Plant Cell Advance Publication. Published on September 17, 2019, doi:10.1105/tpc.19.00275

RESEARCH ARTICLE

Photoreceptor Activity Contributes to Contrasting Responses to Shade in Cardamine and Arabidopsis Seedlings

Maria Jose Molina-Contreras[a1], Sandi Paulišić[a1], Christiane Then[a1,2], Jordi Moreno-Romero[a], Pedro Pastor-Andreu[a], Luca Morelli[a], Irma Roig-Villanova[a3], Huw Jenkins[b], Asis Hallab[c4], Xiangchao Gan[c], Aurelio Gomez-Cadenas[d], Miltos Tsiantis[c], Manuel Rodríguez-Concepción[a], Jaime F. Martínez-García[a,e,g]

[a] Centre for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB-UB, 08193 Barcelona, Spain.

[b] Department of Plant Sciences, University of Oxford, Oxford OX1 3BR, United Kingdom.

[c] Department of Comparative Development and Genetics, Max Planck Institute from Plant Breeding Research, 50829 Cologne, Germany.

[d] Departament de Ciències Agràries i del Medi Natural, Universitat Jaume I, 12071 Castellò de la Plana, Spain.

[e] Institució Catalana de Recerca i Estudis Avançats (ICREA), 08010 Barcelona, Spain.

[g] corresponding author, jaume.martinez@cragenomica.es

[1] These authors contributed equally to this work and are shown in alphabetical order.

[2] Current address, Institute for Epidemiology and Pathogen Diagnostics, Julius Kühn-Institut, Federal Research Institute for Cultivated Plants, 38104 Braunschweig, Germany.

[3] Current address, Escola Superior d'Agricultura de Barcelona, Universitat Politècnica de Catalunya, Campus Baix Llobregat, Castelldefels 08860 Barcelona, Spain.

[4] Current address, IBG-2 Plant Sciences, Forschungszentrum Jülich, Jülich, Germany

Short title: Genetic regulation of shade tolerance

One-sentence summary: The lack of a shade-induced hypocotyl elongation response in *Cardamine hirsuta* results from the enhanced repressor activity of the phytochrome A photoreceptor.

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantcell.org) is: Jaime F. Martinez-Garcia (jaume.martinez@cragenomica.es).

ABSTRACT

Plants have evolved two major ways to deal with nearby vegetation or shade: avoidance and tolerance. Moreover, some plants respond to shade in different ways; for example, *Arabidopsis thaliana* undergoes an avoidance response to shade produced by vegetation, but its close relative *Cardamine hirsuta* tolerates shade. How plants adopt opposite strategies to respond to the same environmental challenge is unknown. Here, using a genetic strategy, we identified the *C. hirsuta slender in shade1* (*sis1*) mutants, which produce strongly elongated hypocotyls in response to shade. These mutants lack the phytochrome A (phyA) photoreceptor. Our findings suggest that *C. hirsuta* has evolved a highly efficient phyA-dependent pathway that suppresses hypocotyl elongation when challenged by shade from nearby vegetation. This suppression relies, at least in part, on stronger phyA activity in *C. hirsuta*; this is achieved by increased *ChPHYA* expression and protein accumulation combined with a stronger specific intrinsic repressor activity. We suggest that modulation of photoreceptor activity is a powerful mechanism in nature to achieve physiological variation (shade tolerance vs. avoidance) for species to colonize different habitats.

INTRODUCTION

Understanding how plants colonize different habitats requires identifying the genetic differences underlying physiological variation between species. In this work, we focus on angiosperm responses to changes in light produced by nearby vegetation, perception of which alerts the plant to potential resource competition by other plants. Nearby vegetation is perceived as changes in light parameters: whereas sunlight has a high red (R) to far-red light (FR) ratio (R:FR, >1.1), proximity to vegetation lowers this ratio (Smith, 1982). Because vegetation specifically reflects FR, proximity to other plants initially results in a mild reduction in R:FR (<0.7) due to the FR enrichment. Eventually, when the vegetation canopy closes, sunlight is filtered by photosynthetic tissues, strongly reducing the intensity of the photosynthetic active radiation (PAR, between 400 to 700 nm, which includes blue and R) while marginally affecting FR. As a result, R:FR resulting from natural canopy shade typically drops to lower values (<0.05) (Casal, 2012; de Wit *et al.*, 2016; Martinez-Garcia *et al.*, 2014; Smith, 1982). In the laboratory, both vegetation proximity and canopy shade can be simulated by providing plants grown under white light (W, high R:FR) varying amounts of supplemental FR (W+FR; low or very low R:FR) while maintaining

total PAR, a treatment known as simulated shade (Casal, 2012; Roig-Villanova and Martinez-Garcia, 2016).

Plants have two main strategies to acclimate to vegetation proximity and shade: avoidance or tolerance. In the early stages of development, shade-avoider species invest energy into promoting elongation to overgrow their neighbors as part of the so-called shade avoidance syndrome (SAS). By contrast, shade-tolerant plants adopt other physiological and metabolic responses to adapt to a highly conservative utilization of resources, commonly accompanied by very low growth rates, i.e., do not involve promotion of elongation growth (Smith, 1982; Valladares and Niinemets, 2008).

