MYCs and PIFs Act Independently in Arabidopsis Growth Regulation.

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Plants adapt their growth, physiology, and development to their environment by perceiving abiotic conditions including light, temperature, and nutrient and water availability, as well as biotic conditions including symbiotic, antagonistic, and commensal biota. Plants depend on light for photosynthesis; to optimize light capture, many plants respond to neighbor shade with increased stem and petiole elongation growth (part of the “shade avoidance” syndrome) in order to compete for light (Casan 2013). Defense and shade avoidance are two fundamental components of plant survival and productivity, and there are often tradeoffs between growth and defense. Recently, MYC2, a major positive regulator of defense, was reported to inhibit elongation during shade avoidance. Here, we further investigate the role of MYC2 and the related MYC3 and MYC4 in shade avoidance, and we examine the relationship between MYC2/3/4 and the PIF family of light-regulated transcription factors. We demonstrate that MYC2/3/4 inhibit both elongation and flowering. Furthermore, using both genetic and transcriptomic analysis we find that MYCs and PIFs generally function independently in growth regulation. However, surprisingly, the myc2/3/4 triple mutant restored the petiole shade avoidance response of myc2 (jin1-2) and myc2/3/4. We theorize that increased petiole elongation in myc2/3/4 could be more due to resource tradeoffs or post-translational modifications rather than interactions with PIF4/5/7 affecting gene regulation.

Plants have a variety of strategies to avoid canopy shade and compete with their neighbors for light, collectively called the shade avoidance syndrome (SAS). Plants also have extensive systems to defend themselves against pathogens and herbivores. Defense and shade avoidance are two fundamental components of plant survival and productivity, and there are often tradeoffs between growth and defense. Recently, MYC2, a major positive regulator of defense, was reported to inhibit elongation during shade avoidance. Here, we further investigate the role of MYC2 and the related MYC3 and MYC4 in shade avoidance, and we examine the relationship between MYC2/3/4 and the PIF family of light-regulated transcription factors. We demonstrate that MYC2/3/4 inhibit both elongation and flowering. Furthermore, using both genetic and transcriptomic analysis we find that MYCs and PIFs generally function independently in growth regulation. However, surprisingly, the myc2/3/4 triple mutant restored the petiole shade avoidance response of myc2 (jin1-2) and myc2/3/4. We theorize that increased petiole elongation in myc2/3/4 could be more due to resource tradeoffs or post-translational modifications rather than interactions with PIF4/5/7 affecting gene regulation.

KEYWORDS
MYC2/3/4
PIF4/5/7
growth-defense tradeoff
shade avoidance
RNA-seq
defense hormones pathways, JA (Moreno et al. 2009; de Wit et al. 2013) and SA. Resistance against a hemi-biotrophic (Pseudomonas syringae pv tomato, Pst) and a necrotrophic (Botrytis cinerea) pathogen is suppressed by shade treatment. Further, shade has been shown to reduce downstream transcriptional responsiveness to JA and SA. Less is understood about how defense pathways regulate growth. In a study of shade avoidance signaling components in adult Arabidopsis, a new link between defense and shade was defined: the JA pathway is important not only for regulating defense but also regulating elongation. Furthermore, growth/defense trade-offs were shown to be uncoupled in the jaz10 phyB and jazQ phyB mutants that showed both robust growth and heightened anti-insect defense (Campos et al. 2016). This uncoupling was attributed, at least in part, to parallel activation of MYC and Phytochrome-Interacting Factor (PIF) transcription factors that are repressed by JAZ and phyB in wild-type plants, respectively (Campos et al. 2016; Cerrudo et al. 2017). The current model of JA/growth interactions focuses on the GA signaling inhibitory DELLA proteins as regulators of the growth/defense trade-off (Leone et al. 2014). The model posits that under sun conditions DELLAs bind the growth-promoting PIF proteins (thereby inhibiting growth) and bind the JA signaling repressor JAZ proteins (allowing increased JA eliciting a defense response). In shade conditions DELLA are degraded, releasing PIFs to promote growth and releasing JAZs to inhibit defense. JA also increases DELLA accumulation, suggesting that JA could inhibit growth via DELLA. JAZ proteins repress MYC2 (Chini et al. 2009), MYC3, and MYC4 transcription factors (Niu et al. 2011; Fernández-Calvo et al. 2011). In the presence of JA, JAZ proteins are degraded allowing MYC2/3/4 to alter transcription of JA regulated genes (Chini et al. 2009; Niu et al. 2011; Fernández-Calvo et al. 2011). Under shade or in phyB mutants, MYC2/3/4 are destabilized and suggest that this destabilization is critical for proper shade avoidance growth (Chico et al. 2014). myc2 knock-out mutants have been found to have constitutively elongated petioles and therefore do not exhibit petiole shade avoidance (Nozue et al. 2015). Thus wild-type MYC2 functions not only to promote defense but also to inhibit growth.

