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

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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 Phytochrome-Interacting Factors (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 Direct-Target Genes (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*, *pifqipif6*, *pifqipif7* and *pifqipif6pif7* 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 (Etiolation-induced only), ES (both Etiolation- and Shade-induced) and S (Shade-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 Etiation-induced only (E) genes (BZIP3, ATHB2, HSD5 and GRF2), Etiation 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 *pif5* (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 *pif5* (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 *pifqif7* 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 deetiolation (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.

Materials and Methods

All seeds were stratified for 4 days at 4° before germination. Germination was induced by 3h of incubation under 30 $\mu\text{mol m}^{-2} \text{s}^{-1}$ WL at 21° followed by a 5 min saturating pulse of FR light. Seedlings were grown for 3 days at 21° in complete darkness or under 30 $\mu\text{mol m}^{-2} \text{s}^{-1}$ WL (R/FR = 6-8). For FR light treatment, seedlings were grown for 3 days in WL before exposing them to simulated shade (30 $\mu\text{mol m}^{-2} \text{s}^{-1}$, R/FR ~ 0.3). R light was defined as 640-680 nm and FR was defined as 710-750nm.

Hypocotyl measurements were performed on seedlings grown at 23° for 2 days in WL and either exposed to simulated shade for 4 days or kept in constant WL for 4 days. Hypocotyl length of 30 seedlings of each genotype were measured for each of three biological replicates.

Chromatin immunoprecipitation experiments were performed as described (Zhang et al., 2013). H3K4me3-enriched DNA fragments from 3-day-old seedlings were immunoprecipitated from 2 g of tissue with polyclonal H3K4me3 antibodies (Millipore, product #07-473) linked to Protein A/G Dynabeads (ThermoFisher). DNA libraries for sequencing were prepared at the Functional Genomics Laboratory at UC Berkeley using an Accel-NGS 2S Plus Library Kit (Swift Biosciences) according to the manufacturer's instructions.

RNA was isolated as described (Zhang et al., 2013). Total RNA was extracted from 3-day-old seedlings using a QIAshredder and RNeasy Plus Mini Kit (Qiagen) according to manufacturer's instructions. RNA libraries for sequencing were prepared at the Functional Genomics Laboratory at UC Berkeley using a KAPA RNA HyperPrep Kit (Roche) according to the manufacturer's instructions.

DNA and RNA libraries were sequenced by the Genomic Sequencing Facility at UC Berkeley. The multiplexed DNA libraries were sequenced by 50-bp single-end sequencing over two lanes on a HiSeq4000. Multiplexed RNA libraries were sequenced by 100-bp paired-end sequencing over two lanes on a HiSeq4000.

For mapping and analysis of ChIP-seq experiments, reads were mapped to the Arabidopsis genome (TAIR10) by BowTie2 (Langmead and Salzberg, 2012) and uniquely-mapping reads were used to call peaks using BayesPeak (Spyrou et al., 2009) (Bioconductor 3.6; binsize = 300, peaks with a PP>0.999 in all 3 biological replicates) or MACS (Zhang et al., 2008). Reads were counted and fold-changes were calculated using DiffBind (Ross-Innes et al., 2012) while VSTs were calculated using DESeq2 (Love et al., 2014).

For mapping and analysis of RNA-seq experiments, reads were mapped to the Arabidopsis genome (TAIR10) by TopHat (Trapnell et al., 2009) (max intron length = 3000, inner mean distance = 200, inner distance standard deviation = 100, minimal allowed intron size = 25). Assembled reads were counted using featureCounts (Liao et al., 2014) and differential expression was determined via DESeq2 (Love et al., 2014) ($\log_2\text{FC} > 1$ or $\log_2\text{FC} < -1$; p-val < 0.05).

Supplemental Data

Supplemental Table S1. List of PIF-induced DTGs identified in this study and whether or not they have been previously identified as a PIF DTG.

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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*, *pifqipif6*, *pifqipif7* and *pifqipif6pif7* 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	CMH1	-	-	-	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

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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)

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