Analyses of the shade-avoider *Arabidopsis thaliana* laid the basis for our knowledge of the genetic components and mechanisms involved in the regulation of the SAS (Casal, 2012; Martinez-Garcia *et al.*, 2010; Roig-Villanova and Martinez-Garcia, 2016). The shade signal is perceived by the phytochrome photoreceptors: phytochrome B (phyB) and phyA have major and antagonistic roles (respectively) in hypocotyl elongation, the most conspicuous *A. thaliana* response to low R:FR (Casal, 2012; Mathews, 2010). Lowering the R:FR to resemble either vegetation proximity or canopy shading, deactivates phyB in wild-type seedlings, resulting in the hypocotyl elongation promotion. By contrast, phyA accumulates and is strongly activated under very low R:FR to prevent excessive seedling elongation (Martinez-Garcia *et al.*, 2014; Yang *et al.*, 2018). Consistent with this, *A. thaliana* phyB-deficient mutants display constitutive shade responses under high R:FR whereas *phyA* mutant seedlings show enhanced hypocotyl elongation only under very low R:FR conditions, which indicates that phyA antagonizes phyB activity under these specific canopy shade conditions (Casal *et al.*, 2014; Martinez-Garcia *et al.*, 2014; Yang *et al.*, 2018; Yanovsky *et al.*, 1995).

SAS responses are mainly initiated because of the interaction of active phytochromes with PHYTOCHROME INTERACTING FACTORs (PIFs), eventually triggering rapid changes in the expression of dozens of genes that implement the SAS responses. Genetic analyses in *A. thaliana* indicate that PIFs, which are basic-helix-loop-helix transcription factors, have a role in positively regulating the shade-triggered hypocotyl elongation. The active form of phyB interacts with PIFs and inhibits their transcriptional activity (Casal,

2012; Martinez-Garcia *et al.*, 2010). After exposure to shade, the proportion of active phyB decreases and PIF activity increases. Enhanced PIF binding to G-boxes of auxin biosynthetic genes (e.g. *YUCCA* genes) then promotes their expression, which results in a rapid (1-4 h) increase in free IAA that is required for the promotion of shade-induced hypocotyl elongation (Bou-Torrent *et al.*, 2014; Hornitschek *et al.*, 2012; Li *et al.*, 2012; Tao *et al.*, 2008). In addition, nuclear-pore complex components and chloroplast-derived signals also prevent an excessive response to shade, providing additional regulatory levels of this response (Gallemi *et al.*, 2016; Ortiz-Alcaide *et al.*, 2019).

There are, however, still major gaps in understanding the genetic and molecular regulation of SAS and, by extension, shade-tolerance traits. Comparative analyses using shade-avoiding and shade-tolerant species is expected to identify regulators of traits associated with shade tolerance habits (Gommers *et al.*, 2013). Indeed, a comparative transcriptomic approach using two *Geranium* species with divergent petiole responses to shade unveiled components that might suppress growth in the shade-tolerant species (Gommers *et al.*, 2017; Gommers *et al.*, 2018). The use of related species but amenable for genetic analyses is expected to push this effort further to find regulatory components used in nature to modulate these divergent responses. This is what we are addressing in this work.

Comparing *A. thaliana* and its close relative *Cardamine hirsuta* to understand the genetic basis for trait diversification between species is a powerful strategy to understand the evolution of morphological traits. Key to this approach is the wide morphological and physiological diversity between these species, such as differences in leaf morphology and seed dispersal mechanism among others (Barkoulas *et al.*, 2008; Hay *et al.*, 2014; Hofhuis *et al.*, 2016; Vlad *et al.*, 2014; Vuolo *et al.*, 2016). Like *A. thaliana*, *C. hirsuta* has a short generation time, small size, inbreeding habit, abundant progeny and ease of large scale cultivation (Hay and Tsiantis, 2016; Hay *et al.*, 2014). It is a diploid species with a small genome and eight chromosomes that has been completely sequenced (Gan *et al.*, 2016). Genetic transformation by floral dipping, a dense genetic map and chemically mutagenized populations, provide the tools to identify the genetic components and molecular mechanisms underlying diversification or morphology and response to environment (Hay and Tsiantis,

2016). *C. hirsuta* is an invasive herbaceous plant that can grow in open sun but it is often found in shaded or semi-shaded areas. Indeed, *C. hirsuta* does not need much light to grow and their stems become purplish (likely to prevent oxidative damage) in strong sun (http://edis.ifas.ufl.edu/pdffiles/EP/EP51100.pdf; http://practicalplants.org/wiki/Cardamine_hirsuta; http://www.asturnatura.com/especie/cardamine-hirsuta.html; http://dnr.wi.gov/topic/Invasives/documents/classification/LR_Cardamine_hirsuta.pdf; https://www.wildfooduk.com/edible-wild-plants/hairy-bittercress/). These observations are consistent with *C. hirsuta* being shade-tolerant (Bealey and Robertson, 1992). In agreement, whereas seedlings of *A. thaliana* elongate in response to shade, those of *C. hirsuta* are unresponsive to the same stimulus (Hay *et al.*, 2014).

The divergent hypocotyl response to shade of *A. thaliana* and *C. hirsuta* species led us to take a comparative approach to understand the genetic basis of the evolution of this physiological trait. We found that *C. hirsuta* has acquired a highly-efficient phyA-dependent pathway that represses hypocotyl elongation and other SAS-associated responses when exposed to simulated shade. After complementing *A. thaliana phyA* mutant plants with endogenous or *C. hirsuta* phyA molecules we concluded that these two photoreceptors are not exchangeable. Differences in phyA intrinsic activity hence contribute to a different response of *C. hirsuta* and *A. thaliana* to shade exposure.