Previous studies revealed the inhibition role of MYC2 in growth during shade avoidance (Nozue et al. 2015) and that shade-mediated accumulation of PIF proteins and the subsequent increase in auxin biosynthesis and signaling are critical for shade avoidance (Lorrain et al. 2008; Nozue et al. 2011; Li et al. 2012; Hornitschek et al. 2012). Since PIF proteins promote growth (Paik et al. 2017), MYC2 inhibits growth (Nozue et al. 2015), and the pair database (Lin et al. 2011) predicts an interaction between MYC2 and PIF4, we hypothesized that MYC2 could inhibit growth by repressing PIF function. In defense signaling, MYC3 and MYC4 heterodimerize with MYC2 and MYC2/3/4 are partially redundant in promoting immunity (Fernández-Calvo et al. 2011), therefore it is possible that this redundancy may also apply to growth inhibition. To address how MYC2 inhibits growth, in this paper, we analyzed shade avoidance phenotypes of the myc2 single (jin1-2) myc3/4 double, myc2/3/4 triple, myc2pif4/5/7 quadruple, and myc2/3/4pif4/5/7 sestuple mutants grown in simulated sun and shade. We found that MYC2/MYC3/MYC4 function redundantly in growth inhibition, and that MYCs and PIFs function in parallel to regulate growth in high red/far-red (R/FR) light conditions. Surprisingly, the pif4/5/7 triple mutant restored the shade avoidance response of jin1-2 and myc2/3/4. RNAseq revealed that a number of genes controlling flowering time were enriched in myc2/3/4 triple mutant, identified tradeoffs between indole-glucosinolate (indo-GS) and indole acetic acid (IAA; auxin) as a possible mechanism for enhanced elongation in myc2/3/4, and were consistent with independent action of MYC2/3/4 and PIF4/5/7. We concluded that myc2/3/4 increased growth could be more due to resource tradeoffs than gene regulation.

**MATERIALS AND METHODS**

**Growth conditions**

For simulated sun condition, white light (cool-white fluorescent light) was supplemented with far-red light provided by LEDs (Orbitec, inc) to obtain R/FR = 2.7 (high red/far-red, high R/FR). For simulated shade condition, white light was supplemented with far-red LEDS to obtain R/FR between 0.3 and 0.4 (low R/FR). Both conditions had 80–120 μE of Photosynthetically Active Radiation (PAR). Plants were grown under long day condition (16 hr light/8 hr dark) at constant temperature (22°C). Ambient light spectrum was measured by Black-Comet (StellarNet, Florida) (Nozue et al. 2015).

**Plant materials**

Arabidopsis seeds: Mutant seeds in the Col-0 background (jin1-2) (Lorenzo et al. 2004), myc2 (jin1-2) myc3 (445B11 GABI-KAT) myc4 (GK 491E10) were obtained from Roberto Solano (Campus Universidad Autonoma), and pif4 (pif4-101, Garlic_114_G06) pif5 (pif6-1, SALK_087012) pif7 (pif7-1) were obtained from Christian Fankhauser (University of Lausanne). jin1-2 pif4/5/7 and myc2/3/4pif4/5/7 were generated by crossing myc2/3/4 with pif4/5/7 and genotyping F2 and F3 generation to recover homozygous plants. Primers used in genotyping are listed in Supplementary Table 1. For phenotyping, Arabidopsis seeds were imbibed with water in 1.5 mL tubes and stored under dark at 4°C for four days. Five seeds were transferred to soil in a 4x9 well flat and placed under simulated sun condition. 7 days after sowing, excess seedlings were removed to leave one well-grown plant per pot, and 13 days after sowing, the plants were either transferred to shade or kept in the sun condition. Genotype positions were randomized in each replicate set.

