\text{C}$ . Plants were grown together, and their mature seeds were harvested at the same time to avoid differences in post-maturation, which can affect seed germination. Seeds of each genotype were harvested as a single bulk that consisted of at least five plants. Seeds were stored in tubes with small holes inside a closed box and maintained in darkness with silica gel at  $4^\circ\text{C}$  until the experiments were performed. *A. thaliana* Columbia-0 (Col-0) and Landsberg *erecta* (*Ler*) were used as wild type (WT). Seeds of *phyB-9* (Col-0 background) and *phyB-5* (*Ler* background) (218790) were obtained from the ABRC ([www.arabidopsis.org/abrc/](http://www.arabidopsis.org/abrc/)).

### Germination Conditions and Light Treatments

Samples of 20 seeds per genotype were sown in clear plastic boxes, each containing 10 ml of 0.8% (w/v) agar in demineralized water. To establish a minimum and equal photo-equilibrium, seeds were imbibed for 2 h in darkness and then irradiated for 20 min with a saturated far-red pulse (FRp; calculated Pfr/P = 0.03,  $42 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) in order to minimize the quantities of Pfr that formed during their development in the mother plant. Seeds were then stratified at  $5^\circ\text{C}$  in darkness for 3 days, prior to the 20 min with a saturated Rp (calculated Pfr/P = 0.87,  $0.05 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) or FRp. After light treatments, the boxes containing the seeds were wrapped again with black plastic bags and incubated at  $25^\circ\text{C}$  for 3 days before germination was determined. The criterion for germination was the emergence of the radicle.

For experiments with hormones, seeds were sown in clear plastic boxes, each containing filter papers imbibed with  $750 \mu\text{l}$  of fluridone  $100 \mu\text{M}$  (Sigma-Aldrich, Steinheim, Germany) or ABA  $1 \mu\text{M}$  supplemented with fluridone  $100 \mu\text{M}$  (Ibarra et al., 2016) until the end of the experiment. We have previously performed calibration curves to determine the optimal ABA concentration to counteract the promotion of germination triggered by fluridone (**Supplementary Figure 1**).

### cDNA Library Preparation and High-Throughput Sequencing

Seed samples were sown in clear plastic boxes, each containing 10 ml of 0.8% (w/v) agar in de-mineralized water. Three biological replicates of each condition were collected 12 h after the corresponding R and FR light pulses. After sampling, seeds were immediately frozen in liquid nitrogen and stored at  $-80^\circ\text{C}$ . RNA was extracted using the Spectrum Plant Total RNA Kit (Sigma-Aldrich, Steinheim, Germany) according to manufacturer's protocol. To estimate the concentration and quality of the samples, spectrophotometry and agarose gel were used, respectively. RNA samples were processed at the Instituto de Agrobiotecnología de Rosario (INDEAR, Rosario, Argentina). Samples were pooled to create six multiplexed DNA libraries, which were pair-end sequenced with an Illumina HiSeq 1500.

### Processing of RNA Sequencing Reads

Sequence reads were aligned with the *A. thaliana* genome TAIR10 (Langmead et al., 2009) with TopHat v2.1.1 (Trapnell et al., 2009) with default parameters, except in the case of the maximum intron length parameter, which was set at 5,000. Count tables for the different feature levels were obtained from bam files using custom R scripts and considering the TAIR10 transcriptome.

### Differential Gene Expression Analysis

Differential gene expression analysis was conducted for genes whose expression was above a minimum threshold level [ $>10$  reads and a read density (RD)  $>0.05$ ] in at least one experimental condition. RD was computed as the number of reads in each gene divided by its effective width. The term effective width corresponds to the sum of the length of all the exons of a given gene. Differential gene expression was estimated using the edgeR package version 3.4.2 (Robinson et al., 2010), and resulting *p*-values were adjusted using a false discovery rate (FDR) criterion (Benjamini and Hochberg, 1995). Genes with FDR values lower than 0.05 and an absolute fold change  $>1.5$  were considered to be differentially expressed (DE). This dataset was labeled as DE genes (**Supplementary Table 1**).

### Differential AS Analysis

For the analysis of differential AS, multiexonic genes were partitioned into features defined as “bins,” corresponding to exonic, intronic, and alternatively spliced regions. We labeled these three kinds of bins as exon bins, intron bins, or AS bins, respectively. In addition, AS bins were further classified as ES, alternative 5' splice site (Alt5'SS), alternative 3' splice site (Alt3'SS), and intron retention (IR). Bins with three or more different AS events in the same subgenic region were labeled as multiple. Read summarization was performed at those three levels: exon, intron, and AS bins. These datasets were then filtered according to several criteria applied at the gene and bin levels. First, defined subgenic regions (i.e., bins) were considered for differential AS analysis only if the genes with which they are associated with were expressed above a minimum threshold level (more than 10 reads per gene and  $\text{RD} > 0.05$ ) in all experimental conditions. Next, bins were considered for differential AS analysis only if they had more than five reads and an RD bin/RD gene ratio  $>0.05$ , in at least one experimental condition. After these filters were applied, reads summarized at the bin level were normalized to the read counts of their corresponding gene. This was done to avoid the influence of changes in gene expression on the differential AS analysis at the bin level. Then, similarly to the approach used for the differential expression analysis, differential AS analysis was conducted at the bin levels using the edgeR package version 3.4.2. Bins with FDR values lower than 0.15 were considered to undergo differential AS. Finally, we restricted the selection of AS bins to those bins for which differential AS analysis was supported by expected changes in the numbers of splice junctions. In order to do this, we obtained information on the number of reads associated with each splice junction, both annotated and novel. Junction coordinates were

extracted from gap containing aligned reads. Junctions with fewer than five reads were discarded. We then computed the metrics PSI (percent spliced-in) and PIR (percent IR), which were used as a final filtering criteria for the AS analysis. PSI was defined as the percentage of the number of junction reads supporting bin inclusion relative to the combined number of reads supporting inclusion and exclusion (Pervouchine et al., 2013). PSI values were computed for ES, Alt5'SS, and Alt3'SS. PIR values, calculated as previously described (Braunschweig et al., 2014), were used for the IR analysis. Briefly, PIR is defined for each experimental condition as the percentage of the number of reads supporting IR (E1I + IE2) relative to the combined number of reads supporting IR and exclusion (E1I + IE2 + 2 exclusion junction [JE1E2]), where E is the exonic bin, I the intronic bin, and J the junction (Mancini et al., 2016). AS, exon, and intron bins were considered to be differentially spliced (DS) if, in addition to fulfilling the filtering criteria described above, the difference in PSI or PIR between experimental conditions was >0.5%. Bins corresponding to alternatively spliced regions identified through novel splice junctions were considered to be differentially alternatively spliced if there was a difference in the PSI value larger than 10% between experimental conditions. This dataset was labeled as DS genes (**Supplementary Table 1**).

## Gene Ontology Analysis

Gene ontology (GO) terms assignment for the DE genes and DS genes datasets were obtained using the BioMaps tool from the virtual plant software (<http://virtualplant.bio.nyu.edu/cgi-bin/vpweb/>). An enrichment test was performed for the following categories: BP (biological process), MF (molecular function), and CC (cellular component). *p*-values were obtained using the Fisher exact test and corrected for multiple testing using FDR. The enrichment factor (EF) was estimated as the ratio between the proportion of genes associated with a particular GO category present in the dataset under analysis, relative to the number of genes in this category in the whole genome (**Supplementary Table 1**). Bubble plots were generated, using a custom script written in R language, for all those categories for which the adjusted *p*-value was lower than 0.01 in at least one dataset.