RESULTS

C. hirsuta seedlings perceive low R:FR but do not elongate

A recent study revealed that different species of the *Tradescantia* genus with divergent tolerance to shade showed clear differences in maximum quantum efficiency of photosystem II (Fv/Fm) upon variations of the growth light (Benkov *et al.*, 2019). In particular, the sun-resistant *T. sillamontana* (a succulent growing in semi-desert regions of Mexico and Peru, hence adapted to high light intensities) was more tolerant to changes in irradiation intensity (i.e. showed a more constant Fv/Fm) than the shade-tolerant *T. fluminensis* (habitant of tropical rainforests and other shaded areas in south-eastern Brazil and hence adapted to grow under low light intensities).

Using a similar experimental system, we aimed to confirm whether *C. hirsuta* is a shade-tolerant plant compared to *A. thaliana* (a broadly accepted shade-avoider). Indeed, when wild-type seedlings of these two species (Ch^{WT} and At^{WT}) were transferred from normal white light (W) to conditions in which PAR was first increased 10-fold (high light, HL) and then reduced 5-fold relative to W (low light, LL) or viceversa, Fv/Fm changes were much more pronounced in Ch^{WT} (Supplemental Figure 1A). The lower capacity of Ch^{WT} to adapt to intense irradiation was confirmed by the bleaching symptoms (e.g., lower chlorophyll contents) observed in Ch^{WT} (but not in At^{WT}) upon transferring to HL (Supplemental Figure 1B). Ch^{WT} seedlings only showed a better performance than At^{WT} when transferred from W to LL. Rapid light curve (RLC) analysis confirmed that Ch^{WT} was better able to maintain its level of photosynthetic activity under LL conditions than At^{WT} (Supplemental Figure 1C), as expected for a shade-tolerant plant (Han *et al.*, 2015).

Besides differentially responding to decreased light quantity, plant species from open habitats show a stronger elongation response to reduced R:FR (i.e. light quality) compared to those from woodland shade habitats (Gommers *et al.*, 2017; Smith, 1982). Further supporting the conclusion that *C. hirsuta* tolerates shade, Ch^{WT} failed to elongate their hypocotyls when exposed to a range of low R:FR treatments (i.e., W+FR), that mimic vegetation proximity (intermediate or low R:FR; 0.09 - 0.07) and canopy shade (very low R:FR; 0.02) (Supplemental Figure 2, Figure 1). W-grown Ch^{WT} hypocotyls, as well as cotyledons, are substantially longer than those of At^{WT} growing under the same conditions. Ch^{WT} hypocotyls were also longer than those of At^{WT} when growing in the dark (Figure 1C), indicating that *C. hirsuta* is overall bigger than *A. thaliana*. More importantly, when treated with growth stimulants, such as gibberellic acid (Hay *et al.*, 2014) or picloram (PIC, a synthetic auxin), hypocotyls of both species elongate (Figure 1D). We therefore concluded that the elongation of *C. hirsuta* hypocotyls is not generally compromised, arguing against the possibility that this species displays a constitutive SAS phenotype.

In *A. thaliana*, exposure to simulated shade also triggers the elongation of leaf petioles. We quantified the elongation response of the petiole and rachis in 2-week-old Ch^{WT} and At^{WT} plants subjected to 7 days of high (W) or low R:FR (W+FR). In agreement with previous studies (de Wit *et al.*, 2015; Kozuka *et al.*,

2010; Sasidharan *et al.*, 2010), shade-treated At^{WT} leaves showed substantially longer petioles than those of plants grown under W. Petiole and rachis length in Ch^{WT}, however, was similar in leaves from plants grown under W or W+FR (Figure 1E, Supplemental Figure 3). These results together suggest that elongation responses to low R:FR are dramatically arrested in *C. hirsuta* plants.

C. hirsuta shows other attenuated responses to shade

Beyond elongation responses, low R:FR triggers a reduction in the levels of photosynthetic pigments, i.e., carotenoids and chlorophylls (Bou-Torrent *et al.*, 2015; Cagnola *et al.*, 2012; Roig-Villanova *et al.*, 2007). While these pigments were also significantly reduced in shade-treated Ch^{WT} seedlings (Figure 1F), the decrease was less prominent than in At^{WT}. These results indicated that not all SAS responses are equally compromised in *C. hirsuta*.

We next used RNA sequencing (RNA-seq) to compare the genome wide expression patterns of 7-day-old At^{WT} and Ch^{WT} whole seedlings in W versus 1 h of simulated shade (W+FR) (Figure 2). Incorporating knowledge about gene orthology, 432 differentially expressed genes (DEGs) were categorized as rapidly regulated by shade in one species or in both. Plotting the W+FR vs. W fold-change in *C. hirsuta* against the same ratio in *A. thaliana* resulted in a linear regression equation with a slope of 0.54 (Figure 2B), which supported that shade-modulated changes in gene expression are also attenuated in *C. hirsuta* compared to *A. thaliana*. In *A. thaliana*, shade treatment induced 246 (fold change>1.5, $p<0.05$,) and repressed 58 genes (fold change<-1.5, $p<0.05$,). In *C. hirsuta*, this same treatment induced 181 and repressed 54 genes (Supplemental Figure 4A, Supplemental Data Sets 1–4). From the set of induced DEGs, 102 responded in both species. They included several of the well-known shade-marker genes in *A. thaliana* and other species, such as *ARABIDOPSIS THALIANA HOMEOBOX PROTEIN 2* (*ATHB2*), *BRASSINOSTEROID-ENHANCED EXPRESSION 1* (*BEE1*), *BES1-INTERACTING MYC-LIKE1* (*BIM1*), *LONG HYPOCOTYL IN FR 1* (*HFR1*) or *XYLOGLUCAN ENDOTRANSGLYCOSYLASE 7* (*XTR7*) (Cifuentes-Esquivel *et al.*, 2013; Karve *et al.*, 2012; Procko *et al.*, 2014; Ueoka-Nakanishi *et al.*, 2011).

