Shade-induced transcription of PIF-Direct-Target Genes precedes H3K4-trimethylation chromatin modifications

Robert H. Calderon[1,2,3*], Jutta Dalton[1,2], Yu Zhang[1,2,4], and Peter H. Quail[1,2]

[1]Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720, USA

[2]Plant Gene Expression Center, Agriculture Research Service, US Department of Agriculture, Albany, CA 94710, USA

[3]Umeå Plant Science Centre, Department of Plant Physiology, Umeå University, 901 87 Umeå, Sweden

[4]US Department of Energy, Joint Genome Institute, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA

* Corresponding author: Robert H. Calderon (robert.calderon@umu.se)

Running title: Shade-induced transcription precedes H3K4me3 rise

Abstract

Plants must sense and adapt to changes in their light environment to survive. Sensing of red and far-red light levels is mediated by the phytochrome (phy) family of photoreceptors, which transduce this information to **P**hytochrome-**I**nteracting **F**actors (PIFs), a small set of DNA-binding transcription factors, that regulate gene expression. The phy photoreceptors are photoreversible in response to changes in red and far-red light levels, and the transcript levels of a set of PIF **D**irect-**T**arget **G**enes (DTGs) also change in a correspondingly photoreversible manner. However, not all PIF DTGs exhibit the same photoreversible pattern. Here, we have integrated previously published data with new transcript and chromatin profiling data to define a comprehensive list of PIF DTGs, and categorize them by their patterns of gene expression. We examine whether the time-dependent, light-signal-induced accumulation of a histone modification, histone 3 lysine 4 trimethylation (H3K4me3), is predictive of specific patterns of PIF-driven gene expression. We find, to the contrary, that H3K4me3 levels appear to accumulate slightly after the transcript levels increase for shade-responsive PIF DTGs, suggesting that these chromatin modifications are not causal in eliciting the transcriptional responses.

Introduction

All organisms must perceive, process and react to environmental cues in order to survive and pass their genetic material onto the next generation. Land plants in particular, given their sessile lifestyle, must quickly perceive these environmental signals and respond accordingly. One particularly well-studied plant signaling system is the phytochrome (phy) family of photoreceptors (phyA to phyE in Arabidopsis), a set of red (R) and far red (FR) light-absorbing chromoproteins that transduce light signals into large-scale changes in gene expression (Tepperman et al., 2001). Upon absorption of R light, the inactive form of the phy molecule (Pr) is photoconverted into the active form (Pfr) and quickly translocates from the cytoplasm to the nucleus, initiating downstream developmental programs, directed by these expression changes (Sakamoto and Nagatani, 1996).

The relative abundance of the Pfr and Pr forms of the phy molecules is determined by the ratio of red to far-red light in the immediate environment. The active Pfr form is favored under white-light illumination where the R/FR ratio is high, while the inactive Pr form is favored in the dark and under vegetative shading, where the R/FR ratio is non-existent or low, respectively (Quail et al., 1995).

Experimental evidence indicates that a critical link between downstream programs and the phy molecules is a subfamily of eight bHLH transcription factors called phy-interacting factors (PIFs) (Ni et al., 1998; Huq and Quail, 2002; Monte et al., 2004; Leivar and Quail, 2011; Pham et al., 2018). The PIFs, in particular PIF1, PIF3, PIF4 and PIF5 (called the PIF quartet), form a set of partially functionally redundant proteins that bind to a consensus sequence in the upstream region of target genes, regulating their transcriptional output (Leivar et al., 2009). The PIF quartet has been shown to physically interact specifically with Pfr, which induces phosphorylation, ubiquitination and degradation of the transcription factor (Ni et al., 2013; 2014; 2017), thereby triggering global changes in target gene expression (Leivar et al., 2009; Leivar and Quail, 2011; Pham et al., 2018). In addition to the PIF quartet, two additional PIFs that play a role in phyB-mediated signaling have been identified: PIF6 and PIF7 (Khanna et al., 2004; Leivar et al., 2008).

Several genome-wide analyses of PIF-binding and PIF-mediated transcriptional regulation have resulted in a complex picture of PIF activity (Leivar et al., 2009; Hornitschek et

al., 2012; Leivar et al., 2012; Oh et al., 2012). Our previous work integrating transcriptional changes with PIF-DNA-binding data defined a set of over 300 Direct Target Genes (DTGs) that are directly, transcriptionally regulated by the PIFs (Zhang et al., 2013; Pfeiffer et al., 2014). PIFs have intrinsic transcriptional activating activity (Dalton et al., 2016) and act as transcriptional activators for many target genes (Huq et al., 2004; Al-Sady et al., 2008; de Lucas et al., 2008). They also act as transcriptional repressors for a different set of target genes (Leivar et al., 2009; Leivar et al., 2012; Liu et al., 2013). Further complicating the picture is the observation that, despite the photoreversible nature of phyB, many PIF direct target genes (DTGs) no longer respond in a photoreversible manner after a period in white light (Leivar et al., 2012).

In an earlier study we identified these non-photoreversible genes by using microarrays to compare the transcriptional responses of *pif*-mutant and wild-type seedlings under various light conditions (Leivar et al., 2009; Leivar et al., 2012). Genes showing a robust, PIF-dependent response were assigned to one of three categories (L, M or R) depending on whether they showed responsiveness in dark-grown seedlings exposed to a red light transition (L), in white-light-grown seedlings exposed to a shade transition (R), or in both cases (M) (Leivar et al., 2012). The differential responsiveness of these three broad sets of PIF DTGs to dark versus shade, both conditions where phy is either inactive, or has reduced activity, and PIFs accumulate, indicates that PIF abundance is not the sole determinant of PIF DTG expression. Recently, components of the plant circadian oscillator have been implicated in modulating some of these changes in gene expression (Martín et al., 2018; Zhang et al., 2020), as have changes in the chromatin environment (Willige et al., 2021).

One way in which chromatin remodeling can modulate the transcriptional output of light-regulated genes is through the enzymatic modification of histones (Fisher and Franklin, 2011; Perrella and Kaiserli, 2016; Bourbousse et al., 2019) with the methylation, acetylation and/or ubiquitination of histones all having been shown to regulate transcription of light-regulated genes (Charron et al., 2009; Bourbousse et al., 2012; Liu et al., 2013). Unique histone modification patterns at the promoters of individual PIF DTGs have the potential to underly the differential responsiveness PIF DTGs under different environmental conditions. The accumulation of one particular mark, histone 3 lysine 4 trimethylation (H3K4me3), at the transcriptional start site (TSS) of genes has been found to strongly correlate with transcriptional activity of those genes (Bernstein et al., 2002).

Here, we have refined the list of PIF DTGs by integrating previously published ChIP binding and RNA-seq data for the PIF quartet, with newly obtained RNA-seq data from both wild-type and a mutant lacking six of the PIFs (PIF1, 3, 4, 5, 6 and 7). In addition, we have used this system to explore the potential role of the epigenetic mark H3K4me3 in mediating these differential patterns of expression of PIF DTGs. Based on this work, we suggest that the PIFs and their DTGs may serve as a model system for exploring the role of epigenetic factors in the regulation of gene expression in plants.

Results

Characterization of *pifqpif6pif7* sextuple mutant

The *pif1pif3pif4pif5* quadruple mutant (hereafter *pifq*) displays a constitutively photomorphogenic phenotype when grown in darkness, indicating that these four PIFs are necessary and sufficient to control de-etiolation in response to R (Leivar et al., 2008; Leivar et al., 2009). The *pifq* mutant does not, however, exhibit a complete lack of responsiveness to simulated shade, supporting the hypothesis that additional factors are required for the complete shade avoidance response (Leivar et al., 2012). PIF7 has been implicated in playing a major role in regulating this process (Li et al., 2012; de Wit et al., 2015; Mizuno et al., 2015) with the quintuple *pifqpif7* mutant showing no statistically-significant shade avoidance response (Zhang et al., 2020).

When we measured the shade avoidance response in the *pifqpif7* mutant, we were still able to detect a small, yet statistically-significant ($p < 0.05$) residual shade avoidance response (**Figure 1**). We then tested whether PIF6 might be responsible for this residual response by generating a sextuple *pifqpif6pif7* mutant and measuring its hypocotyl length in response to simulated shade. This sextuple mutant displayed significantly shorter hypocotyls than the wild-type in response to shade, but no significant decrease relative to the *pifqpif7* quintuple mutant (**Figure 1**).

Generation of a high-confidence list of PIF DTGs

Many PIF direct target genes (DTGs) have been previously observed to be upregulated in the presence of the PIFs while others are downregulated in the presence of the PIFs. For the purposes of this study, we focused only on PIF-induced genes (*i.e.* those genes who appear to require the PIFs for high levels of transcription) because PIFs have been shown to have intrinsic activating activity.

To generate a high-confidence list of PIF-induced DTGs, we integrated the data from a previously published RNA-seq experiment on dark-grown seedlings exposed to 1h of R light (Pfeiffer et al, 2014) with a new RNA-seq time course experiment of white light (WL) grown seedlings exposed to 3h of simulated shade (shade-light). We imposed strict statistically-significant two-fold (SSTF) cutoffs and selected all 764 genes whose expression levels decreased in response to red light and/or increased in response to shade-light, in one or more of all these studies.

Having identified light-responsive genes, we further narrowed our list to include only those genes that show a dependence on PIFs for their expression. We therefore combined the previously published RNA-seq data from the *pifq* mutant grown in darkness, with our newly obtained RNA-seq data, obtained using the *pifqpif6pif7* mutant (*pifS*) grown in WL and exposed to 3h shade-light. We selected only those genes that were SSTF induced in WT relative to their levels in the corresponding *pif* mutant. By filtering out those genes that were not among the 764 light-responsive genes identified above, we were left with 278 PIF-dependent, light-responsive genes.