## Semi-Quantitative Reverse Transcriptase–Polymerase Chain Reaction

Seed samples were sown in clear plastic boxes, each containing 10 ml of 0.8% (w/v) agar in de-mineralized water. Three biological replicates of each condition were collected 12 h after the corresponding R and FR light pulses. After sampling, seeds were immediately frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$ . RNA was extracted using the Spectrum Plant Total RNA Kit (Sigma-Aldrich, Steinheim, Germany) according to manufacturer's protocol. cDNA derived from the extracted RNA was synthesized using M-MLV reverse transcriptase (Promega, Madison, WI, USA) and oligo-dT primers. polymerase chain reaction (PCR) analyses were conducted using a mix with Taq DNA Pol (Inbio Highway, Tandil, Buenos Aires, Argentina), 10 $\times$  buffer, 10 $\times$  polyvinylpyrrolidone

(PVP), 25 mM of MgCl<sub>2</sub>, 10 mM of dNTPs, and gene-specific primers according to the manufacturer's instructions. The PCR program was as follows: 95 $^{\circ}\text{C}$  for 3 min and the respective number of cycles (28–31) at 95 $^{\circ}\text{C}$  for 30 s, 60 $^{\circ}\text{C}$  for 30 s, and 72 $^{\circ}\text{C}$  for 1.5 min. The amplified products were resolved by 1% or 2% agarose gel electrophoreses. The SI, defined as the abundance of the longest splicing isoform relative to the levels of all possible isoforms, was calculated from the relative levels of the corresponding reverse transcriptase (RT)–PCR products quantified using densitometry by the ImageJ software (<https://imagej.net/Welcome>). Gene models and gel images are shown in **Supplementary File 1**. The specific primers used are described in **Supplementary Table 2**.

## Statistical Analysis

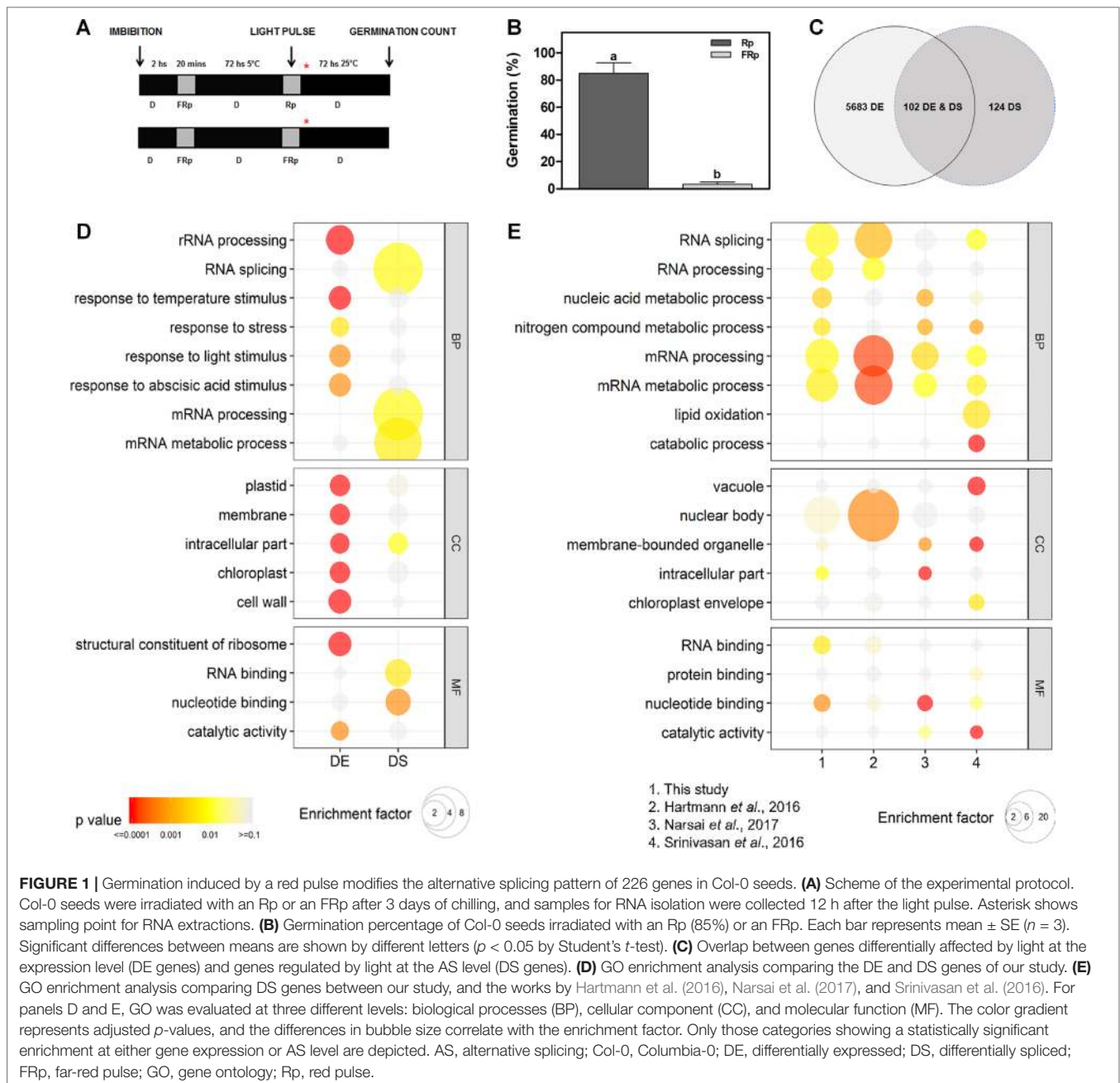
To test for significant differences in the response of the seeds, we conducted two-way analyses of variance (ANOVAs) for each WT and mutant group, using the angular transformation of the percentage of germination and the InfoStat Software version 2017 (Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina). Fisher post-test was used to test differences between genotypes, when significant treatment-by-genotype interactions were observed.

To test for significant differences in the splicing index (SI), we also conducted two-way ANOVAs using the same software, and Fisher post-test was used to test for differences, when significant treatment-by-genotype interactions were observed. When indicated, we used Student's *t*-test.

## RESULTS

### Seed Germination Induced by Red Light Modulates the AS Pattern of 226 genes

WT Col-0 seeds were imbibed in darkness at 5 $^{\circ}\text{C}$  for 3 days prior to irradiation with an Rp or an FRp. Germination was counted after 3 days at 25 $^{\circ}\text{C}$  in darkness (**Figure 1A**). The Rp significantly induces seed germination  $\sim 85\%$  in Col-0 seeds, while the FRp does not promote seed germination (**Figure 1B**), indicating that the phytochrome system is involved in this response. We used this seed population to evaluate changes at the transcriptome level triggered by light, with special focus in AS modulation. Seed samples for RNA isolation were collected 12 h after the light treatments (**Figure 1A**), and R light effects on mRNA levels and AS were analyzed (**Supplementary Table 1**). We identified a total of 5,785 genes whose mRNA levels are significantly affected, either increased or decreased, more than 1.5-fold (FDR < 0.05) in response to the Rp (**Figure 1C** and **Supplementary Table 1**). We defined this group as DE genes. Interestingly, DE genes are enriched in genes associated with response to temperature, light, and ABA stimuli (**Figure 1D** and **Supplementary Table 1**). We then evaluated the effects of R light on AS and identified a total of 226 genes with AS events that are regulated by the light pulse (**Figure 1C** and **Supplementary Table 1**). We defined this group as DS genes. Less than half of the genes whose AS patterns are affected by R light show alterations at the total mRNA levels too (102 genes,



**Figure 1C**). We also observed a strong enrichment in GO categories associated with mRNA processing, RNA splicing, and mRNA metabolic processes among these DS genes (**Figure 1D** and **Supplementary Table 1**). These categories are not enriched among the genes whose mRNA levels, rather than AS patterns, are affected by R light, supporting the idea that light regulates AS patterns mostly through its effect on the AS of splicing factors themselves (**Figure 1C** and **Supplementary Table 1**).