**RNA-seq library preparation and sequencing**

For RNA extraction plants were grown for 13 days under high R/FR until they had 2 cotyledons and 2 expanded true leaves. Half of these plants were treated with shade starting at ZT 6 and the remainder were left in the sun. We prepared four replicates of each sample at 1 hr and 49 hr after sun and shade treatment and three plants were pooled for each replicate. When collecting samples at 1h treatment, cotyledons, leaves, hypocotyls, and roots were removed, leaving apical tissue for samples. When collecting samples at 49h, only apical tissues, and the leaves and petals newly elongated after treatment (leaves 3 and 4) were harvested for samples. Total RNA from the plants was extracted using Trizol (MacRae 2007). Two to five μg of total RNA was used to construct mRNA library using NEBNext Poly(A) mRNA Magnetic Isolation Module (NEB). cDNA libraries were made by using Strand-Specific mRNA-library prep kit for Illumina sequencing (Amaryllis Nucleics). The resulting cDNA libraries were sequenced by HiSeq4000 with 50 bp single end mode (the DNA Technologies and Expression Analysis Cores at the UC Davis Genome Center, supported by NIH Shared Instrumentation Grant 1S10OD010786-01).

**Differential expression analysis and over-representation analysis (ORA)**

Reads were sorted according to barcodes and filtered to remove adaptor contamination by custom Perl scripts (https://github.com/MaloofLab/SAS_defense_transcriptome). Reads were trimmed by Trimmomatic (Bolger et al. 2014) and mapped by Kallisto (Bray et al. 2016) to Arabidopsis TAIR10 cDNA sequences (Supplementary Table S2).
Differentially expressed genes were extracted by edgeR package in R statistical environment (FDR, 0.05). ORA was done by GOseq package (Young et al. 2010) in R statistical environment. GO analysis was done by using GO category database package from Bioconductor org.At.tair.db (Carlson et al.) and ANNOTATE package (Gentleman et al. 2004). For ORA of hormone responsive genes custom categories were used as in (Nozue et al. 2015, 2018).

**Phenotype measurement and analysis**

For scoring leaf phenotypes, 26 day old plants were dissected and leaf images were recorded by a flatbed scanner (Perfection V800 Photo, Epson), and scanned images were measured using ImageJ and the LeafJ plugin as described by Maloof et al. and analyzed as described by Nozue et al. (2015) to determine petiole length, leaf blade length, leaf blade width, and leaf blade area. Days to bolting was used as a measurement of flowering time.

Leaf phenotypes (petiole length, leaf blade length, leaf blade width, leaf blade area) and flowering time (days to bolting) were measured from 3 sets of experiments. Each phenotype was fitted by lme4 (Bates et al. 2014) and lmerTest (Kuznetsova et al. 2017) packages in R, using a model such as:

\[
\text{trait} \sim \text{genotype} + \text{treatment} + \\
\text{genotype : treatment} + (\text{treatment|set}) + \varepsilon
\]

where genotype represent a genotypic line (wildtype or different mutants), treatment is sun or shade condition, genotypetreatment is interaction of “genotype” and “treatment”, (treatment|set) is the random effect associated with the treatment in set of experiments, and \(\varepsilon\) is the error. The model was applied to each trait to calculate coefficient (“sun” value). For leaf traits where we measured across multiple leaves (from leaf 5 to leaf 8) for a...
given trait we treated leaf as a random effect, using the following model

\[
\text{trait} = \text{genotype} + \text{treatment} + \\
\text{genotype : treatment} + (1|\text{leaf}) + (\text{treatment}|\text{set}) + \epsilon
\]

Mutants were considered to have a defect in SAS when the genotype:treatment term was significant \((P < 0.05)\), indicating that the genotype of the plant (mutant vs. wild-type) altered the response to shade. Bootstrap resampling was used to calculate 95% confidence intervals for plotting.