Gene ontology (GO) and MapMan-Bin (MMB) functional prediction of these up-regulated gene group indicated that terms related to auxin were

significantly overrepresented (Supplemental Data Sets 5 and 6), suggesting an early role for auxins in both *A. thaliana* and *C. hirsuta*. Indeed, W+FR treatment for 1 h increased auxin (IAA) levels not only in At^{WT}, as published (Bou-Torrent *et al.*, 2014; Hersch *et al.*, 2014; Hornitschek *et al.*, 2012; Tao *et al.*, 2008), but also in whole Ch^{WT} seedlings (Figure 2C).

Using public transcriptomic data, we identified a group of 13 genes whose expression was induced in *A. thaliana* wild-type seedlings but not in mutants that do not accumulate auxins (*shade avoidance 3-2*, (*sav3-2*) and *pif7-1*) after 1 h of shade treatment (Bou-Torrent *et al.*, 2014; Li *et al.*, 2012; Tao *et al.*, 2008). Based on our RNAseq data, the expression of these genes was significantly upregulated in At^{WT} and, to a lower extent, Ch^{WT} seedlings (Supplemental Figure 5), consistent with the observed increase in IAA content in both species. Since only *A. thaliana* elongates in response to shade exposure, either the observed early changes in gene expression and auxin levels are not reflecting the differences in hypocotyl growth between these species, or the elongation is consequence of differential later events.

In our RNAseq analyses, 55 and 49 DEGs were specifically repressed in either At^{WT} or Ch^{WT} seedlings, respectively, and just 3 genes were repressed in both species. Regarding up-regulated genes, 142 and 79 DEGs were specifically induced either in At^{WT} or in Ch^{WT}, respectively (Supplemental Figure 4A). GO and MMB functional prediction of the 142 DEGs specific for At^{WT} showed genes related to several aspects of plant development, whereas the 79 DEGs specifically induced in Ch^{WT} showed enrichment for genes related with the photosynthetic machinery. Particularly, *C. hirsuta* rapidly responds by inducing the expression of genes encoding components of both photosystems I and II, the NADH dehydrogenase-like complex (involved in chlororespiration) and both small and large subunits of plastidial ribosomes (Supplemental Figure 5B, Supplemental Data Sets 5 and 6). Whether these rapid changes are maintained after prolonged exposure to shade or have any functional relevance is unknown. Nonetheless, these transcriptome differences support that the two mustard species employ alternative strategies to adapt to plant proximity and shade that go further from the modulation of elongation growth.

Comparative approaches have been used before to investigate the differential response to shade of related species. Transcriptomic analyses using

two *Geranium* species that display divergent shade-induced petiole elongation (*G. pyrenaicum* as a shade avoider or responsive, and *G. robertianum* as a shade tolerant) identified a series of 31 up-regulated genes that included a number of candidate regulators of differential shade avoidance (Gommers *et al.*, 2017). In these two species, putatively orthologous transcript groups (OMCL) were defined, and the best BLAST hit with the *A. thaliana* transcriptome was used to name *Geranium* OMCL groups (Gommers *et al.*, 2017). When we compared our lists of shade-regulated genes with the Geranium OMCLs differentially regulated after 2 h of low R:FR in the petioles, we found that the number of genes up-regulated in both shade-tolerant and shade-avoider species was higher for the At^{WT}/Ch^{WT} pair than between the *Geranium* species (Supplemental Figure 4C; Supplemental Data Sets 7 and 8). GO analyses did not identify any function from the lists of genes specifically induced in either *G. pyrenaicum* or *G. robertianum*. Overlap was very limited between the sets of repressed genes. Together, the contrasting rapid shade-induced gene expression changes might either support differences in the early molecular mechanisms between the *Geranium* and mustard groups, or just reflect the differences in tissues (whole seedlings vs. leaf petioles) and/or shade and growth conditions (continuous light vs. photoperiod) between experiments.

We also analyzed the changes in gene expression of *PIF3-LIKE 1* (*PIL1*) and *ATHB2*, two typical shade-marker genes, in response to longer (up to 8 h) exposure to low R:FR. Expression of *PIL1* and *ATHB2* were rapidly induced in both mustard seedlings after simulated shade exposure. However, the relative induction of the expression of these genes was attenuated in Ch^{WT} compared to At^{WT} (Figure 2D). Together, our results indicate that *C. hirsuta* seedlings sense plant proximity and respond molecularly and metabolically to it; however, this signal does not promote hypocotyl elongation in *C. hirsuta* as it does in the shade-avoider *A. thaliana*.

Shade-induced elongation in *C. hirsuta* is repressed

To explain the hypocotyl elongation differences between *A. thaliana* and *C. hirsuta*, we hypothesized two mutually exclusive mechanisms: (i) **uncoupling**: shade perception is specifically unplugged from the endogenous mechanisms of control of hypocotyl elongation, or (ii) **suppression**: there are

mechanisms that strongly suppress the shade-induced elongation of hypocotyls. To distinguish between these possibilities, a genetic screening looking for *C. hirsuta* seedlings with long hypocotyls under simulated shade (> 6 mm long) was carried out, using an EMS-mutagenized population (Vlad *et al.*, 2014). If suppression mechanisms exist, then loss-of-function mutants that unleash shade-induced hypocotyl elongation might be recovered. Indeed, from the various long hypocotyl seedlings identified we focused in two *slender in shade* (*sis*) mutants, shown to be recessive and allelic. After backcrossing these mutants twice with the Ch^{WT} plants, homozygous mutants had slightly longer hypocotyls in W than the wild type, and very long hypocotyls under W+FR. We named the mutants as *sis1-1* and *sis1-2* (Figure 3). These results indicated that (1) loss-of-function (recessive) mutations support the "suppression" mechanisms in *C. hirsuta* to establish shade-tolerance; and (2) a single gene, *SIS1*, is able to repress the elongation response to shade in *C. hirsuta.*