Additionally, we performed a comparative transcriptomic analysis to evaluate if differentially alternatively spliced genes identified here, as responsive to an Rp that induced germination, were also regulated in other developmental processes or conditions,

like seed maturation (Srinivasan *et al.*, 2016), germination induced by a white light/dark photoperiod (Narsai *et al.*, 2017), and/or seedling de-etiolation (Hartmann *et al.*, 2016). We found two common genes affected at the AS level in the four transcriptome studies (*At-RS41* and *At-HYP1*), but a higher number of common genes are found when comparing different combinations of specific transcriptomes with those in our study (**Supplementary Table 1**). We also observed a strong enrichment in GO categories associated with RNA splicing, RNA processing, mRNA processing, and mRNA metabolic processes among these different physiological processes (**Figure 1E** and **Supplementary Table 1**). These data are in agreement with previous studies showing that genes related to

RNA metabolism are among the most affected by AS (Syed et al., 2012; Reddy et al., 2013).

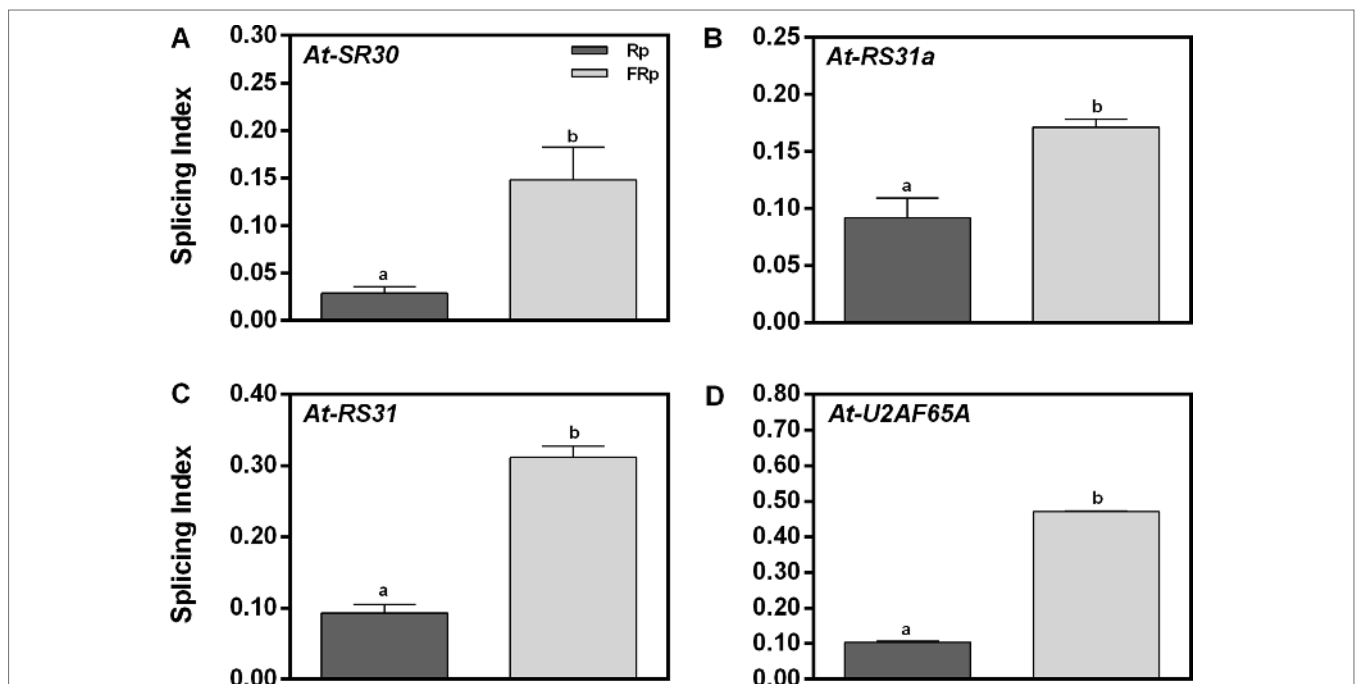
### Red Pulse Reduces the SI of *At-SR30*, *At-RS31a*, *At-RS31*, and *At-U2AF65A*

To validate the RNA sequencing (RNA-seq) data, we selected four genes of our interest and studied their responses by RT-PCR: (a) *At-SR30* and *At-RS31*, which are among the down-regulated and AS affected genes (DE and DS) according to the RNA-seq (Supplementary Table 1), and (b) *At-U2AF65A* and *At-RS31a*, which belong to the down-regulated category (DE) but are known to be light-responsive events in seedlings (Petrillo et al., 2014). All these candidate genes are related to the splicing process itself: three are SR genes (*At-SR30*, *At-RS31a* and *At-RS31*) and one is an auxiliary splicing factor (*At-U2AF65A*). We used the SI, defined as the abundance of the longest splicing isoform relative to the levels of all possible isoforms, as the parameter to evaluate AS changes induced by the Rp. Figure 2 shows that the Rp reduces the SI of *At-SR30*, *At-RS31a*, *At-RS31*, and *At-U2AF65A* between 2- and 5-folds than does the FRp. These results confirm that the AS patterns of these events are modulated by red light when seed germination is induced. Moreover, these results suggest that the total number of affected AS events by red light in germinating seeds is probably higher than those considered in our list (Supplementary Table 1). Further research in this direction is needed to know the actual impact of AS in gene expression regulation by red light during seed germination.

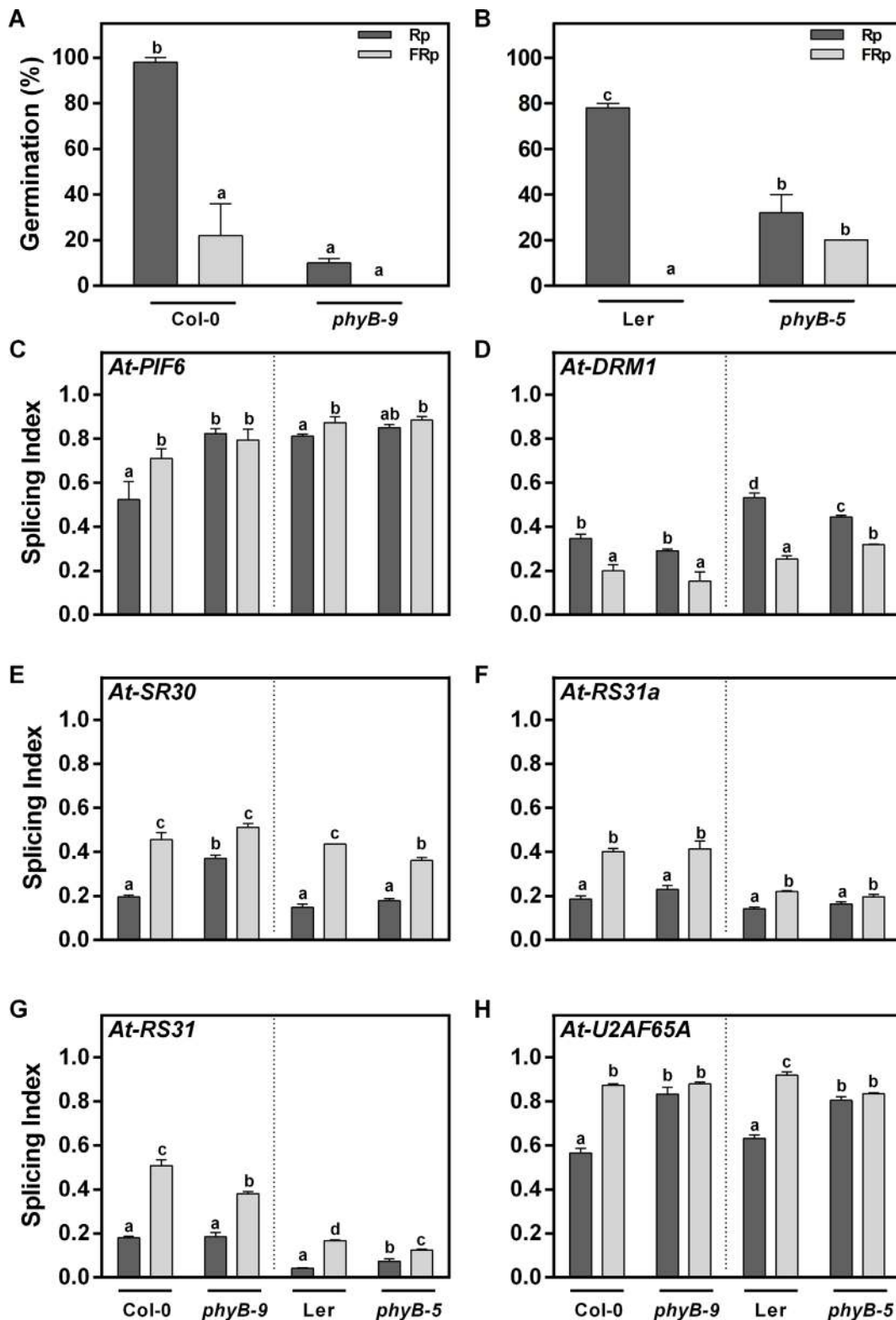
### Phytochrome B Regulates the AS Pattern of *At-PIF6* and *At-U2AF65A*