We next analyzed the promoters of the PIF-dependent light-responsive genes identified in this way and determined whether they were bound by one or more PIFs. We used previously published data to select those genes whose promoters were found to be bound by PIF1, PIF3, PIF4, PIF5 and/or PIF7 (no genome-wide binding data are available for PIF6) (Hornitschek et al., 2012; Oh et al., 2012; Zhang et al., 2013; Pfeiffer et al., 2014; Chung et al., 2020). By selecting only the genes that met all three of our criteria (light-responsiveness, PIF-dependence

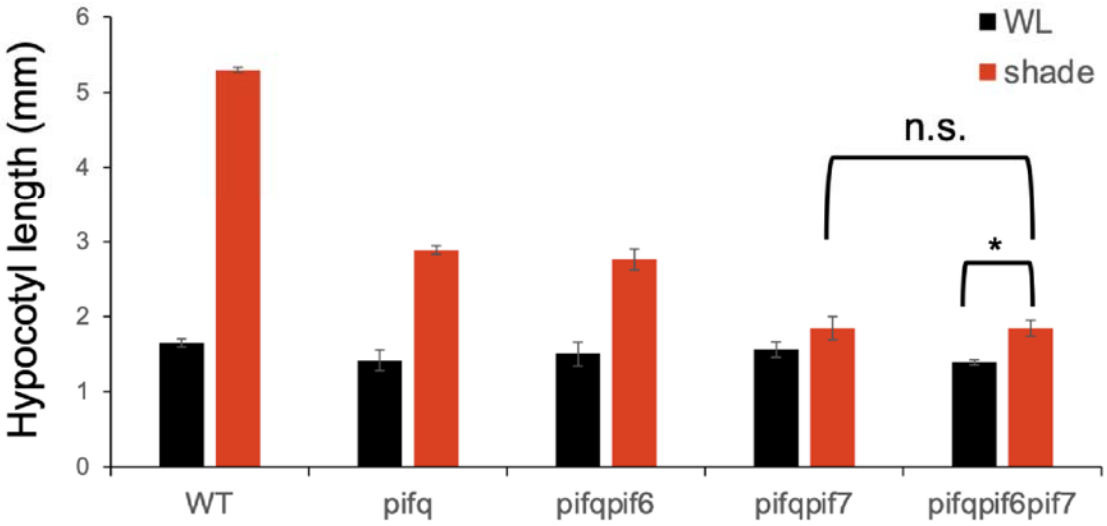

Figure 1: Phenotypic analysis of higher-order *pif* mutants in response to simulated shade. Hypocotyl lengths of wild-type (WT), *pifq, pifqpif6, pifqpif7* and *pifqpif6pif7* mutants grown for 6 days in white light (WL) or 2 days in WL followed by 4 days in simulated shade (shade). Data represent the mean and SE from 30 seedlings. Asterisks indicate that the hypocotyl lengths of shade-treated seedlings are statistically significantly different from the corresponding WLc controls by Student's t test ($P < 0.05$). n.s. indicates "not significantly different" ($P > 0.99$).

and PIF-binding), we obtained 169 candidate PIF-induced, red-light repressed and/or shade-light-induced DTGs **(Table 1**).

Subcategorization of PIF DTGs into E, ES and S classes

As described in Leivar *et al.* (2012), PIF DTGs may be broadly classified into one of three classes: L, M or R (**Figure 2)**. We therefore subdivided our combined 169 shade-light-induced and red-repressed PIF- DTGs into these classes based on their patterns of expression during the D to R, and WL to shade-light transitions (**Table 1**). To avoid any potential confusion between the abbreviations for R class genes and R light, we renamed L, M and R classes as E (**E**tiolation-induced only), ES (both **E**tiolation- and **S**hade-induced) and S (**S**hade-induced only) classes, respectively. As in Leivar et al, 2012, Class E represents genes whose dark-grown wild-type transcript levels are both (a) SSTF higher than those in dark-grown *pifq* and (b) SSTF repressed by the initial red light (R) signal in WT. Although some Class E genes show a degree of re-induction in the shade, this is weaker (*i.e.* non-SSTF) induction and PIF-dependency than initially in the dark (**Figure 2**). Conversely, Class S represents genes that do not exhibit a SSTF response to either (a) the PIFs in dark-grown seedlings or (b) red light exposure, but that do display SSTF induction by shade-light, and PIF-dependent SSTF induction in the shade (**Figure 2**). Finally, Class ES represents those genes that display SSTF reciprocal responsiveness to the onset of the light (R) and shade-light signals and PIF-dependent SSTF induction, both in the dark and in shade-light (**Figure 2**). Using these criteria, we found a total of 24 E genes, 17 ES genes and 128 S genes.

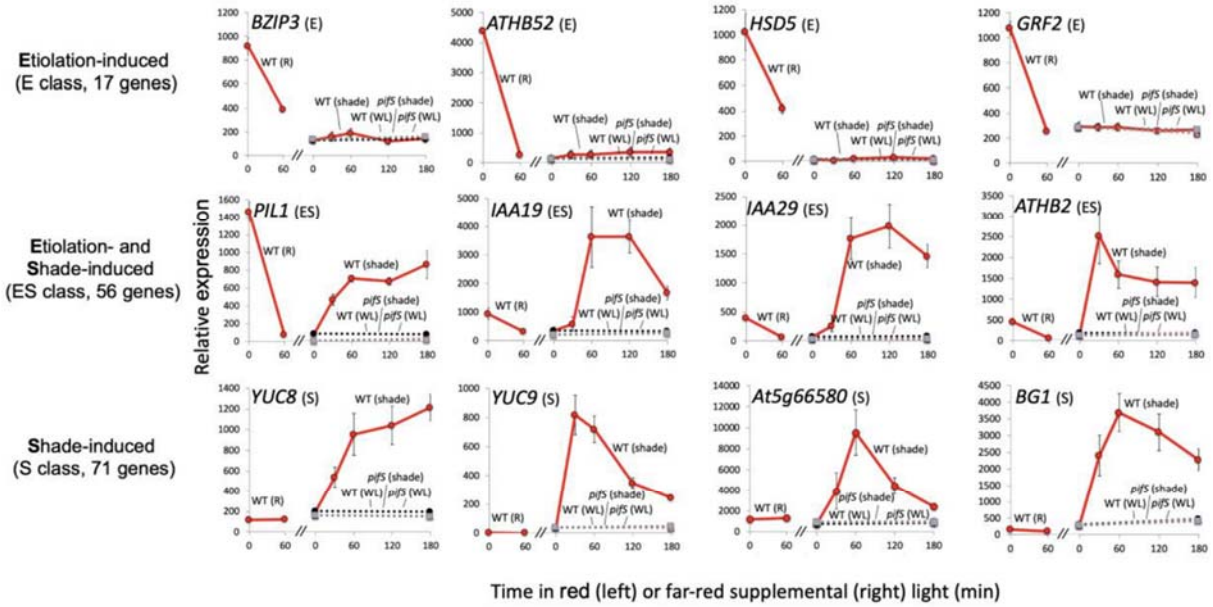

Figure 2: PIF-activated direct target genes (DTGs) can be subdivided into three categories based on their responses to red light and simulated shade. Examples of transcript time course profiles for Etiolation-induced only (E) genes (BZIP3, ATHB2, HSD5 and GRF2), Etiolation and Shade-induced (ES) genes (PIL1, IAA19, IAA29 and ATHB2) and Shade-induced only (S) genes (YUC8, YUC9, At5g02865 and BG1) class genes. Left subpanel shows the effect of 60 min red light on the transcript levels in 3-day-old dark-grown seedlings (wild-type, solid red line). Right subpanel shows the effect of 30, 60, 120 and 180 min of FR-enriched WL or continuous WL on transcript levels in 3-day-old WL-grown seedlings (wild-type, FR: solid red line; *pifS*, FR: dotted black line; wild-type, WL: dotted red line; *pifS*, WL: dotted gray line). Error bars indicate SE.

We observed that a subset of the E, ES and S class genes that we identified above exhibited anomalous transcriptional profiles. We manually removed these 25 genes because they were either highly expressed in WL (6 genes), induced by red light (16 genes), lowly expressed (1 gene) or were otherwise likely to be artifactual (2 genes). The remaining 144 PIF DTGs were then resorted using relaxed cutoffs.

Of the non-anomalous genes first categorized as S class, 38 showed a R-dependent reduction ($p < 0.1$) in transcript levels but were excluded from the ES class because they did not show a SSTF reduction in dark-grown *pifq* mutant relative to WT. These genes were reclassified as ES. Two E class genes were also reclassified as ES genes because they exhibited a statistically-significant upregulation in response to FR despite not being SSTF downregulated in the *pifS* mutant. Ultimately, we were left with 17 E genes, 56 ES genes and 71 S genes **(Table 1**).

A previous report identified 338 PIF DTGs, with 209 of these genes being regulated by PIFs acting as inducers (Pfeiffer et al., 2014). When we compared these genes to our 144 PIF-induced DTGs, we found 45 genes in common (**Table 1)**. Four of these genes were previously identified as PIF-repressed. 34 out of the 45 genes in common were E or ES genes while 11 were classified as S genes.

Examination of potential epigenetic coregulators

We next tested our hypothesis that the differential transcriptional responses of the PIF-activated DTGs to darkness and shade might be due to differences in histone tail modifications. One histone mark, H3K27me3, has already been linked to the light-mediated transcriptional repression (Charron et al., 2009). Because we were focused on loci at which PIFs act as transcriptional activators, we sought to examine the levels of a histone mark associated with active transcription. One such mark, H3K4me3 is both correlated with actively transcribed genes (Bernstein et al., 2002) and inversely correlated with H3K27me3 levels (Zhang et al., 2009). We therefore chose to assay H3K4me3 levels at the transcriptional start sites (TSS) of E, ES and S genes by ChIP-seq. We measured H3K4me3 levels in dark-grown seedlings and in WL-grown seedlings after 0, 30, 60, 120 and 180 min of simulated shade and after 180 min of additional WL. We also measured H3K4me3 levels in WL-grown *pifS* seedlings after 0 and 180 min of simulated shade and after 180 min of additional WL.

H3K4me3 peaks were calculated using BayesPeak (Spyrou et al., 2009) and MACS2 (Zhang et al., 2008) but could only be unambiguously assigned to the transcriptional start sites (TSS) of 102 of the 144 E, ES, and S class genes. To ensure consistency in analysis, we therefore manually assigned peaks to all of the PIF DTGs by creating 300bp windows centered on the TSS.