Data availability
Strains are available upon request. Sequence data are available at the NCBI Short Read Archive under accession number PRJNA512107. Analysis scripts are deposited in github repositories: https://github.com/MaloofLab/SAS_defense_phenotyping, and https://github.com/MaloofLab/SAS_defense_transcriptome. Supplemental material available at figshare: https://doi.org/10.25387/g3.9544832.

RESULTS AND DISCUSSION
MYC2/MYC3/MYC4 function in growth inhibition
MYC2 is a basic helix-loop-helix (bHLH) TF important for JA mediated immune responses that acts semi-redundantly with its homologs, MYC3 and MYC4. myc2 mutants show partially reduced SAS in adult plants, raising the possibility that the MYC2/3/4 redundancy is also true for SAS (Nozue et al. 2015). Therefore, we hypothesized that MYC2/3/4 function redundantly in growth inhibition in the same way as in plant defense. To test this hypothesis, we analyzed the myc3/4 double and myc2/3/4 triple mutant for shade avoidance. To induce shade avoidance responses, we used supplemental far red (FR) LEDs to lower the red/far-red ratio to \(~0.3\) (low R/FR) from the control value of 2.7 (high R/FR). The double mutant, myc3/4, and the triple mutant, myc2/3/4, showed a constitutive “shade” phenotype with increased shade avoidance elongation in two indexes (petiole length or petiole length/blade length ratio) under both high and low R/FR treatment compared to Col (Figure 1). Both myc3/4 and myc2/3/4 mutants displayed increased elongation in high R/FR compared to jin1-2, which is a point mutation mutant of MYC2, indicating that MYC2/3/4, not only function redundantly in plant defense, but also in growth inhibition.

Flowering time of MYC2/3/4
In addition to hypocotyl and petiole elongation, acceleration of flowering time is another aspect of the shade avoidance syndrome. To ask if myc2/3/4 solely affected petiole elongation or instead altered other shade-regulated phenotypes, we measured days to bolting in myc2/3/4 and wild type plants as a measure of flowering time. The myc2/3/4 mutant exhibited significantly earlier flowering phenotype under high R/FR condition, compared to the wild type (Figure 2). As expected, low R/FR treatment caused a significant acceleration of flowering for wild type Col plants as compared to high R/FR. We found that low R/FR also accelerated flowering myc2/3/4, and that the magnitude of this effect was similar to the effect of low R/FR on wild type. Thus, while myc2/3/4 mutations do affect flowering time, this effect is independent of low R/FR.

MYCs and PIFs act in parallel to regulate petiole growth in high R/FR
Having established that MYCs inhibit petiole elongation, we hypothesized that MYCs may inhibit growth by repressing PIFs function. This idea is based on the fact that PIF proteins accumulate under shade increasing auxin biosynthesis and signaling pathway which are critical for shade avoidance elongation (Lorrain et al. 2008; Nozue et al. 2011; Hornitschek et al. 2012; Leivar and Monte 2014). Since the myc mutants show a constitutive shade phenotype we reasoned that if PIFs are required for the mutant myc phenotype that pif mutants should be epistatic to myc mutants in high R/FR conditions. Therefore, we created strains that combined mutations in the three PIF genes critical for shade regulated elongation with various myc mutants. Specifically, we constructed a jin1-2/pif4/5/7 quadruple mutant strain and a myc2/3/4/pif4/5/7 sextuple mutant strain. In high R/FR, the MYC and PIF genes appear to act additively (Figure 3). On their own, the myc mutants are longer than wildtype and the pif triple mutant is shorter. The myc, pif quadruple and sextuple mutants have intermediate phenotypes. For example, the sextuple mutant was longer than pif4/5/7, but shorter than myc2/3/4, suggesting that MYC2/3/4 and PIF4/5/7 regulate petiole elongation through parallel pathways.