R-light-induced germination can be mediated by different phytochromes (Botto et al., 1996; Hennig et al., 2002; Arana et al., 2014), and both phyA and phyB are the main photoreceptors. The phyB contribution to AS regulation is still unknown. Thus, we analyzed germination of Col-0 and *Ler* WT seeds and *phyB-9* (Col-0 background) and *phyB-5* (*Ler* background) mutant seeds under our experimental conditions. The FRp does not promote seed germination in any of the analyzed genotypes (Figures 3A, B). On the contrary, the Rp induces 98% of germination in Col-0 seeds (Figure 3A) and 78% in *Ler* seeds (Figure 3B), while *phyB-9* and *phyB-5* mutant seeds germinate at 10% and 32%, respectively, suggesting that phyB is the main phytochrome controlling seed germination under these conditions (Figures 3A, B).

We then asked whether the light-induced AS changes could be subjected to phyB regulation. The Rp reduces the SI of *At-PIF6*, a phytochrome interacting factor, and increases the SI of *At-DRM1* (DORMANCY-ASSOCIATED PROTEIN 1, AT1G28330), a dormancy-related gene in Col-0 and *Ler* seeds (Figures 3C, D). The phyB is responsible for the AS changes of *At-PIF6* since light differences associated with the SI are lost in both *phyB-5* and *phyB-9* mutant seeds (Figure 3C). In opposition, the AS pattern of *At-DRM1* is not regulated by the phyB (Figure 3D). With respect to the splicing related factors, the SI of *At-SR30*, *At-RS31a*, and *At-RS31* are significantly



**FIGURE 2 |** Germination and alternative splicing regulation by red/far-red light. (A–D) Germination induced by an Rp reduces the splicing index (SI) of *At-U2AF65A*, *At-SR30*, *At-RS31*, and *At-RS31a* in Col-0 seeds. We selected these genes from the RNA-seq data based on two simultaneous criteria: (1) genes involved in RNA splicing and (2) genes previously documented to have AS (Petrillo et al., 2014; Shikata et al., 2014). RNA Col-0 samples were harvested 12 h after the Rp or FRp. AS alterations as SI in response to the Rp or FRp are shown. Gene expression levels were analyzed by RT-PCR, and each PCR product was quantified by densitometry using ImageJ. Each bar represents mean  $\pm$  SE ( $n = 3$ ). Significant differences between means are shown by different letters ( $p < 0.05$  by Student's  $t$ -test). AS, alternative splicing; Col-0, Columbia-0; FRp, far-red pulse; Rp, red pulse; RT-PCR, reverse transcriptase–polymerase chain reaction.



**FIGURE 3** | *phyB* differentially regulates the AS pattern of *At-U2AF65A* and *At-PIF6*. **(A–B)** Germination percentages of *Col-0*, *phyB-9*, *Ler*, and *phyB-5* irradiated with an Rp or an FRp. **(C–H)** AS alterations quantified as splicing index (SI) in response to the Rp or FRp are shown. Seed samples were harvested 12 h after the Rp or FRp. Splice variants of the indicated genes were analyzed by RT-PCR, and each PCR product was quantified by densitometry using ImageJ. Each bar represents mean  $\pm$  SE ( $n = 3$ ). Means with the same letters are not significantly different ( $p > 0.05$  by ANOVA followed by Fisher post-test). ANOVA, analysis of variance; AS, alternative splicing; *Col-0*, Columbia-0; FRp, far-red pulse; Rp, red pulse; RT-PCR, reverse transcriptase–polymerase chain reaction.

reduced in response to the Rp in both Col-0 and *Ler* seeds. Even though *phyB* mutants show some changes in the SI of these AS events, similar differences between Rp and FRp are still observed in both *phyB-9* and *phyB-5* mutant seeds, suggesting the AS control of these splicing factors by light does not involve phyB regulation (Figures 3E–G). On the other hand, even though the Rp significantly changes the SI of *At-U2AF65A* in Col-0 and *Ler* seeds (~30%), this difference is completely abolished in the *phyB* mutant seeds in both genetic backgrounds (Figure 3H). We conclude that phyB regulates the AS pattern of *At-U2AF65A* and *At-PIF6* in *Arabidopsis* seeds germinating with an Rp.

## Red Light Perception and Alternative Splicing Are Directly Linked

We have clearly shown that red light promotes seed germination and affects AS (Figures 1–3). One remaining question is whether these AS changes are directly triggered by the red-light signal or if they are a consequence of the germination process *per se*. We hypothesized that if light is directly controlling the AS response, changes in splicing patterns should not be affected by different levels of germination under the same light condition (Rp or FRp). Since ABA is a known hormone regulating seed germination, we sowed WT Col-0 seeds in water (control); fluridone (F), an inhibitor of ABA synthesis; and fluridone supplemented with ABA (F + ABA). The Rp induces 100% germination in water and F but only 40% in F + ABA. The FRp reduces the germination to ~40%, ~80%, and 0%, respectively (Figure 4A). As expected, the F and F + ABA treatments modulate the germination response induced by red light. Hence, we used these seeds to analyze the AS patterns of *At-PIF6*, *At-DRM1*, *At-SR30*, *At-RS31a*, *At-RS31*, and *At-U2AF65A*. We found that the SI of the six genes changes significantly between seeds exposed to Rp and FRp in water, and these changes in the SI are still present in F and F + ABA-treated seeds (Figures 4B–G). These results clearly show that changes in AS patterns induced by light are independent of the level of germination. This conclusion is also supported by our previous results showing that the *phyB* mutant, which presents reduced germination under Rp, displayed similar AS changes than the WT seeds for the vast majority of the analyzed events (Figure 3). Taken altogether, our data suggest that the Rp directly controls AS processes and that these effects are independent of the level of germination induced by light.