To test whether H3K4me3 levels at the TSS of a given locus might be predictive of the transcriptional activity at that locus, we used DiffBind (Ross-Innes et al., 2012) and DESeq2 (Love et al., 2014) to quantify the H3K4me3 peaks and measure differences between time points. Because the changes in magnitude of H3K4me3 levels were far smaller than the changes in transcript abundance (**Figure 3**), we used DESeq2 to calculate variance-stabilizing transformations (VSTs) across the time course experiment for both H3K4me3 levels and transcript levels. This enables comparison of relative changes in H3K4me3 levels to the corresponding changes in transcription for a given gene or class of genes (**Figure 4**).

As expected, H3K4me3 levels for E class genes were higher in D than in WL and simulated shade (**Figure 3**). On average, H3K4me3 levels for ES and S class genes increase over the course of the shade treatment and this increase is attenuated in the *pifS* mutant **(Figure 4**). In both classes, however, the increase only occurs after 60 minutes of FR while an increase in transcript level abundance is already visible after 30 minutes of FR. Both classes also exhibit a transient reduction in H3K4me3 levels after 30 minutes of FR.

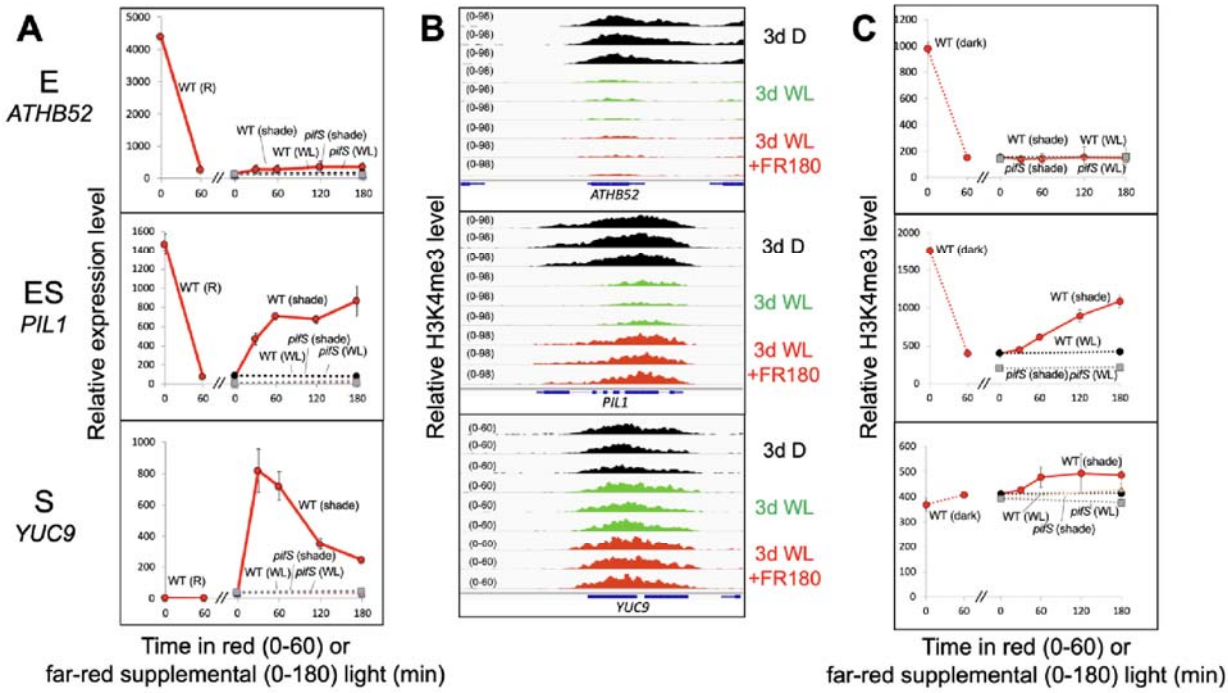

Figure 3: Transcript levels are broadly correlated with H3K4me3 levels for PIF DTGs belonging to Etiolation-induced only (E), Etiolation and Shade-induced (ES) and Shade-induced only (S) classes. A) Average relative transcript levels as measured by RNA-seq of ATHB52 (E class, top), PIL1 (ES class, middle) and YUC9 (S class, bottom). Left subpanel shows the effect of 60 min red light on the transcript levels in 3-day-old dark-grown seedlings (wild-type, solid red line). Right subpanel shows the effect of 30, 60, 120 and 180 min of FR-enriched WL or continuous WL on transcript levels in 3-day-old WL-grown seedlings (wild-type, FR: solid red line; *pifS*, FR: dotted black line; wild-type, WL: dotted red line; *pifS*, WL: dotted gray line). Error bars indicate SE. **B)** H3K4me3 enrichment as measured by ChIP-seq of ATHB52 (top), PIL1 (middle) and YUC9 (bottom) in 3-day-old dark-grown seedlings (3d D, black), 3-day-old WL-grown seedlings (3d WL, green) and 3-day-old WL-grown seedlings after 180 min of FR-enriched WL (3d WL +FR180, red). Data from each of three biological replicates are shown. **C)** Average relative H3K4me3 levels of ATHB52 (top), PIL1 (middle) and YUC9 (bottom). Left subpanel shows the levels in 3-day-old dark-grown seedlings and the levels in 3-day-old WL-grown seedlings (wild-type, connected by dashed red line). Right subpanel shows the effect of 30, 60, 120 and 180 min of FR-enriched WL or continuous WL on transcript levels in 3-day-old WL-grown seedlings (wild-type, FR: solid red line; *pifS*, FR: dotted black line; wild-type, WL: dotted red line; *pifS*, WL: dotted gray line). Error bars indicate SE.

Discussion

We have established the PIFs and their DTGs as a model system to explore the role of epigenetic factors in the regulation of gene expression in shade avoidance. Specifically, we have integrated our newly obtained data with previously published data and have now identified a set of 144 "high-confidence" PIF-induced DTGs. Additionally, we have categorized them into three classes (E, ES and S) based on their transcriptional behavior in mutant seedlings and their responses to light. While 130 of these genes had been previously described as PIF DTGs, the

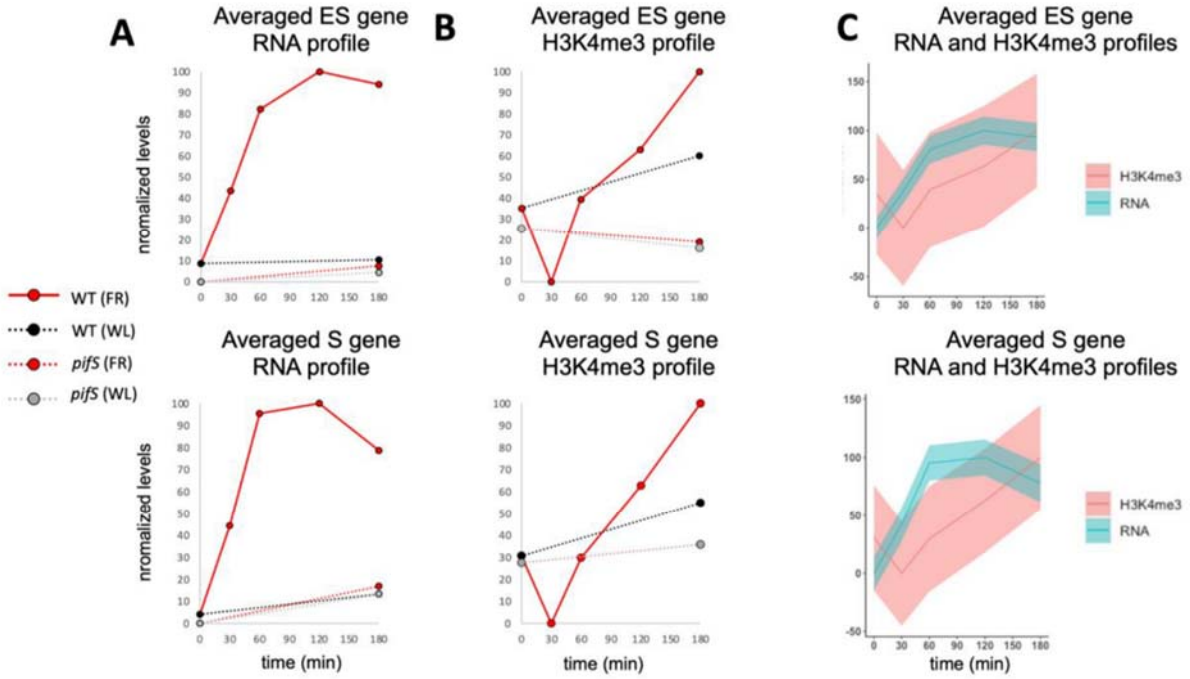

Figure 4: Shade-induced, PIF-dependent increases in transcription precede corresponding increases in H3K4me3 for ES class and S class genes. A) Normalized levels of the average transcript profile for all ES class genes (top) or S class genes during 180 minute shade-light (FR, red) or white-light (WL, black/gray) treatment for wild-type (WT, solid red/dashed black) or *pifS* (dashed red/dashed gray). **B)** Normalized levels of the average H3K4me3 profile for all ES class genes (top) or S class genes during 180 minute shade-light (FR, red) or white-light (WL, black/gray) treatment for wild-type (WT, solid red/dashed black) or *pifS* (dashed red/dashed gray). **C)** Overlays of the RNA (yellow) and H3K4me3 (blue) profiles from WT seedlings during the FR treatment. Shaded variance indicate normalized SE.

work presented here has identified 14 additional genes, which, to our knowledge, are newly implicated as direct targets of the PIFs (**Supplemental Table 1**). Our set of PIF-induced DTGs is nearly 50% smaller than a similar list generated in our group (Pfeiffer et al., 2014). This could be due, at least in part, to the fact that the previous study focused on dark-grown seedlings while we have focused on shade-responsiveness and incorporated additional datasets obtained from shade-exposed seedlings.