Figure 2 Flowering time of myc2/3/4. Significant differences were evaluated using a linear mixed-effects model with genotype, treatment, and the genotype-by-treatment interaction as fixed effects. “H” denotes high R/FR and “L” denotes low R/FR. Black asterisks indicate difference between Col in high R/FR and Col in low R/FR. Dashed lines indicate other comparisons being made. Red dashed lines show comparisons between mutant and Col in high R/FR and red asterisks indicate significant differences from Col. Blue dashed lines show comparisons between mutant and Col for the response to low R/FR (low R/FR – high R/FR) and blue asterisks indicate significant differences from Col (in this case there was no significant difference in response to low R/FR between Col and myc2/3/4). Flowering time is days to bolting from 9 to 12 plants per genotype/condition from 2 independent experimental trials. Error bars show 95% bootstrap confidence interval. * \(P < 0.05\); ** \(P < 0.01\); *** \(P < 0.001\).
**pif4/5/7 rescues the shade avoidance response in myc2/3/4**

Under low R/FR conditions pif4/5/7 mutant plants had shorter petioles than wild type, as we had also observed in high R/FR (Figure 3, 4). However, the pif4/5/7 petiole elongation response to low R/FR was comparable to the wild type Col response, indicating that, surprisingly, the petiole shade avoidance response still exists in the pif4/5/7 mutant (Figure 4). pif4/5/7 mutants have been previously reported to elongate petioles in response to low R/FR, however, with a reduced response that we did not observe in our study (de Wit et al. 2015); we (Nozue et al. 2015) also previously reported a reduced response of pif4/5 mutant petioles to low R/FR. We have verified the genotype of the pif4/5/7 plants used in the current study; the discrepancies between this study and previous reports likely indicate sensitivity to undetermined environmental factors for this phenotype. The pif/myc quadruple and sextuple mutants also showed an interesting phenotype under low R/FR. Specifically, we found that pif4/5/7 rescued the petiole shade avoidance response of both jin1-2 and myc2/3/4. That is, both jin1-2/pif4/5/7 and myc2/3/4/pif4/5/7 showed a petiole elongation shade response indistinguishable from wild type and pif4/5/7. One possible explanation is that petiole length could already be near its maximum in the jin1-2 and myc2/3/4 mutants such that it is not physically possible for these plants to respond to the shade cue with additional elongation. Under this scenario, removing PIF4/5/7 function shortens the myc mutant petioles such that they are below their physical limit and can elongate in response to the low R/FR cue.

**Transcriptome Analysis of myc2/3/4 mutants**

Overall, our genetic experiments suggested that MYCs and PIFs regulate elongation via parallel pathways. To test this finding at the molecular level and to identify possible direct and indirect downstream targets of MYC2/3/4 related to elongation, we performed RNA-seq to compare the transcript profile of wild type and myc2/3/4 at 1 h and 49 h treatment in high R/FR and low R/FR. The number of differentially expressed genes (DEGs; Table 1), the overlap between them (Figure 5), and the clustering of the DEGs (Figure 6) are summarized and discussed in detail below.

In the differentially expressed gene set of wild type Col, auxin-activated signaling pathway genes were up regulated in Col after 1h of low R/FR treatment, including known shade-responsive genes, IAA19, IAA29, ATHB2, matching expectation and showing that our low R/FR treatment worked (Table 1; Supplementary Table S3). Previous work has shown that glucosinolate (GS) biosynthesis genes are direct targets of MYC2/3/4 in defense signaling (Schweizer et al. 2013). We found that most of these genes were down regulated in myc2/3/4 in 2-week-old plants (our experiment) as well, even though the previous study used leaves of 4-week-old plants (Figure 7; Supplementary Table S4), consistent with results that MYC2/3/4 directly activates transcription of GS biosynthesis genes. Thus, overall treatment and genotype effects are as expected.