As a first step to explore *SIS1* identity, we determined whether light perception was altered in *sis1* mutants by analyzing hypocotyl length after de-etiolation under monochromatic lights. We noticed that Ch^{WT} seedlings were quite hyposensitive to R compared to At^{WT} (Figure 3B), suggesting that an attenuated phyB signaling might result in a constitutive SAS hypocotyl response, causing the observed suppression of the shade-induced hypocotyl elongation. Considering the relationship between the attenuated responsiveness to R and the strength of the shade-induced hypocotyl elongation of the weak *phyB-4* and strong *phyB-1 A. thaliana* mutant seedlings (Figure 3C,D), the hyposensitivity to R observed in Ch^{WT} might contribute but is not enough to fully suppress the shade-induced hypocotyl elongation in this species. Therefore, additional components are required to establish the shade-tolerant hypocotyl habit in *C. hirsuta*. Indeed, mutant *sis1* seedlings, although slightly hyposensitive to R and blue light, were fully blind to FR compared to Ch^{WT} seedlings (Figure 3B).

A very similar pattern of response was also shown by *A. thaliana* phyA-deficient *phyA-501* seedlings (Figure 3B) (Li *et al.*, 2011), which suggested that *sis1* seedlings might be deficient in phyA activity or signaling. Sequencing of the *C. hirsuta PHYA* (*ChPHYA*) gene from *sis1-1* and *sis1-2* plants showed point mutations (transitions) that introduced either a nonsense mutation in Gln935 (in

sis1-1) or a missense mutation in the conserved Gly913 (in *sis1-2*) (Figure 3E, Supplemental Figure 6A). Immunoblot analyses using a specific monoclonal antibody against phyA (073D), indicated that only *sis1-1* was lacking phyA (Figure 3D). Consistent with this, *C. hirsuta* lines with reduced activity of phyA by overexpressing an RNA interference construct directed towards the *ChPHYA* gene (lines 35S:RNAi-ChPHYA) also resulted in a *sis* phenotype (Supplemental Figure 6B-D). Together, these results indicated that *sis1* are *C. hirsuta phyA* deficient mutants (for clarity, we will keep the *sis1* mutant name along the manuscript to distinguish it from the *phyA* mutants from *A. thaliana*). They also suggested that shade tolerance in *C. hirsuta* might be caused by the existence of a phyA-dependent suppression mechanism that represses the hypocotyl elongation response to shade.

Molecular analyses showed that the relative induction of *PIL1* and *ATHB2* expression was enhanced in both *sis1* mutants compared to Ch^{WT} seedlings after more than 4 h of simulated shade exposure (Figure 4). This relatively late effect of *ChPHYA* absence (*sis1*) on gene expression is consistent with what was observed in *A. thaliana phyA* mutants (Ciolfi *et al.*, 2013). We also measured the levels of photosynthetic pigments (carotenoids and chlorophylls) after long-term exposure to low R:FR, in wild-type and phyA-deficient *A. thaliana* and *C. hirsuta* seedlings. Simulated shade triggered a stronger decrease in the accumulation of these pigments in *phyA-501*, *sis1-1* and *sis1-2* seedlings compared to wild-type controls (Figure 4B), hence indicating that phyA represses this trait in both species, likely to avoid exaggerated losses of photosynthetic pigments in response to vegetation proximity and shade.

Phytochrome A represses the shade-induced hypocotyl elongation in *A. thaliana* caused by the deactivation of phyB only under conditions that mimic closed canopies, i.e., under very low R:FR (Casal *et al.*, 2014; Martinez-Garcia *et al.*, 2014; Yanovsky *et al.*, 1995). Indeed, *A. thaliana phyA* deficient mutants behaved almost like At^{WT} seedlings under various shade mimicking conditions except for the lowest R:FR tested (Figure 4C). By contrast, *C. hirsuta sis1* mutants behaved differently than its Ch^{WT} under all the low R:FR applied (Figure 4D), indicating that phyA has a broader role in suppressing the shade-induced hypocotyl elongation in *C. hirsuta* than in *A. thaliana*.

C. hirsuta has higher phyA activity than *A. thaliana*

Our results suggested the possibility that phyA activity is higher in the shade-tolerant *C. hirsuta* than in the shade-avoider *A. thaliana*. Higher phyA activity can be achieved by at least two alternative and non-exclusive ways: higher phyA levels and/or higher specific (intrinsic) activity of the photoreceptor. To analyze these possibilities, we first aimed to compare *PHYA* expression levels in At^{WT} and Ch^{WT} seedlings. Data extracted from our RNAseq experiment indicated that the expression of several commonly-used reference genes, such as *EF1α* or *YLS8* (Gallemi *et al.*, 2017b; Hornitschek *et al.*, 2009; Kohnen *et al.*, 2016), was within the same range (Supplemental Table 1). Then, we quantified *PHYA* expression levels in At^{WT} and Ch^{WT} seedlings growing under W or W+FR (Figure 5) using primers that recognize the sequence of the target gene (*PHYA*) and three normalizer genes (*EF1*α, *SPC25*, *YLS8*) in both species (Supplemental Figure 7). Expression of *PHYA* was significantly higher in *C. hirsuta* than in *A. thaliana* seedlings (two-way ANOVA tests, $p<0.05$) in seedlings of different ages grown under W or W+FR conditions (Figure 5B).