Our results also suggest that PIF6 appears to play no significant role in mediating the shade-avoidance response (**Figure 1**), consistent with its proposed role in mediating seed dormancy and development (Penfield et al., 2010). In slight contrast to previously published results from our group (Zhang et al., 2020), we find a small but statistically-significant increase in hypocotyl length in the *pifqpif7* mutant in response to 4 days of simulated shade. However, the statistical significance of this difference, as assayed by the Student's t test, yielded a p-value of 0.04034, a value just barely satisfying our criteria for significance ($p < 0.05$). It is also worth noting that the results presented here were obtained on 2-day-old seedlings exposed to simulated shade while our previous experiments were performed on 3-day-old seedlings exposed to simulated shade. Nonetheless, any residual shade avoidance response observed under our

conditions could be due to the presence of yet other members of the PIF-subfamily, like PIF8 or PIL1 (PIF2) (Leivar and Quail, 2011; Pham et al., 2018), or to other light-responsive transcription factors.

The results obtained from our integrated RNA-seq and ChIP-seq analyses showed no clear predictive patterns when observing H3K4me3 levels at the TSS of PIF DTGs in any of the three classes. We thus found no evidence of a contribution of H3K4me3 in determining the transcriptional responsiveness of PIF DTGs. To the contrary, we found that, on average, transcript levels of shade-induced PIF DTGs rise before their corresponding H3K4me3 levels rise (**Figure 4**). This is consistent with previous reports indicating that high levels of transcription from a given locus leads to trimethylation of H3K4me3 (Le Martelot et al., 2012; Kuang et al., 2014). Also in agreement with our results, a recent study in Arabidopsis examining transcriptional and epigenetic changes over a 12 hour time course in response to wounding found that H3K4me3 increases broadly correlate with transcriptional increases between 1 and 3 hours after wounding (Rymen et al., 2019). Surprisingly, we actually observed a transient decrease in H3K4me3 levels at the TSS of shade-induced genes (**Figure 4**). This decrease was not observed in response to wounding, most probably because H3K4me3 levels were first measured after 60 minutes and the transient decrease we observe was only visible 30 minutes after the signal was generated. To our knowledge, this phenomenon has not been described previously and merits further investigation.

Our results indicate that, at least in the case of shade-induced PIF-DTGs, H3K4me3 plays little to no role in causing the responsiveness of these genes to different light conditions. H3K4me3 is one of many histone modifications implicated in the epigenetic regulation of transcription and further experiments may reveal which, if any, of these other modifications might be involved in determining how a given PIF DTG responds to different types of illumination. In fact, in a recent parallel investigation of the role of H3K9 acetylation in the dark-to-red-light-induced expression of PIF-repressed DTGs, we found that H3K9 acetylation temporally parallels transcriptional induction, raising the possibility that light-induced transcriptional and chromatin-remodeling processes are mechanistically intertwined, at least during seedling deetiolotion (Gonzalez-Grandio et al, 2021, bioRxiv). These differing results could be due to differences between PIF-induced versus PIF-repressed genes, histone methylation versus acetylation or the regulatory role(s) of H3K9 versus H3K4. In addition to chromatin changes, recently published reports have also led to a model in which the PIFs act as the primary DNA-binding regulators of DTGs and that their transcriptional activating activity is modulated via protein-protein interactions with *trans* factors including components of the circadian oscillator like TOC1, PRR5 and PRR7 (Soy et al., 2016; Martín et al., 2018; Zhang et al., 2020). It is also possible that blue light photoreceptors including cryptochromes and phototropins play a role in determining how PIF DTGs respond to the different light conditions used in this study. Cryptochromes (CRYs) in particular have been observed to interact with members of the PIF family (Pedmale et al., 2016). CRYs were also recently discovered to interact with and regulate the activity of mRNA modifying enzymes, thereby providing a direct link between blue light signals and transcript abundance (Wang et al., 2021). This interaction was localized in photobodies, light-induced nuclear speckles in which phy molecules have also been found, raising the possibility that these photoreceptors coordinate to nucleate the formation and/or regulate the activity of large RNA (or chromatin) modifying enzyme complexes. Such photoregulated complexes could conceivably contribute to the differential transcriptional responses we observed for E, ES and S genes, a possibility which merits further exploration.

298
299 **Materials and Methods**
300 All seeds were stratified for 4 days at 4º before germination. Germination was induced by
301 3h of incubation under 30 µmol m^{-2} s^{-1} WL at 21º followed by a 5 min saturating pulse of FR
302 light. Seedlings were grown for 3 days at 21º in complete darkness or under 30 µmol m^{-2} s^{-1} WL
303 (R/FR = 6-8). For FR light treatment, seedlings were grown for 3 days in WL before exposing
304 them to simulated shade (30 µmol m^{-2} s^{-1}, R/FR ~ 0.3). R light was defined as 640-680 nm and
305 FR was defined as 710-750nm.
306 Hypocotyl measurements were performed on seedlings grown at 23º for 2 days in WL
307 and either exposed to simulated shade for 4 days or kept in constant WL for 4 days. Hypocotyl
308 length of 30 seedlings of each genotype were measured for each of three biological replicates.
309 Chromatin immunoprecipitation experiments were performed as described (Zhang et al.,
310 2013). H3K4me3-enriched DNA fragments from 3-day-old seedlings were immunoprecipitated
311 from 2 g of tissue with polyclonal H3K4me3 antibodies (Millipore, product #07-473) linked to
312 Protein A/G Dynabeads (ThermoFisher). DNA libraries for sequencing were prepared at the
313 Functional Genomics Laboratory at UC Berkeley using an Accel-NGS 2S Plus Library Kit
314 (Swift Biosciences) according to the manufacturer's instructions.
315 RNA was isolated as described (Zhang et al., 2013). Total RNA was extracted from 3-
316 day-old seedlings using a QIAshredder and RNeasy Plus Mini Kit (Qiagen) according to
317 manufacturer's instructions. RNA libraries for sequencing were prepared at the Functional
318 Genomics Laboratory at UC Berkeley using a KAPA RNA HyperPrep Kit (Roche) according to
319 the manufacturer's instructions.
320 DNA and RNA libraries were sequenced by the Genomic Sequencing Facility at UC
321 Berkeley. The multiplexed DNA libraries were sequenced by 50-bp single-end sequencing over
322 two lanes on a HiSeq4000. Multiplexed RNA libraries were sequenced by 100-bp paired-end
323 sequencing over two lanes on a HiSeq4000.
324 For mapping and analysis of ChIP-seq experiments, reads were mapped to the
325 Arabidopsis genome (TAIR10) by BowTie2 (Langmead and Salzberg, 2012) and uniquely-
326 mapping reads were used to call peaks using BayesPeak (Spyrou et al., 2009) (Bioconductor 3.6;
327 binsize = 300, peaks with a PP>0.999 in all 3 biological replicates) or MACS (Zhang et al.,
328 2008). Reads were counted and fold-changes were calculated using DiffBind (Ross-Innes et al.,
329 2012) while VSTs were calculated using DESeq2 (Love et al., 2014).
330 For mapping and analysis of RNA-seq experiments, reads were mapped to the
331 Arabidopsis genome (TAIR10) by TopHat (Trapnell et al., 2009) (max intron length = 3000,
332 inner mean distance = 200, inner distance standard deviation = 100, minimal allowed intron size
333 = 25). Assembled reads were counted using featureCounts (Liao et al., 2014) and differential
334 expression was determined via DESeq2 (Love et al., 2014) ($\log_2FC > 1$ or $\log_2FC < -1$; p-val <
335 0.05).
336
337 **Supplemental Data**
338
339 **Supplemental Table S1.** List of PIF-induced DTGs identified in this study and whether or not
340 they have been previously identified as a PIF DTG.
341
342 **Funding**

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Author Contributions

RHC and PHQ. designed the research. RHC, JD and YZ performed research. RHC, JD, YZ and PHQ analyzed data. RHC and PHQ wrote the paper.

Figure 1. Phenotypic analysis of higher-order *pif* mutants in response to simulated shade. Hypocotyl lengths of wild-type (WT), *pifq, pifqpif6, pifqpif7* and *pifqpif6pif7* mutants grown for 6 days in white light (WL) or 2 days in WL followed by 4 days in simulated shade (shade). Data represent the mean and SE from 30 seedlings. Asterisks indicate that the hypocotyl lengths of shade-treated seedlings are statistically significantly different from the corresponding WLc controls by Student's t test ($P < 0.05$). n.s. indicates "not significantly different" ($P > 0.99$).

Figure 2. PIF-activated direct target genes (DTGs) can be subdivided into three categories based on their responses to red light and simulated shade. Examples of transcript time course profiles for Etiolation-induced only (E) genes (BZIP3, ATHB2, HSD5 and GRF2), Etiolation and Shade-induced (ES) genes (PIL1, IAA19, IAA29 and ATHB2) and Shade-induced only (S) genes (YUC8, YUC9, At5g02865 and BG1) class genes. Left subpanel shows the effect of 60 min red light on the transcript levels in 3-day-old dark-grown seedlings (wild-type, solid red line). Right subpanel shows the effect of 30, 60, 120 and 180 min of FR-enriched WL or continuous WL on transcript levels in 3-day-old WL-grown seedlings (wild-type, FR: solid red line; *pifS*, FR: dotted black line; wild-type, WL: dotted red line; *pifS*, WL: dotted gray line). Error bars indicate SE.

Figure 3. Transcript levels are broadly correlated with H3K4me3 levels for PIF DTGs belonging to Etiolation-induced only (E), Etiolation and Shade-induced (ES) and Shade-induced only (S) classes. A) Average relative transcript levels as measured by RNA-seq of ATHB52 (E class, top), PIL1 (ES class, middle) and YUC9 (S class, bottom). Left subpanel shows the effect of 60 min red light on the transcript levels in 3-day-old dark-grown seedlings (wild-type, solid red line). Right subpanel shows the effect of 30, 60, 120 and 180 min of FR-enriched WL or continuous WL on transcript levels in 3-day-old WL-grown seedlings (wild-type, FR: solid red line; *pifS*, FR: dotted black line; wild-type, WL: dotted red line; *pifS*, WL: dotted gray line). Error bars indicate SE. **B)** H3K4me3 enrichment as measured by ChIP-seq of ATHB52 (top), PIL1 (middle) and YUC9 (bottom) in 3-day-old dark-grown seedlings (3d D, black), 3-day-old WL-grown seedlings (3d WL, green) and 3-day-old WL-grown seedlings after 180 min of FR-enriched WL (3d WL +FR180, red). Data from each of three biological replicates are shown. **C)** Average relative H3K4me3 levels of ATHB52 (top), PIL1 (middle) and YUC9

(bottom). Left subpanel shows the levels in 3-day-old dark-grown seedlings and the levels in 3-day-old WL-grown seedlings (wild-type, connected by dashed red line). Right subpanel shows the effect of 30, 60, 120 and 180 min of FR-enriched WL or continuous WL on transcript levels in 3-day-old WL-grown seedlings (wild-type, FR: solid red line; *pifS*, FR: dotted black line; wild-type, WL: dotted red line; *pifS*, WL: dotted gray line). Error bars indicate SE.