## DISCUSSION

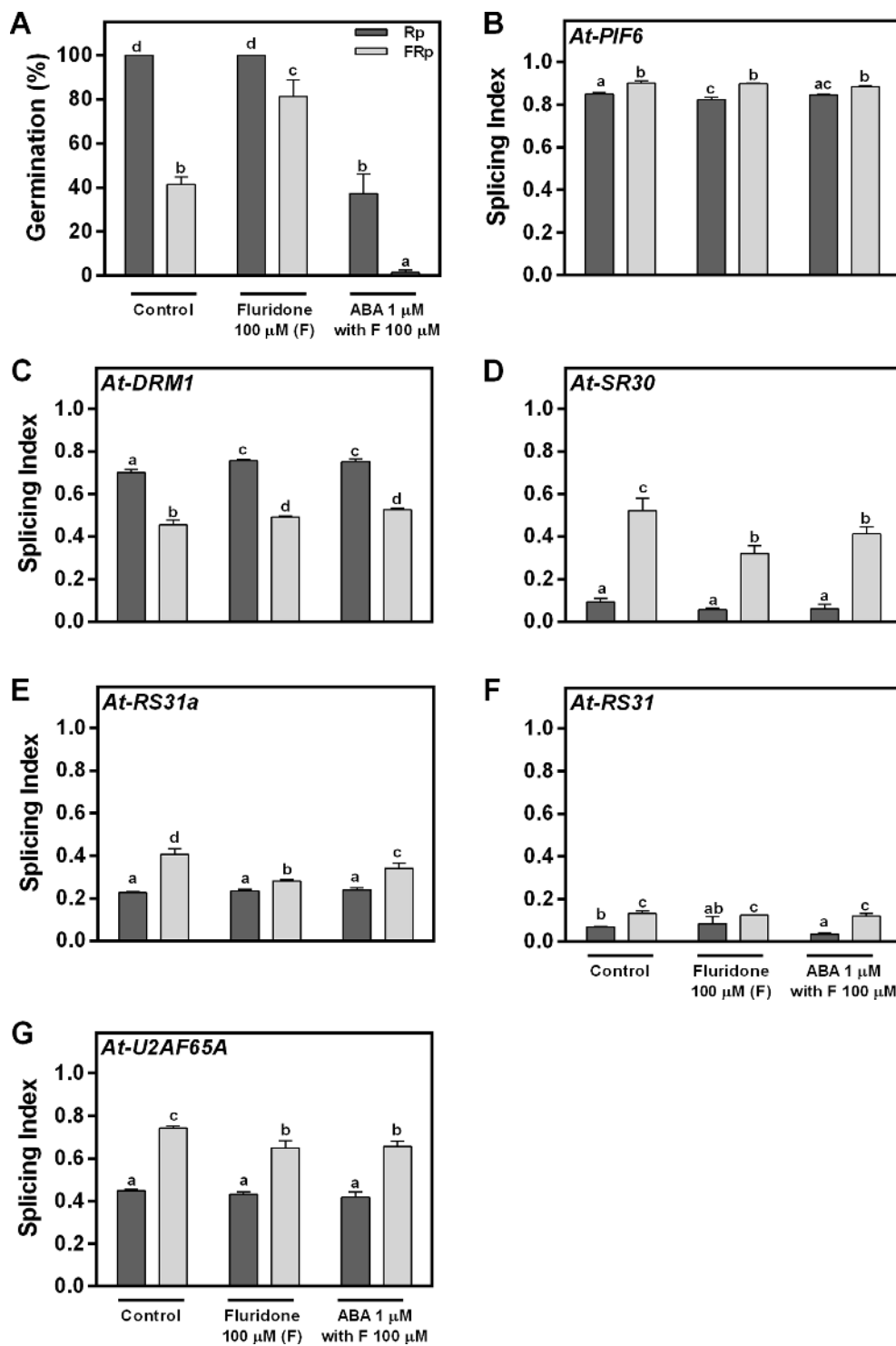
Seed dormancy and germination are processes of extreme relevance for a successful seedling establishment in the field. Light is one of the most important and best characterized environmental factors involved in the relief of seed dormancy to promote seed germination (Benech-Arnold et al., 2000). Plants possess a wide variety of photoreceptors capable of sensing this environmental cue (van Gelderen et al., 2018). The *Arabidopsis* genome encodes for five different phytochromes, and phyB is the one with the most prominent role in controlling the R/FR reversible response inducing seed germination (Shinomura et al., 1994; Botto et al., 1995). Moreover, light shapes plants' transcriptomes by affecting

every possible level of gene expression regulation (Petrillo et al., 2015; Merchante et al., 2017; Kaiserli et al., 2018; Godoy Herz et al., 2019). AS, a powerful mechanism that allows rapid changes in transcriptome and proteome complexity during development and in response to changes in the environment (Reddy et al., 2013), is also dramatically affected by this environmental cue at different developmental stages. Nowadays, it is becoming evident that AS substantially increases transcriptome complexity and plays an important role in modulating gene expression in response to internal and external cues (Lightfoot et al., 2008; Matsumura et al., 2009; Sanchez et al., 2010; Martin-Trillo et al., 2011; James et al., 2012; Jones et al., 2012; Rosloski et al., 2013). While the information concerning the molecular basis of light-induced germination is abundant at the level of gene expression/transcription (Penfield et al., 2005; Oh et al., 2006; Oh et al., 2007; Oh et al., 2009; Gabriele et al., 2010; Park et al., 2012; Ibarra et al., 2013), that concerning global studies describing light effects on AS has only recently started to appear. Here, we provide evidence showing that (i) Rp triggers AS changes in some splicing factors, light-signaling components, and dormancy/germination regulators; (ii) Rp exerts these effects on AS through the action of phyB in some few events; and (iii) AS events are regulated by R/FR light, but this regulation is not directly associated with the intensity of germination response.

We identified 226 genes with AS events regulated by light (Figure 1C and Supplementary Table 1) and that the Rp reduces the SI of *At-SR30*, *At-RS31a*, *At-RS31*, and *At-U2AF65A* (Figure 2). Since *At-SR30*, *At-RS31*, and *At-RS31a* are members of the RS subfamily (like *At-RS41*), these results may have implications for all the SR genes in *Arabidopsis*. Previous studies have evaluated light effects on AS at a global level in *Physcomitrella patens* and in etiolated *A. thaliana* seedlings using RNA-seq (Shikata et al., 2014; Wu et al., 2014). These studies found several hundreds of light-regulated AS events, many of which were associated with genes encoding splicing factors and light-signaling components. Interestingly, in both reports, the effects of brief light treatments on AS were modulated to a great extent, although not exclusively, by the phytochromes (Shikata et al., 2014; Wu et al., 2014). Furthermore, it was previously shown that *RRC1* (REGULATOR OF CHROMOSOME CONDENSATION 1), an SR-like protein, is required for normal seedling development under R light (Shikata et al., 2014) and, more recently, that *RRC1* interacts with SFPS (SPLICING FACTOR FOR PHYTOCHROME SIGNALING) and phyB to coordinately regulate the splicing of genes involved in light signaling and circadian clock pathways to promote photomorphogenesis in *A. thaliana* (Xin et al., 2019). The fact that we found common genes whose AS is regulated in different developmental processes (Shikata et al., 2014; Hartmann et al., 2016; Srinivasan et al., 2016; Narsai et al., 2017; this study) strongly points towards the existence and relevance of an AS regulatory network that is active throughout the whole life cycle of a plant.