Higher expression of *PHYA* in *C. hirsuta* might result in higher phyA protein levels, contributing to an increased phyA activity in this species. Our immunoblot analyses showed that PHYA protein levels were significantly higher in *C. hirsuta* than *A. thaliana* etiolated seedlings (Figure 5D). More importantly, whereas PHYA levels almost disappear after 6 h of W exposure in both species, *C. hirsuta* seedlings maintained higher PHYA levels than *A. thaliana* when exposed to W+FR for 6-10 h (Figure 5C-D). Together, these results support that PHYA levels in *C. hirsuta* are generally higher than in *A. thaliana* seedlings, even under shade conditions. This observation is consistent with the strongest difference in hypocotyl length under W of wild-type and phyA-deficient seedlings from *C. hirsuta* compared to *A. thaliana* (Figure 4C-D, Supplemental Figure 6D) (Martinez-Garcia *et al.*, 2014). Furthermore, transgenic overexpression of *PHYA* has been shown to attenuate shade-triggered hypocotyl elongation in *A. thaliana* seedlings and stem elongation in other species (Heyer *et al.*, 1995; Robson *et al.*, 1996; Roig-Villanova *et al.*, 2006).

To compare AtphyA and ChphyA specific (intrinsic) activities, complementation analyses of the *A. thaliana phyA-501* mutant were carried out

with the *AtPHYA* or *ChPHYA* genes under the control of the endogenous promoter of *AtPHYA* (*pAtPHYA:AtPHYA* or *pAtPHYA:ChPHYA*, respectively). The resulting lines were named as *phyA>AtPHYA* and *phyA>ChPHYA* (Figure 6). We obtained a total of 5 independent *phyA>AtPHYA* lines and 7 independent *phyA>ChPHYA* lines with different transcript and protein levels (Supplemental Figure 8). To estimate PHYA protein levels we used etiolated seedlings, as phyA is photolabile. Because *PHYA* expression is repressed by light via phyA and phyB (Canton and Quail, 1999), RNA was extracted from seedlings either grown in the dark or under W+FR (Supplemental Figure 8A). *PHYA* expression in seedlings grown in these two conditions correlated positively in both *phyA>AtPHYA* (R^2=0.79) and *phyA>ChPHYA* lines (R^2=0.79) (Supplemental Figure 8B). The slope of these equations, however, was significantly higher ($p<0.05$) for *phyA>AtPHYA* (7.49) than *phyA>ChPHYA* (2.81) lines. Specifically, *phyA>AtPHYA* and *phyA>ChPHYA* lines with comparable *PHYA* expression levels in the dark showed lower *PHYA* expression under simulated shade when complemented by *ChPHYA* (*phyA>ChPHYA*) compared to *AtPHYA* (*phyA>AtPHYA*). These results pointed to a stronger activity for the ChphyA protein in repressing its own (*PHYA*) expression.

For the comparison of AtphyA and ChphyA activities, we initially studied their effect on the promotion of the shade-induced hypocotyl elongation in transgenic lines. At^{WT} and *phyA-501* seedlings were incorporated as controls. In these experiments, the difference in hypocotyl length between seedlings grown under W+FR vs. W (Hyp_{W+FR}-Hyp_W) provided values indicative of the complementation level (or phyA biological activity) for the response analyzed. Consequently, in these analyses, the lower the Hyp_{W+FR}-Hyp_W value, the higher the phyA activity. Opposite to that observed with transcript levels (Supplemental Figure 8C), Hyp_{W+FR}-Hyp_W correlated well with ChPHYA but not with AtPHYA protein levels (Supplemental Figures 8D). These results together indicate that the two photoreceptors are not fully exchangeable and suggest different intrinsic qualities (i.e., biological activity) between the phyA receptors of *A. thaliana* and *C. hirsuta*.

When lines with comparable PHYA protein levels were selected (Figure 6B), the response to shade (Hyp_{W+FR}-Hyp_W) was more strongly attenuated by

ChPHYA (Figure 6C-D). As an additional way to test for phyA activity, we estimated hypocotyl elongation in seedlings etiolated (Hyp_D) and deetiolated under monochromatic FR (Hyp_{FR}). In this case, the higher the difference between these two values (Hyp_D-Hyp_{FR}), the stronger the activity of phyA. Similar to the shade response analyses, ChphyA showed a stronger activity than AtphyA in deetiolating seedlings under FR (Figure 6E-F). A good correlation between these two phyA-mediated responses was also found when all the lines were considered together (Supplemental Figure 8E), reinforcing our interpretation that ChphyA is intrinsically more active than AtphyA.

The expression of dozens of auxin-responsive genes is repressed by phyA after just 1 h of very low R:FR treatment (Yang *et al.*, 2018). As an additional and complementary test of phyA biological activity different from hypocotyl elongation we evaluated the repressive effect of AtphyA and ChphyA on the expression of these genes. First, we selected *1-AMINO-CYCLOPROPANE-1-CARBOXYLATE SYNTHASE 8* (*ACS8*), *GRETCHEN HAGEN 3.3* (*GH3.3*), *INDOLE-3-ACETIC ACID INDUCIBLE 19* (*IAA19*) and *IAA29*, four auxin-responsive genes described as repressed phyA targets (Yang *et al.*, 2018). As expected, the shade-induced expression of these genes was attenuated in At^{WT} compared to *phyA-501* seedlings, but under our shade conditions the differences were most obvious after long exposure to W+FR (Figure 7).