**Flowering related genes are enriched in myc2/3/4 differentially expressed genes**

In accordance with the early-flowering phenotype of myc2/3/4, we also found that a number of flowering related genes were up regulated in myc2/3/4 at both 1 and 49 h treatment, compared to the wild-type Col. These genes had not previously been identified as being up regulated in myc2/3/4 mutants, likely because the previous study harvested 4-week-old plants that had finished bolting. Up regulated genes include SOC1 (SUPPRESSOR OF OVEREXPRESSION OF CONSTANS)/AGL 20 (AGAMOUS LIKE 20) (Borner et al. 2000; Onouchi et al. 2000; Samach et al. 2000; Lee et al. 2000) which is an integrator in flower development, AGL24 (AGAMOUS-LIKE 24) (Michaels et al. 2003; Fujita et al. 2003) and LFY (LEAFY)(Weigel et al. 1992), which act downstream of SOC1 and are positively regulated by SOCI (Samach et al. 2000; Lee et al. 2000, 2008; Moon et al. 2003; Liu et al. 2007, 2008), API(APETALA1) (Mandel et al. 1992), which is positively regulated by LFY (Wagner et al. 1999) and negatively regulates AGL24 (Yu et al. 2004), and FUL (FRUITFUL)(Gu et al. 1998), which is the downstream of FT (FLOWERING LOCUS T) and is positively regulated by FT (Teper-Bammolker and Samach 2005).
MYC2/3/4 have been demonstrated to inhibit flowering by repressing FT (Wang et al. 2017). FT was not detected to be differentially expressed in our condition (Supplementary table S4, S5, S6, S7, S8). The reason could be that the expression of FT is induced by CO (CONSTANS) protein, which only accumulates in the late afternoon in long day (Samach et al. 2000), while the tissue was collected at ZT7 in our experiment when FT might not be expressed. Overall, the flowering genes that we detected as being upregulated in myc2/3/4 mutants are consistent with induction through FT and support the findings of Wang et al. 2017.

Transcriptome analysis reveals a possible mechanism for increased elongation in myc2/3/4

Because of the role of MYC2/3/4 in inhibiting petiole elongation, we expected growth-related genes, such as auxin (indoleacetic acid, IAA) biosynthetic genes and IAA-regulated genes, to be upregulated in the myc2/3/4 mutant. However, contrary to our expectation, IAA biosynthetic genes including SUR1, SUR2, CYP79B2/3 and TRP2 were down regulated in myc2/3/4. Since MYC2/3/4 regulate glucosinolate biosynthesis, one explanation for our finding is

| GENOTYPE  | TIME POINT | NO. OF UP-REGULATED GENES | NO. OF DOWN-REGULATED GENES |
|-----------|------------|----------------------------|-----------------------------|
| Col       | 1 h        | 53                         | 98                          |
| Col       | 49 h       | 60                         | 136                         |
| myc2/3/4  | 1 h        | 24                         | 51                          |
| myc2/3/4  | 49 h       | 56                         | 139                         |
the known relationship between indole-GS and auxin biosynthesis. Specifically, indole-GS contributes to auxin biosynthesis via the metabolic intermediates indole-3-acetaldoxime (IAOx) and indole-3-acetonitrile (IAN). CYP79B2/B3 are involved in formation of IAOx from TRP, SUR1 and SUR2 are involved in the biosynthesis of indole-GS from IAOx, and indole-GS can be digested by myrosinases to form IAN (Halkier and Gershenzon 2006; Malka and Cheng 2017). It has been reported that inactivation of GS biosynthesis genes acting post IAOx, such as SUR1, SUR2 and UGT74B1, leads to elevated IAA level along with impaired indole GS (Delarue et al. 1998; Bak et al. 2001; Malka and Cheng 2017). Thus, SUR1 and SUR2 balance GS and IAA biosynthesis. SUR1 and SUR2 were down-regulated in our study in the myc2/3/4 triple mutant, and furthermore, the results showed that the known genes down regulated by IAA including CYP79B2/3 and GSTF11 were down-regulated in our study (Figure 8; Supplementary Table S9). Previous studies have shown that MYC2 binds to the SUR2 promoter (Schweizer et al. 2013) and have shown that low R/FR reduces the SUR2 reaction product I3M in a JAZ10 dependent manner (Cargnel et al. 2014) suggesting that low R/FR inactivates GS synthesis via JAZ / MYC2 interactions (Cargnel et al. 2014). In addition, myc2 mutants have been found to have increased auxin content (Huang et al. 2017). Therefore, it is possible that free IAA level also increases in myc2/3/4 mutant and leads to petiole elongation in the mutant. Genes classified as up-regulated by IAA

Figure 5 Differentially expressed genes in Col and myc2/3/4 under different experimental conditions. (A) Differentially expressed genes at 1h high R/FR and low R/FR treatments. (B) Differentially expressed genes at 49h high R/FR and low R/FR treatments. “Col up” and “Col down” indicate genes up-regulated and down-regulated, respectively, in Col under low R/FR. “myc2/3/4 up” and “myc2/3/4 down” indicate genes up-regulated and down-regulated, respectively, in myc2/3/4 under low R/FR. All genes are differentially expressed at P < 0.05. For differentially expressed genes in each genotype, the corresponding genotype under high R/FR condition was used as reference.