Figure 4. Shade-induced, PIF-dependent increases in transcription precede corresponding increases in H3K4me3 for ES class and S class genes. A) Normalized levels of the average transcript profile for all ES class genes (top) or S class genes during 180 minute shade-light (FR, red) or white-light (WL, black/gray) treatment for wild-type (WT, solid red/dashed black) or *pifS* (dashed red/dashed gray). **B)** Normalized levels of the average H3K4me3 profile for all ES class genes (top) or S class genes during 180 minute shade-light (FR, red) or white-light (WL, black/gray) treatment for wild-type (WT, solid red/dashed black) or *pifS* (dashed red/dashed gray). **C)** Overlays of the RNA (yellow) and H3K4me3 (blue) profiles from WT seedlings during the FR treatment. Shaded variance indicate normalized SE.

Table 1 List of all candidate PIF DTGs and categorization into etiolation (E), shade (S) or etiolation and shade (ES) responsive genes.

locus	name	R60	pifQ	FR30	FR60	FR120	FR180	pS	PIF bound	Original class	New class	Group	Pfeiffer class	known PIF DTG	ANOM
AT5G02260	EXP9	-1.31	-2.99	-	-	-	-	-	157	E	E	1	Ind	yes	N/A
AT5G02580	At5g02580	-2.99	-1.39	-	-	-	-	-	13457	E	E	1	Ind	yes	N/A
AT5G67020	At5g67020	-2.28	-1.21	-	-	-	-	-	7	E	E	1		yes	N/A
AT5G02190	PCS1	-2.27	-1.19	-	-	-	-	-	57	E	E	1	Ind	yes	N/A
AT1G07090	LSH6	-1.09	-1.14	-	-	-	-	-	13457	E	E	1	Ind	yes	N/A
AT1G67265	DVL3	-4.26	-2.16	-	-	-	-	-	1345	E	E	1	Ind	yes	N/A
AT1G60060	At1g60060	-2.14	-2.03	-	-	-	-	-	4	E	E	1		yes	N/A
AT5G15830	BZIP3	-1.18	-1.78	-	-	-	-	-	5	E	E	1		yes	N/A
AT4G37740	GRF2	-2.01	-1.66	-	-	-	-	-	14	E	E	1	Ind	yes	N/A
AT5G50175	At5g50175	-1.68	-1.65	-	-	-	-	-	14	E	E	1	Ind	yes	N/A
AT4G36010	At4g36010	-2.30	-1.64	-	-	-	-	-	134	E	E	1	Ind	yes	N/A
AT4G10020	HSD5	-1.23	-1.64	-	-	-	-	-	14	E	E	1	Ind	yes	N/A
AT3G25730	EDF3	-2.91	-1.55	-	-	-	-	-	3	E	E	1		yes	N/A
AT3G28340	GATL10	-1.00	-1.48	-	-	-	-	-	15	E	E	1	Ind	yes	N/A
AT1G58410	At1g58410	-1.35	-1.28	-	-	-	-	-	14	E	E	1		yes	N/A
AT3G53200	MYB27	-2.40	-1.07	-	-	-	-	-	14	E	E	1	Ind	yes	N/A
AT5G53980	ATHB52	-4.07	-1.01	-	-	-	-	-	35	E	E	1	Ind	yes	N/A
AT2G42870	PAR1	-2.31	-	-	-	-	2.14	-2.34	13457	S	ES	2		yes	N/A
AT3G59900	ARGOS	-1.01	-	-	1.62	1.90	1.84	-2.13	14	S	ES	2		yes	N/A
AT2G44910	ATHB-4	-2.10	-	3.95	1.83	1.27	-	-1.50	13457	S	ES	2		yes	N/A
AT5G28300	GT2L	-2.17	-	-	-	***	1.19	-1.47	13457	S	ES	2		yes	N/A
AT1G13260	RAV1	-2.51	-	-	-	1.22	-	-1.32	1345	S	ES	2		yes	N/A

AT5G44260	TZF5	-3.69	-	-	-	2.60	2.85	-3.01	7	S	ES	2		yes	N/A
AT5G02760	APD7	-1.70	-	-	3.23	3.53	2.95	-2.50	45	S	ES	2	Rep	yes	N/A
AT5G62280	At5g62280	-1.96	-	-	2.70	3.11	2.74	-2.34	7	S	ES	2		yes	N/A
AT5G46330	FLS2	-1.52	-	-	-	-	1.62	-1.74	57	S	ES	2		yes	N/A
AT2G44080	ARL	-2.04	-	-	-	1.68	1.46	-1.41	45	S	ES	2		yes	N/A
AT3G60390	HAT3	-1.51	-	1.42	1.18	-	-	-	1345	S	ES	3		yes	N/A
AT5G25190	ESE3	-1.83	-	-	1.24	2.13	***	-	1345	S	ES	3		yes	N/A
AT1G25560	EDF1	-1.64	-	-	-	1.25	1.05	-	145	S	ES	3		**no**	N/A
AT3G60520	At3g60520	-1.50	-	-	-	1.49	-	-	45	S	ES	3		yes	N/A
AT4G28240	BGL1	-1.45	-	-	***	1.24	1.04	-	457	S	ES	3		yes	N/A
AT2G45210	SAUR36	-1.19	-1.25	-	-	***	1.05	-	15	E	ES	1	Ind	yes	N/A
AT2G43060	IBH1	-1.56	-1.44	-	-	1.04	-	-	13457	ES	ES	1 & 3	Ind	yes	N/A
AT5G02540	At5g02540	-1.63	-1.49	-	2.50	5.38	6.26	-5.74	13457	ES	ES	1 & 2	Ind	yes	N/A
AT3G15540	IAA19	-1.46	-1.63	-	3.31	3.37	2.20	-2.62	1345	ES	ES	1 & 2	Ind	yes	N/A
AT3G21330	At3g21330	-3.11	-1.65	2.52	4.25	4.00	3.90	-3.52	1345	ES	ES	1 & 2	Ind	yes	N/A
AT5G63650	SNRK2.5	-1.75	-1.68	-	-	1.78	2.30	-2.67	1345	ES	ES	1 & 2	Ind	yes	N/A
AT5G07010	ST2A	-2.97	-1.71	-	-	-	2.06	-2.87	13457	ES	ES	1 & 2	Ind	yes	N/A
AT5G01790	At5g01790	-1.41	-1.74	-	-	1.62	1.69	-1.56	145	ES	ES	1 & 2	Ind	yes	N/A
AT1G10550	XTH33	-1.05	-1.75	-	-	1.21	***	-1.25	13457	ES	ES	1 & 2	Ind	yes	N/A
AT3G61830	ARF18	-1.63	-1.78	-	-	-	1.04	-1.08	3	ES	ES	1 & 2	Ind	yes	N/A
AT4G16780	ATHB-2	-2.97	-1.97	3.69	3.03	2.91	2.85	-2.69	13457	ES	ES	1 & 2	Ind	yes	N/A
AT4G35720	At4g35720	-2.68	-2.27	-	-	1.67	1.38	-1.95	1345	ES	ES	1 & 2	Ind	yes	N/A
AT4G14130	XTR7	-2.50	-2.41	-	-	2.17	3.82	-3.98	13457	ES	ES	1 & 2	Ind	yes	N/A
AT4G32280	IAA29	-2.47	-2.56	-	4.51	4.72	4.25	-5.40	1345	ES	ES	1 & 2	Ind	yes	N/A
AT5G65800	ACS5	-2.04	-2.73	-	2.84	-	-	-	145	ES	ES	1 & 2	Ind	yes	N/A
AT4G31380	FLP1	-2.18	-2.99	-	3.00	3.82	3.22	-4.20	1457	ES	ES	1 & 2		yes	N/A
AT2G46970	PIL1	-4.26	-5.56	2.35	2.95	2.94	3.25	-4.62	13457	ES	ES	1 & 2	Ind	yes	N/A
AT5G05965	At5g05965	-1.97	-1.20	-	-	***	-	-1.62	1345	E	ES	1	Ind	yes	N/A
AT5G09970	CYP78A7	***	-	-	1.31	1.78	1.01	-1.98	1	S	ES	2		yes	N/A
AT1G21050	At1g21050	***	-	-	1.49	1.49	1.58	-1.43	1357	S	ES	2		yes	N/A
AT5G59010	BSK1	***	-	-	-	1.30	***	-1.28	145	S	ES	2		yes	N/A
AT3G61460	BRH1	***	-	-	1.35	1.39	1.29	-1.21	13457	S	ES	2		yes	N/A
AT1G21830	At1g21830	***	-	-	1.13	1.10	***	-1.05	1345	S	ES	2		yes	N/A
AT3G50340	At3g50340	***	-	-	2.48	2.17	1.41	-1.32	5	S	ES	2		yes	N/A
AT1G54120	At1g54120	***	-	-	1.58	-	-	-	15	S	ES	3		**no**	N/A
AT4G22780	ACR7	***	-	-	1.08	-	-	-	145	S	ES	3		yes	N/A
AT2G28400	At2g28400	***	-	-	-	1.15	-	-	3	S	ES	3		yes	N/A
AT4G25260	PMEI7	***	-2.16	-	-	1.47	1.25	-1.62	145	S	ES	2	Ind	yes	N/A
AT5G46240	KAT1	***	-	1.55	2.15	1.77	1.62	-1.77	14	S	ES	2		yes	N/A
AT5G18030	SAUR21	***	-	2.63	3.37	2.46	2.10	-1.53	357	S	ES	2		yes	N/A