The expression of the same genes was next quantified in seedlings from the various *phyA>AtPHYA* and *phyA>ChPHYA* lines grown for 24 h under W+FR. When plotting transcript levels of phyA target genes as a function of *PHYA* expression in these lines, the clouds of data corresponding to *phyA>ChPHYA* lines (red) were separated from that of *phyA>AtPHYA* lines (blue) (Figure 7B). Importantly, the expression of all phyA target genes tested was overall lower in *phyA>ChPHYA* than *phyA>AtPHYA* lines, indicating that ChphyA repressed more efficiently gene expression than AtphyA (Figure 7B). Consistent with this conclusion, the expression of these and other phyA target genes (Yang *et al.*, 2018) was attenuated in shade-induced seedlings of Ch^{WT} compared to At^{WT} (Supplemental Figure 9). Together, these data further support that ChphyA is intrinsically more active than AtphyA.

DISCUSSION

Currently, the genetic basis of shade tolerance is poorly understood. To address this open question, we have focused on comparative analyses of the hypocotyl response to shade in young seedlings of two related mustards, *A. thaliana* and *C. hirsuta*. *S*hade avoidance and tolerance are ecological concepts originated from the natural habitats of plants species (Callahan *et al.*, 1997). Hence, defining the shade habit of a species is difficult because shade tolerance is not an absolute value but a relative concept; indeed, plants may exhibit different strategies during the juvenile and adult phases of their lives (Valladares and Niinemets, 2008). Despite the uncertainty, *A. thaliana* is generally considered as shade avoider and it is a model broadly used to study the SAS hypocotyl response, but there is little information referring to its physiological shade-responsiveness habit. *C. hirsuta*, by contrast, has been previously described as a shade tolerant species whose hypocotyls are unresponsive to shade (Bealey and Robertson, 1992; Hay *et al.*, 2014), but little is known about other shade response mechanisms. Here we confirm that, as expected for a shade-tolerant species, *C. hirsuta* showed a much better capacity to acclimate to LL than to HL compared to *A. thaliana* (Supplemental Figure 1). Most strikingly, *C. hirsuta* seedlings failed to elongate in response to simulated proximity or canopy shade (Figure 1). Such a dramatic hypocotyl elongation response compared to *A. thaliana* makes these two related species good candidates for comparative analyses of divergent responses to shade.

Our comparative and genetic analyses suggest that the absence of a shade-induced hypocotyl elongation in *C. hirsuta* is not caused by defects on the rapid biosynthesis of auxin in seedlings (Figure 2). Although we cannot exclude local defects in auxin biosynthesis (e.g., in hypocotyls) that might be masked by collecting whole seedlings, our conclusion is consistent with the lack of effect of phyA on the rapid shade-induced biosynthesis of auxin (Yang *et al.*, 2018). On the contrary, we favor that the differences in hypocotyl elongation between these species is the result of a suppression mechanisms sustained by the stronger activity of the ChphyA photoreceptor, likely enhanced by the attenuated ChphyB activity (Figure 3B). A stronger intrinsic (specific) repressor activity of ChphyA would result in a strong suppression of the elongation of *C. hirsuta* seedlings when exposed to shade (Figure 8). The underlying

mechanism likely relies, at least partly, upon suppression of auxin signaling via phyA directly binding and stabilizing AUX/IAA proteins, as it has been shown in *A. thaliana* (Yang *et al.*, 2018). In this scenario, ChphyA seems to suppress not auxin biosynthesis but signaling more strongly than AtphyA, as deduced from the results with transgenic lines (Figure 7B) but also from the stronger repression in shade of auxin-responsive genes with a putative role in auxin-signaling (e.g., several *IAA* and *SAUR* genes) detected in Ch^{WT} compared to At^{WT} (Supplemental Figure 9).

AtphyA and ChphyA might achieve different activities by changes in particular residues that could alter susceptibility to post-translational modifications. For instance, phyA stability, Pfr to Pr reversion rate upon shade treatment or/and interaction with protein partners (e.g., PIF1/PIF3, FHY1/FHL, AUX/IAA) affect phyA activity in *A. thaliana* (Dieterle *et al.*, 2005; Genoud *et al.*, 2008; Kim *et al.*, 2004; Oka *et al.*, 2012; Seo *et al.*, 2004; Sheerin *et al.*, 2015). These intrinsic differences might be also enhanced by changes in protein abundance of phyA or/and other components in its signaling pathway specifically acting in light-grown seedlings (see below). Comparison of the amino acid sequences of AtphyA and ChphyA, however, did not point to any obvious specific residue or region that could be responsible for the observed intrinsic differences in activity (Supplemental Figure 10). This is an issue that would need future research.

The genetic mechanisms underlying physiological evolution remain largely unknown, but changes in the timing, location and levels of gene expression (i.e., *cis*-regulatory evolution of key genes) have caused much of morphological evolution changes (Carroll, 2008). Our data on *PHYA* expression and PHYA protein levels (Figure 5) agree with this view, but they go a step beyond by showing that differences in protein (ChphyA and AtphyA) intrinsic activities also contribute to differential responses to shade (Figure 6-7). As both components (levels vs. intrinsic activity) are intimately connected (e.g., phyA represses its own expression in a light-dependent manner), at this stage it is difficult to quantify the specific contribution of each one. Moreover, additional components might contribute: while we show that phyA is a central component of a range of regulators that can be modulated in nature to implement shade tolerance, the observation that none of the *phyA>ChPHYA* lines display a

shade-tolerant habit (Supplemental Figure 8D) strongly suggests that additional downstream components of the shade-regulatory network are also participating in suppressing this response in *C. hirsuta* (e.g., differences in phyB activity). Indeed, it cannot be excluded that the mutant screen, despite identifying an important regulator, did not establish the causal difference between the two species in terms of shade-induced hypocotyl elongation. Nonetheless, our results unveil the importance of modulating photoreceptor activity as a powerful evolutionary mechanism in nature to achieve physiological variation between species, hence enabling the colonization of new, different habitats. In addition, searching for variability in phyA function could provide a suitable tool to modify the impact of neighbors' cues in crops to minimize yield losses.