Figure 6 Heatmap of differentially expressed genes in Col and myc2/3/4 under different experimental conditions. The differentially expressed genes in myc2/3/4 include all genes that are differentially expressed between high and low R/FR within genotype, and between Col and myc2/3/4 at P < 0.05.
were not differentially expressed in our experiment, however most such genes are only transiently activated during shade avoidance (Nozue 2018), so would not serve as good indicators of long-term IAA growth promotion. In summary, the RNAseq results point to an increase of IAA via GS/IAA tradeoffs as a possible explanation for increased elongation in the myc2/3/4 mutants.

Besides the possible increase of IAA in myc2/3/4, MYC2 has been reported to suppress the activity of COP1 (CONSTITUTIVE PHOTOMORPHOGENIC 1) in promoting HY5 (ELONGATED HYPOCOTYL 5) degradation and was found to be required for COP1 suppression of hypocotyl elongation in JA signaling pathway (Zheng et al. 2017). In our RNA-seq data, COP1 was up-regulated in myc2/3/4 at both 1h and 49h treatment (Supplementary Table S5, S6). It is possible that MYC2/3/4 inhibits petiole growth in adult plants through COP1. However, HY5 only accumulates in plants younger than 7-days old (Hardtke et al. 2000), so this mechanism seems unlikely. It is possible that MYC2/3/4 affect petiole elongation by inhibiting the activity of COP1 in degrading other targets, such as HYH (HY5-HOMOLOG) (Holm et al. 2002).

Consistent with the idea that MYCs and PIFs act independently in growth regulation, PIF genes were not differentially expressed in myc2/3/4. We also examined whether there was overlap between PIF target genes and genes differentially expressed in myc2/3/4. Since PIF genes promote elongation and elongation is also promoted in myc2/3/4 mutants, if PIFs and MYCs regulate a common growth pathway we would expect positive PIF targets to also be upregulated in myc2/3/4. We tested this idea and found that while 6 of 39 positive PIF targets were differentially expressed in myc2/3/4

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**Figure 7** Over-represented GO term in differentially expressed genes in Col and myc2/3/4 under different experimental conditions. For each GO term, all genes enriched at \( P < 0.05 \). “Col up” column indicates the over-represented GO terms in up-regulated genes compared to Col under high R/FR. “myc2/3/4 up” and “myc2/3/4 down” columns indicate the over-represented GO terms in up- and down-regulated genes, respectively, compared to Col.
relative to Col (significant overlap, $P = 0.002$), these genes were down-regulated in $\text{myc2/3/4}$ and/or less induced by low R/FR (Supplementary Figure S1). Similarly, three of ten negative PIF targets were differentially expressed in $\text{myc2/3/4}$, but two of these three were upregulated. Since the PIF targets are regulated opposite to PIF4/5/7 action, the opposite regulation may indicate negative regulation.

CONCLUSIONS

Our genetic experiments show that PIF4/5/7 and MYC2/3/4 act additively to control petiole growth in high R/FR but that in low R/FR, PIF4/5/7 function are required for the $\text{myc2/3/4}$ constitutive petiole shade avoidance phenotype. Our RNA-seq experiments support the hypothesis that PIF4/5/7 and MYC2/3/4 can regulate growth independently. We propose at least three possible mechanisms for $\text{MYC2/3/4}$ regulation of petiole growth that are not mutually exclusive. One possibility is that the $\text{MYC2/3/4}$ increased growth is more due to energetic tradeoffs rather than gene regulation. The second possibility is that impaired GS biosynthesis in $\text{myc2/3/4}$ mutants elevates IAA levels. Third, the loss of $\text{MYC2/3/4}$ function in the triple mutant could relieve its inhibition on COPI activity, leading to increased growth.

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