AT4G38860	SAUR16	***	-	-	1.13	-	-	-	135	S	ES	3		yes	N/A
AT1G02400	GA2OX6	***	-	-	-	1.89	-	-	1345	S	ES	3		yes	N/A
AT3G05640	EGR1	***	-	-	-	1.17	-	-	457	S	ES	3		**no**	N/A
AT3G62070	At3g62070	***	-1.33	-	1.53	1.05	-	-	5	S	ES	3		**no**	N/A
AT1G29430	SAUR62	***	-	1.18	2.22	1.02	-	-	5	S	ES	3		**no**	N/A
AT1G75450	CKX5	***	-1.66	-	-	1.68	2.04	-1.96	13457	S	ES	2	Ind	yes	N/A
AT5G18060	SAUR23	***	-1.73	-	3.00	2.53	2.14	-2.18	157	S	ES	2	Ind	yes	N/A
AT4G37770	ACS8	***	-2.29	-	4.62	4.64	4.96	-5.52	7	S	ES	2		yes	N/A
AT4G13790	SAUR25	***	-3.24	-	4.15	-	-	-	15	S	ES	3	Ind	yes	N/A
AT3G62090	PIF6	***	-3.57	-	-	3.99	3.85	-6.90	13457	S	ES	2	Ind	yes	N/A
AT3G12820	MYB10	***	-4.10	-	-	2.34	2.97	-2.25	4	S	ES	2		yes	N/A
AT3G21320	At3g21320	-	-	-	6.54	7.28	7.16	-7.75	13457	S	S	2		yes	N/A
AT5G22500	FAR1	-	-	-	-	2.31	3.50	-3.44	145	S	S	2		yes	N/A
AT4G28720	YUC8	-	-	1.36	2.19	2.36	2.55	-2.88	13457	S	S	2		yes	N/A
AT1G04180	YUC9	-	-	4.42	4.24	3.25	2.68	-2.71	1345	S	S	2		yes	N/A
AT5G18050	SAUR22	-	-	-	3.89	3.34	3.01	-2.58	157	S	S	2		yes	N/A
AT1G02350	At1g02350	-	-	-	3.31	2.81	2.98	-2.20	13457	S	S	2		yes	N/A
AT5G66080	APD9	-	-	-	1.26	1.47	1.62	-1.88	1457	S	S	2		yes	N/A
AT3G23030	IAA2	-	-	1.29	2.32	2.29	1.98	-1.73	1345	S	S	2		yes	N/A
AT5G47370	HAT2	-	-	1.40	3.40	2.34	1.62	-1.54	1345	S	S	2		yes	N/A
AT4G14560	IAA1	-	-	-	3.10	2.19	1.89	-1.54	134	S	S	2		yes	N/A
AT5G25460	DGR2	-	-	-	-	***	1.12	-1.25	1357	S	S	2		yes	N/A
AT1G36940	At1g36940	-	-	-	-	1.06	***	-1.20	15	S	S	2		yes	N/A
AT3G23050	IAA7	-	-	-	-	-	1.13	-1.01	1345	S	S	2		yes	N/A
AT2G23170	GH3.3	-	-	-	2.30	3.57	3.29	-3.14	3	S	S	2		yes	N/A
AT5G12050	BG1	-	-	2.93	3.56	3.36	2.87	-2.28	57	S	S	2		yes	N/A
AT1G76610	At1g76610	-	-	-	2.44	2.51	2.04	-1.96	7	S	S	2		yes	N/A
AT1G29465	At1g29465	-	-	-	1.77	2.75	2.50	-1.63	5	S	S	2		**no**	N/A
AT1G75500	WAT1	-	-	-	***	1.39	1.34	-1.42	7	S	S	2		yes	N/A
AT1G21980	PIP5K1	-	-	-	***	***	1.24	-1.28	3	S	S	2		yes	N/A
AT1G31880	BRX	-	-	-	1.32	1.52	1.09	-1.10	4	S	S	2		yes	N/A
AT4G39800	MIPS1	-	-	-	-	1.41	1.28	-1.03	5	S	S	2		yes	N/A
AT1G67900	At1g67900	-	-	-	2.61	2.06	1.36	-1.03	7	S	S	2		yes	N/A
AT5G16023	DVL1	-	-	-	2.48	-	-	-	1345	S	S	3		yes	N/A
AT5G39860	PRE1	-	-	-	2.42	-	-	-	157	S	S	3		yes	N/A
AT4G34760	SAUR50	-	-	-	1.08	***	-	-	145	S	S	3		yes	N/A
AT1G49780	PUB26	-	-	***	1.06	***	-	-	13457	S	S	3		**no**	N/A
AT4G32290	At4g32290	-	-	-	1.04	***	***	-	15	S	S	3		**no**	N/A
AT5G43890	YUC5	-	-	2.14	-	-	-	-	145	S	S	3		yes	N/A
AT3G62100	IAA30	-	-	-	2.60	2.32	-	-	134	S	S	3		**no**	N/A

AT4G37390	GH3.2	-	-	-	1.29	2.04	-	-	1345	S	S	3	Rep	yes	N/A
AT1G75490	At1g75490	-	-	-	-	1.99	-	-	145	S	S	3	Rep	yes	N/A
AT4G24275	At4g24275	-	-	-	1.11	1.79	1.04	-	14	S	S	3		**no**	N/A
AT4G27280	CMI1	-	-	-	1.84	1.61	-	-	1	S	S	3		yes	N/A
AT4G27310	BBX28	-	-	-	-	1.54	1.66	-	13457	S	S	3		yes	N/A
AT5G59220	HAI1	-	-	-	-	1.50	-	-	1345	S	S	3	Ind	yes	N/A
AT1G60190	PUB19	-	-	-	-	1.45	-	-	13457	S	S	3		yes	N/A
AT2G40610	EXP8	-	-	-	-	1.44	-	-	1345	S	S	3		yes	N/A
AT5G54510	GH3.6	-	-	-	***	1.27	1.04	-	135	S	S	3		yes	N/A
AT2G45420	LBD18	-	-	-	-	1.24	-	-	1	S	S	3		**no**	N/A
AT5G60840	At5g60840	-	-	-	***	1.12	***	-	13457	S	S	3		yes	N/A
AT4G09890	At4g09890	-	-	-	1.30	1.12	-	-	145	S	S	3		yes	N/A
AT5G62220	GT18	-	-	-	-	1.09	-	-	15	S	S	3		yes	N/A
AT3G19380	PUB25	-	-	-	***	1.08	-	-	1345	S	S	3		yes	N/A
AT3G44310	NIT1	-	-	-	-	1.07	1.25	-	13457	S	S	3		yes	N/A
AT5G16200	At5g16200	-	-	-	-	1.03	-	-	15	S	S	3		**no**	N/A
AT1G21910	DREB26	-	-	-	-	1.01	***	-	15	S	S	3		yes	N/A
AT3G03850	SAUR26	-	-	-	3.17	-	-	-	5	S	S	3		yes	N/A
AT3G03840	SAUR27	-	-	-	2.89	-	-	-	5	S	S	3		yes	N/A
AT2G18010	SAUR10	-	-	-	4.65	3.72	3.53	-	5	S	S	3		yes	N/A
AT3G03830	SAUR28	-	-	-	4.00	2.87	-	-	5	S	S	3		yes	N/A
AT4G34770	SAUR1	-	-	-	2.31	2.13	-	-	5	S	S	3		yes	N/A
AT1G29460	SAUR65	-	-	-	2.83	1.99	1.58	-	5	S	S	3		yes	N/A
AT1G29500	SAUR66	-	-	-	2.53	1.91	-	-	35	S	S	3		yes	N/A
AT3G55840	At3g55840	-	-	-	-	1.83	-	-	5	S	S	3		yes	N/A
AT5G18020	SAUR20	-	-	1.98	2.37	1.82	1.42	-	357	S	S	3		yes	N/A
AT1G29440	SAUR63	-	-	-	2.31	1.73	-	-	5	S	S	3		yes	N/A
AT1G29450	SAUR64	-	-	-	2.51	1.70	-	-	5	S	S	3		yes	N/A
AT1G52565	At1g52565	-	-	-	-	1.68	-	-	5	S	S	3		**no**	N/A
AT1G69160	WIP1	-	-	-	1.41	1.26	1.20	-	5	S	S	3	Rep	yes	N/A
AT5G18010	SAUR19	-	-1.10	-	3.28	2.68	-	-2.39	145	S	S	2	Ind	yes	N/A
AT3G50350	At3g50350	-	-1.16	-	1.12	1.99	1.15	-	5	S	S	3		**no**	N/A
AT3G50800	At3g50800	-	-1.20	1.97	2.47	2.77	2.25	-2.03	13457	S	S	2	Ind	yes	N/A
AT1G04240	IAA3	-	-1.22	-	1.66	-	-	-	1457	S	S	3	Ind	yes	N/A
AT1G76240	At1g76240	-	-1.26	-	-	1.01	1.04	-1.01	15	S	S	2	Ind	yes	N/A
AT1G18400	BEE1	-	-1.30	-	2.50	2.35	1.80	-1.60	1345	S	S	2	Ind	yes	N/A
AT5G66580	At5g66580	-	-1.32	2.35	3.63	2.60	1.67	-	13457	S	S	3	Ind	yes	N/A
AT3G28857	PRE5	-	-1.79	-	3.28	-	-	-	145	S	S	3	Ind	yes	N/A
AT2G14960	GH3.1	-	-1.90	-	-	1.41	-	-	13	S	S	3	Ind	yes	N/A
AT1G06080	ADS1	-	-2.01	-	-	3.18	3.94	-4.13	4	S	S	2		yes	N/A