The R/FR reversible response of seed germination is mainly mediated by phyB (Shinomura et al., 1994; Botto et al., 1995). Interestingly, we found that changes on some AS events (*At-PIF6* and *At-U2AF65A* out of six genes analyzed) are mediated by this photoreceptor (Figures 3C, H). However, the observation that the SI values of *At-SR30*, *At-RS31a*, *At-RS31*, and *At-DRM1* were not altered by the absence of phyB suggests that other stable





**FIGURE 4 |** Red light perception and alternative splicing are directly linked. **(A)** Germination percentages of Col-0 seeds imbibed in water (control), fluridone, or ABA supplemented with fluridone and irradiated with an Rp or an FRp. **(B–G)** AS alterations for each hormone condition quantified as splicing index (SI) in response to the Rp or FRp are shown. Seed samples were harvested 12 h after the Rp or FRp. Splice variants of the indicated genes were analyzed by RT-PCR, and each PCR product was quantified by densitometry using ImageJ. Each bar represents mean  $\pm$  SE ( $n = 3$ ). Means with same letters are not significantly different ( $p > 0.05$  by ANOVA followed by Fisher post-test). ABA, abscisic acid; ANOVA, analysis of variance; AS, alternative splicing; Col-0, Columbia-0; FRp, far-red pulse; Rp, red pulse; RT-PCR, reverse transcriptase–polymerase chain reaction.

phytochromes, mediating the R/FR response, might be responsible for the regulation of the AS pattern of these genes. Interestingly, *At-U2AF65A* and *At-RS31* AS is regulated by a common light pathway in seedlings, involving chloroplast's retrograde signals (Petrillo et al., 2014). Hence, these results suggest seeds can have completely different gene regulatory networks. Further research is needed to know whether other photoreceptors, or other signaling pathways (i.e., retrograde signals from organelles), could be involved in the control of AS patterns in light-germinating seeds. Moreover, since red light promotes seed germination (Figure 1B), we could argue that AS changes are a consequence of the germination process. However, we provide compelling evidence showing that Rp and FRp can directly affect AS regulation since the analyzed AS changes are not correlated with the intensity of germination response (Figure 4). As previously shown, retrograde signals arising from the organelles are important for triggering AS changes during the transition from darkness to light in *Arabidopsis* seedlings (Petrillo et al., 2014; Riegler et al., 2018). Taking this into account, it is possible to think that retrograde signals arising from different organelles could be associated with signals from photoreceptors to fine-tune the AS process in some genes during light induction of seed germination.

Finally, we conclude that AS is a source of gene expression diversity that potentially leads to different proteins involved in the promotion of seed germination by light, and a proper regulation of this process might be of key relevance for the adjustment of seed germination in the correct place and time. The next step would be to determine which signaling pathway(s) is (are) controlling the splicing of these events and identify the targets of the splicing factors regulated by light. Moreover, the question whether these changes in the AS of different splicing regulators are physiologically relevant is still open. Unraveling these AS regulatory networks will help us understand the mechanisms through which these genes may be responsible in the promotion of germination in crop seeds.

## DATA AVAILABILITY

All datasets for this study are included in the manuscript and the Supplementary files. The RNA-seq data is available at the Gene Expression Omnibus (GEO) with this accession GSE134019

## REFERENCES

- Arana, M. V., Sánchez-Lamas, M., Strasser, B., Ibarra, S. E., Cerdán, P. D., and Sánchez, R. A. (2014). Functional diversity of phytochrome family in the control of light and gibberellin-mediated germination in *Arabidopsis*. *Plant Cell Environ.* 37, 2014–2023. doi: 10.1111/pce.12286
- Benech-Arnold, R. L., Sánchez, R. A., Forcella, F., Kruk, B. C., and Ghersa, C. M. (2000). Environmental control of dormancy in weed seed banks in soil. *Field Crops Res.* 67, 105–122. doi: 10.1016/S0378-4290(00)00087-3
- Benjamini, Y., and Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. Ser. B.* 57, 289–300. doi: 10.1111/j.2517-6161.1995.tb02031.x
- Bentsink, L., Jowett, J., Hanhart, C. J., and Koornneef, M. (2006). Cloning of *DOG1*, a quantitative trait locus controlling seed dormancy in

## AUTHOR CONTRIBUTIONS

EP and JB conceived the project. RT, LS, and EP performed the experiments. CH and MY processed the transcriptome data. MS-S performed the GO analyses. RT, EP, and JB analyzed and interpreted the data and wrote the manuscript. All authors read and approved the final version of the manuscript.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2019.01076/full#supplementary-material>

**SUPPLEMENTARY FIGURE 1 |** Calibration curves for the hormone experiments.

**SUPPLEMENTARY TABLE 1 |** Differential gene expression and alternative splicing analyses.

**SUPPLEMENTARY TABLE 2 |** Primers used in the study.

**SUPPLEMENTARY FILE 1 |** Gene models and gel images.

*Arabidopsis*. *Proc. Natl. Acad. Sci. U. S. A.* 103, 17042–17047. doi: 10.1073/pnas.0607877103

- Botto, J. F., Sánchez, R. A., and Casal, J. J. (1995). Role of phytochrome B in the induction of seed germination by light in *Arabidopsis thaliana*. *J. Plant Physiol.* 146, 307–312. doi: 10.1016/S0176-1617(11)82059-6
- Botto, J. F., Scopel, A. L., Ballaré, C. L., and Sánchez, R. A. (1998a). The effect of light during and after soil cultivation with different tillage implements on weed seedling emergence. *Weed Sci.* 46, 351–357. doi: 10.1017/S0043174500089530
- Botto, J. F., Sánchez, R. A., and Casal, J. J. (1998b). Burial conditions affect light responses of *Datura ferox* seeds. *Seed Sci Res.* 8, 423–429. doi: 10.1017/S0960258500004384
- Botto, J. F., Sánchez, R. A., Whitelam, G. C., and Casal, J. J. (1996). Phytochrome A mediates the promotion of seed germination by very low fluences of light and canopy shade-light in *Arabidopsis*. *Plant Physiol.* 110, 439–444. doi: 10.1104/pp.110.2.439