AT5G66590	At5g66590	-	-2.06	-	1.40	1.27	1.03	-1.05	13457	S	S	2	Ind	yes	N/A
AT1G16850	At1g16850	-	-2.82	-	-	1.03	-	-	135	S	S	3	Ind	yes	N/A
AT2G31980	CYS2	-1.68	-3.00	-	-	-	-	-	37	E	ANOM	1		N/A	High in WL
AT1G10560	PUB18	-1.51	-2.06	-	-	-	-	-	13457	E	ANOM	1	Ind	N/A	High in WL
AT1G11960	At1g11960	-1.32	-1.21	-	-	-	-	-	37	E	ANOM	1		N/A	High in WL
AT3G61680	PLIP1	-1.32	-1.05	-	-	-	-	-	7	E	ANOM	1		N/A	High in WL
AT1G77200	At1g77200	-2.31	-1.80	-	-	1.29	-	-	1345	ES	ANOM	1 & 3	Ind	N/A	High in WL
AT1G36060	TG	-1.04	-1.29	-	-	-	-	-	5	E	ANOM	1	Ind	N/A	High in WL
AT1G02340	HFR1	-	-3.82	2.56	3.05	3.41	3.44	-3.18	13457	S	ANOM	2	Ind	N/A	R-induced
AT3G54200	NHL39	-	-1.04	-	-	2.04	1.73	-1.77	1345	S	ANOM	2		N/A	artifactual
AT1G69570	CDF5	-	-	-	2.21	2.32	2.08	-1.55	13457	S	ANOM	2		N/A	artifactual
AT4G01680	MYB55	-	-	-	-	1.13	1.28	-1.22	145	S	ANOM	2		N/A	R-induced
AT1G18710	MYB47	-	-	-	1.35	1.36	-	-1.02	1	S	ANOM	2		N/A	R-induced
AT2G33380	RD20	-	-3.08	-	-	2.66	2.39	-2.24	34	S	ANOM	2	Ind	N/A	R-induced
AT1G80130	At1g80130	-	-	-	-	1.60	2.26	-2.04	5	S	ANOM	2		N/A	R-induced
AT5G54470	BBX29	-	-	-	2.69	2.81	2.88	-	1345	S	ANOM	3	Ind	N/A	R-induced
AT1G09350	GOLS3	-	-	-	1.91	2.10	-	-	13	S	ANOM	3		N/A	R-induced
AT5G66110	HIPP27	-	-1.15	-	-	1.65	1.67	-	1	S	ANOM	3	Ind	N/A	low
AT3G16800	EGR3	-	-	-	1.07	1.38	1.49	-	1457	S	ANOM	3		N/A	R-induced
AT1G73480	MAGL4	-	-	-	-	1.37	-	-	145	S	ANOM	3		N/A	R-induced
AT3G29575	AFP3	-	-1.45	-	-	1.33	-	-	13457	S	ANOM	3	Ind	N/A	R-induced
AT3G22830	HSFA6B	-	-2.29	-	-	1.19	-	-	145	S	ANOM	3		N/A	R-induced
AT3G57540	REM4.1	-	-	-	-	1.08	-	-	1	S	ANOM	3		N/A	R-induced
AT2G29440	GST24	-	-2.56	-	-	1.04	-	-	1	S	ANOM	3	Ind	N/A	R-induced
AT1G78440	GA2OX1	-	-	-	2.22	2.38	2.53	-	57	S	ANOM	3		N/A	R-induced
AT2G46790	PRR9	-	-	-	-	1.85	2.28	-	3	S	ANOM	3		N/A	R-induced
AT1G09250	AIF4	-	-	-	-	1.28	-	-	7	S	ANOM	3		N/A	R-induced

locus	gene locus
name	gene name, if present
R60	log2FC of transcript levels after exposure of 3-day-old dark-grown seedlings exposed to 60 minutes of R light
pifQ	log2FC of transcript levels in 3-day-old dark-grown pifq mutant seedlings relative to WT
FR30	log2FC of transcript levels in 3-day-old WL-grown seedlings exposed to 30 min supplemental FR
FR60	log2FC of transcript levels in 3-day-old WL-grown seedlings exposed to 60 min supplemental FR
FR120	log2FC of transcript levels in 3-day-old WL-grown seedlings exposed to 120 min supplemental FR
FR180	log2FC of transcript levels in 3-day-old WL-grown seedlings exposed to 180 min supplemental FR
pS	log2FC of transcript levels in 3-day-old WL-grown pifS relative to 3-day-old WL-grown WT exposed to 180 min supplemental FR
PIF bound	confirmed binding by PIF1, PIF3, PIF4, PIF5 and/or PIF7
Original class	original categorized class (E, ES or S)
New class	New class after resorting: E, ES, S or anomalous (ANOM)
Group	Initial Group categorization (see below)
ANOM	rationale for inclusion in "anomalous" category
Pfeiffer class	If described in Pfeiffer et al., 2014, Mol Plant: Induced or Repressed

known PIF DTG	From Supplemental Table 1
-	indicates no SSTF changes in transcript levels
***	indicates statistically-significant 1.5-fold change (p-val < 0.1; used only for recategorization)
4.72	indicates statistically-significant 26.4-fold (2^4.72101552) change for the indicated comparison
Group 1	genes SSTF downregulated by R light **AND** SSTF downregulated in pifq **AND** PIF-bound (1, 3, 4, 5 and/or 7)
Group 2	genes SSTF upregulated by FR light (30, 60, 120 and/or 180min) **AND** SSTF downregulated in pifS **AND** PIF-bound (1, 3, 4, 5 and/or 7)
Group 3	genes SSTF upregulated by FR light (30, 60 and/or 120min) **AND** PIF-bound (PIF1, 3, 4, 5 and/or 7)

Parsed Citations

Al-Sady B, Kikis EA, Monte E, Quail PH (2008) Mechanistic duality of transcription factor function in phytochrome signaling. Proc Natl Acad Sci U S A 105: 2232-2237
Google Scholar: Author Only Title Only Author and Title

Bernstein BE, Humphrey EL, Erlich RL, Schneider R, Bouman P, Liu JS, Kouzarides T, Schreiber SL (2002) Methylation of histone H3 Lys 4 in coding regions of active genes. Proc Natl Acad Sci U S A 99: 8695-8700
Google Scholar: Author Only Title Only Author and Title

Bourbousse C, Ahmed I, Roudier F, Zabulon G, Blondet E, Balzergue S, Colot V, Bowler C, Barneche F (2012) Histone H2B monoubiquitination facilitates the rapid modulation of gene expression during Arabidopsis photomorphogenesis. PLoS Genet 8: e1002825
Google Scholar: Author Only Title Only Author and Title

Bourbousse C, Barneche F, Laloi C (2019) Plant Chromatin Catches the Sun. Front Plant Sci 10: 1728
Google Scholar: Author Only Title Only Author and Title

Charron JB, He H, Elling AA, Deng XW (2009) Dynamic landscapes of four histone modifications during deetiolation in Arabidopsis. Plant Cell 21: 3732-3748
Google Scholar: Author Only Title Only Author and Title

Chung BYW, Balcerowicz M, Di Antonio M, Jaeger KE, Geng F, Franaszek K, Marriott P, Brierley I, Firth AE, Wigge PA (2020) An RNA thermoswitch regulates daytime growth in Arabidopsis. Nat Plants 6: 522-532
Google Scholar: Author Only Title Only Author and Title

Dalton JC, Bätz U, Liu J, Curie GL, Quail PH (2016) A Modified Reverse One-Hybrid Screen Identifies Transcriptional Activation Domains in PHYTOCHROME-INTERACTING FACTOR 3. Front Plant Sci 7: 881
Google Scholar: Author Only Title Only Author and Title

de Lucas M, Davière JM, Rodríguez-Falcón M, Pontin M, Iglesias-Pedraz JM, Lorrain S, Fankhauser C, Blázquez MA, Titarenko E, Prat S (2008) A molecular framework for light and gibberellin control of cell elongation. Nature 451: 480-484
Google Scholar: Author Only Title Only Author and Title

de Wit M, Ljung K, Fankhauser C (2015) Contrasting growth responses in lamina and petiole during neighbor detection depend on differential auxin responsiveness rather than different auxin levels. New Phytol 208: 198-209
Google Scholar: Author Only Title Only Author and Title

Fisher AJ, Franklin KA (2011) Chromatin remodelling in plant light signalling. Physiol Plant 142: 305-313
Google Scholar: Author Only Title Only Author and Title

Hornitschek P, Kohnen MV, Lorrain S, Rougemont J, Ljung K, López-Vidriero I, Franco-Zorrilla JM, Solano R, Trevisan M, Pradervand S, Xenarios I, Fankhauser C (2012) Phytochrome interacting factors 4 and 5 control seedling growth in changing light conditions by directly controlling auxin signaling. Plant J 71: 699-711
Google Scholar: Author Only Title Only Author and Title

Huq E, Al-Sady B, Hudson M, Kim C, Apel K, Quail PH (2004) Phytochrome-interacting factor 1 is a critical bHLH regulator of chlorophyll biosynthesis. Science 305: 1937-1941
Google Scholar: Author Only Title Only Author and Title

Huq E, Quail PH (2002) PIF4, a phytochrome-interacting bHLH factor, functions as a negative regulator of phytochrome B signaling in Arabidopsis. EMBO J 21: 2441-2450
Google Scholar: Author Only Title Only Author and Title

Khanna R, Huq E, Kikis EA, Al-Sady B, Lanzatella C, Quail PH (2004) A novel molecular recognition motif necessary for targeting photoactivated phytochrome signaling to specific basic helix-loop-helix transcription factors. Plant Cell 16: 3033-3044
Google Scholar: Author Only Title Only Author and Title

Kuang Z, Cai L, Zhang X, Ji H, Tu BP, Boeke JD (2014) High-temporal-resolution view of transcription and chromatin states across distinct metabolic states in budding yeast. Nat Struct Mol Biol 21: 854-863
Google Scholar: Author Only Title Only Author and Title

Langmead B, Salzberg SL (2012) Fast gapped-read alignment with Bowtie 2. Nat Methods 9: 357-359
Google Scholar: Author Only Title Only Author and Title