- Botto, J. F., Scopel, A. L., and Sánchez, R. A. (2000). Water constrains on the photoinduction on weed seed germination during tillage. *Funct. Plant Biol.* 27, 463–471. doi: 10.1071/PP99199
- Braunschweig, U., Barbosa-Morais, N. L., Pan, Q., Nachman, E. N., Alipanahi, B., Gonatopoulos-Pournatzis, T., et al. (2014). Widespread intron retention in mammals functionally tunes transcriptomes. *Genome Res.* 24, 1774–1786. doi: 10.1101/gr.177790.114
- Casal, J. J., and Yanovsky, M. J. (2005). Regulation of gene expression by light. *Int. J. Dev. Biol.* 49, 501–511. doi: 10.1387/ijdb.051973jc
- Finkelstein, R., Reeves, W., Ariizumi, T., and Steber, C. (2008). Molecular aspects of seed dormancy. *Ann. Rev. Plant Biol.* 59, 387–415. doi: 10.1146/annurev.arplant.59.032607.092740
- Fouquet, R., Martin, F., Fajardo, D. S., Gault, C. M., Gomez, E., Tseung, C. W., et al. (2011). Maize *rough endosperm3* encodes an RNA splicing factor required for endosperm cell differentiation and has a nonautonomous effect on embryo development. *Plant Cell.* 23, 4280–4297. doi: 10.1105/tpc.111.092163
- Gabriele, S., Rizza, A., Martone, J., Circelli, P., Costantino, P., and Vittorioso, P. (2010). The Dof protein DAG1 mediates PIL5 activity on seed germination by negatively regulating GA biosynthetic gene *AtGA3ox1*. *Plant J.* 61, 312–323. doi: 10.1111/j.1365-313X.2009.04055.x
- Galvao, V. C., and Fankhauser, C. (2015). Sensing the light environment in plants: photoreceptors and early signaling steps. *Curr. Opin. Neurobiol.* 34C, 46–53. doi: 10.1016/j.conb.2015.01.013
- Godoy Herz, M. A., Kubaczka M. G., Brzyzek G., Servi, L., Krzyszton M., Simpson, C., et al. (2019). Light regulates plant alternative splicing through the control of transcriptional elongation. *Mol. Cell.* 73, 1066–1074
- Graeber, K., Linkies, A., Steinbrecher, T., Mummehoff, K., Tarkowska, D., Turecková, V., et al. (2014). *DELAY OF GERMINATION 1* mediates a conserved coat-dormancy mechanism for the temperature- and gibberellin-dependent control of seed germination. *Proc. Natl. Acad. Sci. U. S. A.* 111, 3571–3580. doi: 10.1073/pnas.1403851111
- Hartmann, L., Drewe-Boß, P., Wießner, T., Wagner, G., Geue, S., Lee, H. C., et al. (2016). Alternative splicing substantially diversifies the transcriptome during early photomorphogenesis and correlates with the energy availability in *Arabidopsis*. *Plant Cell.* 28, 2715–2734. doi: 10.1105/tpc.16.00508
- Hennig, L., Stoddart, W. M., Dieterle, M., Whitelam, G. C., and Schäfer, E. (2002). Phytochrome E controls light-induced germination of *Arabidopsis*. *Plant Physiol.* 128, 194–200. doi: 10.1104/pp.010559
- Ibarra, S. E., Auge, G., Sánchez, R. A., and Botto, J. F. (2013). Transcriptional programs related to phytochrome A function in *Arabidopsis* seed germination. *Mol. Plant.* 6, 1261–1273. doi: 10.1093/mp/sst001
- Ibarra, S. E., Tognacca, R. S., Dave, A., Graham, I. A., Sanchez, R. A., and Botto, J. F. (2016). Molecular mechanisms underlying the entrance in secondary dormancy of *Arabidopsis* seeds. *Plant Cell Environ.* 39, 213–221. doi: 10.1111/pce.12607
- James, A. B., Syed, N. H., Bordage, S., Marshall, J., Nimmo, G. A., Jenkins, G. I., et al. (2012). Alternative splicing mediates responses of the *Arabidopsis* circadian clock to temperature changes. *Plant Cell.* 24, 961–981. doi: 10.1105/tpc.111.093948
- Jones, M. A., Williams, B. A., McNicol, J., Simpson, C. G., Brown, J. W. S., and Harmer, S. L. (2012). Mutation of *Arabidopsis SPLICEOSOMAL TIMEKEEPER LOCUS1* causes circadian clock defects. *Plant Cell.* 24, 4066–4082. doi: 10.1105/tpc.112.104828
- Jung, K. H., Bartley, L. E., Cao, P., Canlas, P. E., and Ronald, C. P. (2009). Analysis of alternatively spliced rice transcripts using microarray data. *Rice* 2, 44–55. doi: 10.1007/s12284-008-9020-9
- Juntawong, P., and Bailey-Serres, J. (2012). Dynamic light regulation of translation status in *Arabidopsis thaliana*. *Front. Plant Sci.* 3, 66. doi: 10.3389/fpls.2012.00066
- Kaiserli, E., Perrella, G., and Davidson, M. L. (2018). Light and temperature shape nuclear architecture and gene expression. *Curr. Opin. Plant Biol.* 45 (Pt A), 103–111. doi: 10.1016/j.pbi.2018.05.018
- Langmead, B., Trapnell, C., Pop, M., and Salzberg, S. L. (2009). Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol.* 10, R25.
- Lightfoot, D. J., Malone, K. M., Timmis, J. N., and Orford, S. J. (2008). Evidence for alternative splicing of MADS-box transcripts in developing cotton fibre cells. *Mol. Genet. Genomics.* 279, 75–85. doi: 10.1007/s00438-007-0297-y
- Liu, M. J., Wu, S. H., Wu, J. F., Lin, W. D., Wu, Y. C., Tsai, T. Y., et al. (2013). Translational landscape of photomorphogenic *Arabidopsis*. *Plant Cell.* 25, 3699–3710. doi: 10.1105/tpc.113.114769
- Liu, M. J., Wu, S. H., and Chen, H. M. (2012). Widespread translational control contributes to the regulation of *Arabidopsis* photomorphogenesis. *Mol. Syst. Biol.* 8, 566. doi: 10.1038/msb.2011.97
- Mancini, E., Sanchez, S. E., Romanowski, A., Schlaen, R. G., Sanchez-Lamas, M., Cerdán, P. D., et al. (2016). Acute effects of light on alternative splicing in light-grown plants. *Photochem. Photobiol.* 92, 126–133. doi: 10.1111/php.12550
- Mano, S., Hayashi, M., and Nishimura, M. (1999). Light regulates alternative splicing of hydroxypyruvate reductase in pumpkin. *Plant J.* 17, 309–320. doi: 10.1046/j.1365-313X.1999.00378.x
- Martin-Trillo, M., Grandio, E. G., Serra, F., Marcel, F., Rodriguez-Buey, M. L., Schmitz, G., et al. (2011). Role of tomato *BRANCHED1-like* genes in the control of shoot branching. *Plant J.* 67, 701–714. doi: 10.1111/j.1365-313X.2011.04629.x
- Matsumura, H., Kitajima, H., Akada, S., Abe, J., Minaka, N., and Takahashi, R. (2009). Molecular cloning and linkage mapping of cryptochrome multigene family in soybean. *Plant Genome* 2, 271–281. doi: 10.3835/plantgenome.2009.06.0018
- Merchante, C., Stepanova, A. N., and Alonso, J. M. (2017). Translation regulation in plants: an interesting past, an exciting present and a promising future. *Plant J.* 90, 628–653. doi: 10.1111/tbj.13520
- Nakabayashi, K., Bartsch, M., Ding, J., and Soppe, W. J. J. (2015). Seed dormancy in *Arabidopsis* requires self-binding ability of DOG1 protein and the presence of multiple isoforms generated by alternative splicing. *PLOS Genet.* 11, e1005737. doi: 10.1371/journal.pgen.1005737
- Nakabayashi, K., Bartsch, M., Xiang, Y., Miatton, E., Pellengahr, S., Yano, R., et al. (2012). The time required for dormancy release in *Arabidopsis* is determined by *DELAY OF GERMINATION1* protein levels in freshly harvested seeds. *Plant Cell.* 24, 2826–2838. doi: 10.1105/tpc.112.100214
- Narsai, R., Gouil, Q., Secco, D., Srivastava, A., Karpievitch, Y. V., Liew, L. C., et al. (2017). Extensive transcriptomic and epigenomic remodelling occurs during *Arabidopsis thaliana* germination. *Genome Biol.* 18, 172. doi: 10.1186/s13059-017-1302-3
- Oh, E., Kang, H., Yamaguchi, S., Park, J., Lee, D., Kamiya, Y., et al. (2009). Genome-wide analysis of genes targeted by PHYTOCHROME INTERACTING FACTOR 3-LIKE5 during seed germination in *Arabidopsis*. *Plant Cell.* 21, 403–419. doi: 10.1105/tpc.108.064691
- Oh, E., Yamaguchi, S., Hu, J., Utsuke, J., Jung, B., Paik, I., et al. (2007). PIL5, a phytochrome-interacting bHLH protein, regulates gibberellin responsiveness by binding directly to the GAI and RGA promoters in *Arabidopsis* seeds. *Plant Cell.* 19, 1192–1208. doi: 10.1105/tpc.107.050153
- Oh, E., Yamaguchi, S., Kamiya, Y., Bae, G., Chung, W. I., and Choi, G. (2006). Light activates the degradation of PIL5 protein to promote seed germination through gibberellin in *Arabidopsis*. *Plant J.* 47, 124–139. doi: 10.1111/j.1365-313X.2006.02773.x
- Paik, I., Yang, S., and Choi, G. (2012). Phytochrome regulates translation of mRNA in the cytosol. *Proc. Natl. Acad. Sci. U. S. A.* 109, 1335–1340. doi: 10.1073/pnas.1109683109
- Park, E., Park, J., Kim, J., Nagatani, A., Lagarias, J. C., and Choi, G. (2012). Phytochrome B inhibits binding of phytochrome-interacting factors to their target promoters. *Plant J.* 72, 537–546. doi: 10.1111/j.1365-313X.2012.05114.x
- Penfield, S., Josse, E. M., Kannangara, R., Gilday, A. D., Halliday, K. J., and Graham, I. A. (2005). Cold and light control seed germination through the bHLH transcription factor SPATULA. *Curr. Biol.* 15, 1998–2006.
- Penfield, S., Josse, E. M., and Halliday, K. J. (2010). A role for an alternative splice variant of *PIF6* in the control of *Arabidopsis* primary seed dormancy. *Plant Mol. Biol.* 73, 89–95. doi: 10.1007/s11103-009-9571-1
- Pervouchine, D. D., Knowles, D. G., and Guigo, R. (2013). Intron-centric estimation of alternative splicing from RNA-seq data. *Bioinformatics* 29, 273–274. doi: 10.1093/bioinformatics/bts678
- Petrillo, E., Godoy Herz, M. A., Barta, A., Kalyna, M., and Kornbliht, A. R. (2015). Let there be light: regulation of gene expression in plants. *RNA Biol.* 11, 1215–1220. doi: 10.4161/15476286.2014.972852
- Petrillo, E., Godoy Herz, M. A., Fuchs, A., Reifer, D., Fuller, J., Yanovsky, M. J., et al. (2014). A chloroplast retrograde signal regulates nuclear alternative splicing. *Science* 344, 427–430. doi: 10.1126/science.1250322
- Reddy, A. S. N., Marquez, Y., Kalyna, M., and Barta, A. (2013). Complexity of the alternative splicing landscape in plants. *Plant Cell.* 25, 3657–3683. doi: 10.1105/tpc.113.117523

- Riegler, S., Servi, L., Fuchs, A., Godoy Herz, M. A., Kubaczka, M. G., Venhuizen, P., et al. (2018). Light remote control of alternative splicing in roots through TOR kinase. Pre-print at bioRxiv. Available at: <https://www.biorxiv.org/content/10.1101/472126v2>.
- Robinson, M. D., McCarthy, D. J., and Smyth, G. K. (2010). edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*. 26, 139–140. doi: 10.1093/bioinformatics/btp616
- Rosloski, S. M., Singh, A., Jali, S. S., Balasubramanian, S., Weigel, D., and Grbic, V. (2013). Functional analysis of splice variant expression of MADS AFFECTING FLOWERING 2 of *Arabidopsis thaliana*. *Plant Mol. Biol.* 81, 57–69. doi: 10.1007/s11103-012-9982-2
- Sanchez, S. E., Petrillo, E., Beckwith, E. J., Zhang, X., Rugnone, M. L., Hernando, C. E., et al. (2010). A methyl transferase links the circadian clock to the regulation of alternative splicing. *Nature*. 468, 112–116. doi: 10.1038/nature09470
- Scopel, A. L., Ballaré, C. L., and Sánchez, R. A. (1991). Induction of extreme light sensitivity in buried weed seeds and its role in the perception of soil cultivations. *Plant Cell Environ.* 14, 501–508. doi: 10.1111/j.1365-3040.1991.tb01520.x
- Shikata, H., Hanada, K., Ushijima, T., Nakashima, M., Suzuki, Y., and Matsushita, T. (2014). Phytochrome controls alternative splicing to mediate light responses in *Arabidopsis*. *Proc. Natl. Acad. Sci. U. S. A.* 111, 18781–18786. doi: 10.1073/pnas.1407147112
- Shikata, H., Shibata, M., Ushijima, T., Nakashima, M., Kong, S. G., Matsuoka, K., et al. (2012). The RS domain of *Arabidopsis* splicing factor RRC1 is required for phytochrome B signal transduction. *Plant J.* 70, 727–738. doi: 10.1111/j.1365-313X.2012.04937.x
- Shinomura, T., Nagatani, A., Chory, J., and Furuya, M. (1994). The induction of seed germination in *Arabidopsis thaliana* is regulated principally by phytochrome B and secondarily by phytochrome A. *Plant Physiol.* 104, 363–371. doi: 10.1104/pp.104.2.363
- Shinomura, T., Nagatani, A., Hanzawa, H., Kubota, M., Watanabe, M., and Furuya, M. (1996). Action spectra for phytochrome A- and B-specific photoinduction of seed germination in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. U. S. A.* 93, 8129–8133. doi: 10.1073/pnas.93.15.8129
- Simpson, C. G., Fuller, J., Maronova, M., Kalyna, M., Davidson, D., McNicol, J., et al. (2008). Monitoring changes in alternative precursor messenger RNA splicing in multiple gene transcripts. *Plant J.* 53, 1035–1048. doi: 10.1111/j.1365-313X.2007.03392.x
- Srinivasan, A., Jiménez-Gómez, J. M., Fornara, F., Soppe, W. J. J., and Brambilla, V. (2016). Alternative splicing enhances transcriptome complexity in desiccating seeds. *JIPB*. 58, 947–958. doi: 10.1111/jipb.12482
- Sugliani, M., Brambilla, V., Clerckx, E. J. M., Koornneef, M., and Soppe, W. J. J. (2010). The conserved splicing factor SUA controls alternative splicing of the developmental regulator ABI3 in *Arabidopsis*. *Plant Cell*. 22, 1936–1946. doi: 10.1105/tpc.110.074674
- Syed, N. H., Kalyna, M., Marquez, Y., Barta, A., and Brown, J. W. S. (2012). Alternative splicing in plants—coming of age. *Trends Plant Sci.* 17, 616–623. doi: 10.1016/j.tplants.2012.06.001
- Trapnell, C., Pachter, L., and Salzberg, S. L. (2009) TopHat: discovering splice junctions with RNA-Seq. *Bioinformatics* 25, 1105–1111.
- Tsai, H. L., Li, Y. H., Hsieh, W. P., Lin, M. C., Ahn, J. H., and Wu, S. H. (2014). HUA ENHANCER1 is involved in posttranscriptional regulation of positive and negative regulators in *Arabidopsis* photomorphogenesis. *Plant Cell*. 26, 2858–2872. doi: 10.1105/tpc.114.126722
- van Gelderen, K., Kang, C., and Pierik, R. (2018). Light signaling, root development, and plasticity. *Plant Physiol.* 176, 1049–1060. doi: 10.1104/pp.17.01079
- Wang, Y., Fan, X., Lin, F., He, G., Terzaghi, W., Zhu, D., et al. (2014). *Arabidopsis* noncoding RNA mediates control of photomorphogenesis by red light. *Proc. Natl. Acad. Sci. U. S. A.* 111, 10359–10364. doi: 10.1073/pnas.1409457111
- Wu, H. P., Su, Y. S., Chen, H. C., Chen, Y. R., Wu, C. C., Lin, W. D., et al. (2014). Genome-wide analysis of light-regulated alternative splicing mediated by photoreceptors in *Physcomitrella patens*. *Genome Biol.* 15, R10. doi: 10.1186/gb-2014-15-1-r10
- Xin, R., Kathare, P. K., and Huq, E. (2019). SFPS forms a complex with the SR-like protein RRC1. *Plant Cell*. doi: 10.1105/tpc.18.00786
- Yakir, E., Hilman, D., Hassidim, M., and Green, R. M. (2007). CIRCADIAN CLOCK ASSOCIATED1 transcript stability and the entrainment of the circadian clock in *Arabidopsis*. *Plant Physiol.* 145, 925–932. doi: 10.1104/pp.107.103812.

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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