Le Martelot G, Canella D, Symul L, Migliavacca E, Gilardi F, Liechti R, Martin O, Harshman K, Delorenzi M, Desvergne B, Herr W, Deplancke B, Schibler U, Rougemont J, Guex N, Hernandez N, Naef F, Consortium C (2012) Genome-wide RNA polymerase II profiles and RNA accumulation reveal kinetics of transcription and associated epigenetic changes during diurnal cycles. PLoS Biol 10: e1001442
Google Scholar: Author Only Title Only Author and Title

Leivar P, Monte E, Al-Sady B, Carle C, Storer A, Alonso JM, Ecker JR, Quail PH (2008) The Arabidopsis phytochrome-interacting factor PIF7, together with PIF3 and PIF4, regulates responses to prolonged red light by modulating phyB levels. Plant Cell 20: 337-352
Google Scholar: Author Only Title Only Author and Title

Leivar P, Monte E, Oka Y, Liu T, Carle C, Castillon A, Huq E, Quail PH (2008) Multiple phytochrome-interacting bHLH transcription factors repress premature seedling photomorphogenesis in darkness. Curr Biol 18: 1815-1823
Google Scholar: Author Only Title Only Author and Title

Leivar P, Quail PH (2011) PIFs: pivotal components in a cellular signaling hub. Trends Plant Sci 16: 19-28
Google Scholar: Author Only Title Only Author and Title

Leivar P, Tepperman JM, Cohn MM, Monte E, Al-Sady B, Erickson E, Quail PH (2012) Dynamic antagonism between phytochromes and PIF family basic helix-loop-helix factors induces selective reciprocal responses to light and shade in a rapidly responsive transcriptional network in Arabidopsis. Plant Cell 24: 1398-1419
Google Scholar: Author Only Title Only Author and Title

Leivar P, Tepperman JM, Monte E, Calderon RH, Liu TL, Quail PH (2009) Definition of early transcriptional circuitry involved in light-induced reversal of PIF-imposed repression of photomorphogenesis in young Arabidopsis seedlings. Plant Cell 21: 3535-3553
Google Scholar: Author Only Title Only Author and Title

Li L, Ljung K, Breton G, Schmitz RJ, Pruneda-Paz J, Cowing-Zitron C, Cole BJ, Ivans LJ, Pedmale UV, Jung HS, Ecker JR, Kay SA, Chory J (2012) Linking photoreceptor excitation to changes in plant architecture. Genes Dev 26: 785-790
Google Scholar: Author Only Title Only Author and Title

Liao Y, Smyth GK, Shi W (2014) featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics 30: 923-930
Google Scholar: Author Only Title Only Author and Title

Liu X, Chen CY, Wang KC, Luo M, Tai R, Yuan L, Zhao M, Yang S, Tian G, Cui Y, Hsieh HL, Wu K (2013) PHYTOCHROME INTERACTING FACTOR3 associates with the histone deacetylase HDA15 in repression of chlorophyll biosynthesis and photosynthesis in etiolated Arabidopsis seedlings. Plant Cell 25: 1258-1273
Google Scholar: Author Only Title Only Author and Title

Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 15: 550
Google Scholar: Author Only Title Only Author and Title

Martín G, Rovira A, Veciana N, Soy J, Toledo-Ortiz G, Gommers CMM, Boix M, Henriques R, Minguet EG, Alabadí D, Halliday KJ, Leivar P, Monte E (2018) Circadian Waves of Transcriptional Repression Shape PIF-Regulated Photoperiod-Responsive Growth in Arabidopsis. Curr Biol 28: 311-318.e315
Google Scholar: Author Only Title Only Author and Title

Mizuno T, Oka H, Yoshimura F, Ishida K, Yamashino T (2015) Insight into the mechanism of end-of-day far-red light (EODFR)-induced shade avoidance responses in Arabidopsis thaliana. Biosci Biotechnol Biochem 79: 1987-1994
Google Scholar: Author Only Title Only Author and Title

Monte E, Tepperman JM, Al-Sady B, Kaczorowski KA, Alonso JM, Ecker JR, Li X, Zhang Y, Quail PH (2004) The phytochrome-interacting transcription factor, PIF3, acts early, selectively, and positively in light-induced chloroplast development. Proc Natl Acad Sci U S A 101: 16091-16098
Google Scholar: Author Only Title Only Author and Title

Ni M, Tepperman JM, Quail PH (1998) PIF3, a phytochrome-interacting factor necessary for normal photoinduced signal transduction, is a novel basic helix-loop-helix protein. Cell 95: 657-667
Google Scholar: Author Only Title Only Author and Title

Oh E, Zhu JY, Wang ZY (2012) Interaction between BZR1 and PIF4 integrates brassinosteroid and environmental responses. Nat Cell Biol 14: 802-809
Google Scholar: Author Only Title Only Author and Title

Pedmale UV, Huang SC, Zander M, Cole BJ, Hetzel J, Ljung K, Reis PAB, Sridevi P, Nito K, Nery JR, Ecker JR, Chory J (2016) Cryptochromes Interact Directly with PIFs to Control Plant Growth in Limiting Blue Light. Cell 164: 233-245
Google Scholar: Author Only Title Only Author and Title

Penfield S, Josse EM, Halliday KJ (2010) A role for an alternative splice variant of PIF6 in the control of Arabidopsis primary seed dormancy. Plant Mol Biol 73: 89-95
Google Scholar: Author Only Title Only Author and Title

Perrella G, Kaiserli E (2016) Light behind the curtain: photoregulation of nuclear architecture and chromatin dynamics in plants.

New Phytol 212: 908-919
Google Scholar: Author Only Title Only Author and Title

Pfeiffer A, Shi H, Tepperman JM, Zhang Y, Quail PH (2014) Combinatorial complexity in a transcriptionally centered signaling hub in Arabidopsis. Mol Plant 7: 1598-1618
Google Scholar: Author Only Title Only Author and Title

Pham VN, Kathare PK, Huq E (2018) Phytochromes and Phytochrome Interacting Factors. Plant Physiol 176: 1025-1038
Google Scholar: Author Only Title Only Author and Title

Quail PH, Boylan MT, Parks BM, Short TW, Xu Y, Wagner D (1995) Phytochromes: photosensory perception and signal transduction. Science 268: 675-680
Google Scholar: Author Only Title Only Author and Title

Ross-Innes CS, Stark R, Teschendorff AE, Holmes KA, Ali HR, Dunning MJ, Brown GD, Gojis O, Ellis IO, Green AR, Ali S, Chin SF, Palmieri C, Caldas C, Carroll JS (2012) Differential oestrogen receptor binding is associated with clinical outcome in breast cancer. Nature 481: 389-393
Google Scholar: Author Only Title Only Author and Title

Rymen B, Kawamura A, Lambolez A, Inagaki S, Takebayashi A, Iwase A, Sakamoto Y, Sako K, Favero DS, Ikeuchi M, Suzuki T, Seki M, Kakutani T, Roudier F, Sugimoto K (2019) Histone acetylation orchestrates wound-induced transcriptional activation and cellular reprogramming in Arabidopsis. Commun Biol 2: 404
Google Scholar: Author Only Title Only Author and Title

Sakamoto K, Nagatani A (1996) Nuclear localization activity of phytochrome B. Plant J 10: 859-868
Google Scholar: Author Only Title Only Author and Title

Soy J, Leivar P, González-Schain N, Martín G, Diaz C, Sentandreu M, Al-Sady B, Quail PH, Monte E (2016) Molecular convergence of clock and photosensory pathways through PIF3-TOC1 interaction and co-occupancy of target promoters. Proc Natl Acad Sci U S A 113: 4870-4875
Google Scholar: Author Only Title Only Author and Title

Spyrou C, Stark R, Lynch AG, Tavaré S (2009) BayesPeak: Bayesian analysis of ChIP-seq data. BMC Bioinformatics 10: 299
Google Scholar: Author Only Title Only Author and Title

Tepperman JM, Zhu T, Chang HS, Wang X, Quail PH (2001) Multiple transcription-factor genes are early targets of phytochrome A signaling. Proc Natl Acad Sci U S A 98: 9437-9442
Google Scholar: Author Only Title Only Author and Title

Trapnell C, Pachter L, Salzberg SL (2009) TopHat: discovering splice junctions with RNA-Seq. Bioinformatics 25: 1105-1111
Google Scholar: Author Only Title Only Author and Title

Wang X, Jiang B, Gu L, Chen Y, Mora M, Zhu M, Noory E, Wang Q, Lin C (2021) A photoregulatory mechanism of the circadian clock in Arabidopsis. Nat Plants 7: 1397-1408
Google Scholar: Author Only Title Only Author and Title

Willige BC, Zander M, Yoo CY, Phan A, Garza RM, Trigg SA, He Y, Nery JR, Chen H, Chen M, Ecker JR, Chory J (2021) PHYTOCHROME-INTERACTING FACTORs trigger environmentally responsive chromatin dynamics in plants. Nat Genet 53: 955-961
Google Scholar: Author Only Title Only Author and Title

Zhang X, Bernatavichute YV, Cokus S, Pellegrini M, Jacobsen SE (2009) Genome-wide analysis of mono-, di- and trimethylation of histone H3 lysine 4 in Arabidopsis thaliana. Genome Biol 10: R62
Google Scholar: Author Only Title Only Author and Title

Zhang Y, Liu T, Meyer CA, Eeckhoute J, Johnson DS, Bernstein BE, Nusbaum C, Myers RM, Brown M, Li W, Liu XS (2008) Model-based analysis of ChIP-Seq (MACS). Genome Biol 9: R137
Google Scholar: Author Only Title Only Author and Title

Zhang Y, Mayba O, Pfeiffer A, Shi H, Tepperman JM, Speed TP, Quail PH (2013) A quartet of PIF bHLH factors provides a transcriptionally centered signaling hub that regulates seedling morphogenesis through differential expression-patterning of shared target genes in Arabidopsis. PLoS Genet 9: e1003244
Google Scholar: Author Only Title Only Author and Title

Zhang Y, Pfeiffer A, Tepperman JM, Dalton-Roesler J, Leivar P, Gonzalez Grandio E, Quail PH (2020) Central clock components modulate plant shade avoidance by directly repressing transcriptional activation activity of PIF proteins. Proc Natl Acad Sci U S A 117: 3261-3269
Google Scholar: Author Only Title Only Author and Title