MATERIALS AND METHODS

Plant material and plant growth conditions

Plants of *Arabidopsis thaliana* Col-0 (At^{WT}) *phyA-501* (in Col-0 background), *phyB-1*, *phyB-4* (both phyB deficient lines are in L*er* background), and *Cardamine hirsuta*, of the reference Oxford (Ox) accession (Ch^{WT}), have been described (Hay *et al.*, 2014; Martinez-Garcia *et al.*, 2014; Reed *et al.*, 1993). Plant growth conditions have been described elsewhere (Gallemi *et al.*, 2016; Martinez-Garcia *et al.*, 2014). Normal light conditions refer to W produced by cool-white vertical fluorescent tubes (PAR of 20-24 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Low and high light conditions corresponded to PAR values of 4 and 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively. Shade treatments in seedlings were provided by enriching W (R:FR of 2.5) with different intensities of FR LEDs (730-nm peak; Philips Greenpower Research modules) to produce the indicated R:FR (0.091 to 0.021) without altering PAR. Light spectra are presented in Supplemental Figure 2. For estimating petiole and rachis length, rosette plants were grown under long day (LD, 16 h light, 8 h dark) photoperiods, in which W was generated by cool-white horizontal fluorescent tubes (PAR of ~100 $\mu mol\ m^{-2}\ s^{-1}$, R:FR of 3.0); for shade treatments, W was supplemented with FR (W+FR, PAR of ~100 $\mu mol\ m^{-2}\ s^{-1}$, R:FR of 0.05). Fluence rates were measured with a Spectrosense2 meter associated with a 4-channel sensor (Skye instruments Ltd., www.skyinstruments.com) which measures PAR (400-700 nm) and 10 nm windows in the blue (464-473 nm), R (664-673 nm) and FR (725-734 nm)

regions (Gallemi *et al.*, 2017b). Light spectra were generated using a Flame Model Spectrometer with Sony Detector (FLAME-S) (https://oceanoptics.com).

Hypocotyl, petiole and rachis measurements

For hypocotyl measurement, about 30 seeds of each genotype were germinated on the plates for observing the seedling phenotype and at least 20 seedlings were measured for quantification of hypocotyl length. All experiments were repeated at least three times with consistent results. Hypocotyl measurements from all the different experiments were averaged. For petiole measurement, about 30 seeds of each genotype were germinated under continuous W. One week later, 20 seedlings in a similar stage of development were transferred to individual pots and moved to a LD growth chamber (R:FR of 3.0). After one week, half of the rosette plants stayed under W and the other half were moved to a W+FR shelf (R:FR of 0.05). After one week of differential R:FR treatment, leaves were harvested and petiole was measured; in the case of complex leaves from *C. hirsuta*, rachises were measured, covering the distance from the base of the leaf until the base of the main leaflet (Supplemental Figure 3). At least 8 leaves were measured for quantification of petiole and rachis length for each leaf number. Experiments were repeated four times with consistent results. Petiole and rachis measurements from all four experiments were averaged.

Photosynthetic pigment quantification and chlorophyll fluorescence.

Whole 7-day-old seedlings of the indicated genotypes and grown under W or W+FR (Figures 1 and 4) or transferred to HL conditions (Supplemental Figure 1B) were harvested, ground in liquid nitrogen, and the resulting powder was used for quantification of chlorophylls and carotenoids spectrophotometrically or by HPLC, as described (Bou-Torrent *et al.*, 2015).

Fluorescence measurements were carried out on seedlings grown under different light regimes using a MAXI-PAM fluorometer (Heinz Walz GmbH). For every measurement the whole cotyledons of 7 seedlings were considered. Maximum quantum yield of photosystem II (PSII), Fv/Fm, was calculated as (Fm-Fo)/Fm, where Fm and Fo are respectively the maximum and the minimum fluorescence of dark-adapted samples. For dark acclimation, plates were

incubated for at least 30 minutes in darkness to allow the full relaxation of photosystems. Rapid light curves (RLCs) were constructed with 10 incremental steps of actinic irradiance (E; 0, 1, 21, 56, 111, 186, 281, 396, 531, 701 µmol photons m^{-2} s^{-1}). For each step, the effective quantum yield of PSII (ΔF/Fm') was monitored every min and relative electron transport rate (rETR) was calculated as E×ΔF/Fm'. The light response was characterized by fitting iteratively, using MS Excel Solver, the model of Platt (1980) to rETR versus E curves. The fit was very good in all the cases (r>0.98).

Expression analyses by RT-qPCR and RNA-seq

RNA was extracted from whole seedlings of *A. thaliana* and *C. hirsuta* (grown as detailed in each experiment, three biological replicates per time point, each biological replicate composed of 30-40 seedlings) using commercial kits (RNAeasy Plant Mini kit; Qiagen; www.qiagen.com; or the semi-automatic Maxwell SimplyRNA kit; Promega; www.promega.com). For real-time qPCR analysis, two micrograms of RNA were reverse-transcribed using the M-MLV Reverse Transcriptase (Invitrogen, www.lifetechnologies.com) or Transcriptor First Strand cDNA synthesis (Roche, lifescience.roche.com). Reference genes used were *UBQ10*, *EF1α*, *SPC25* or/and *YLS8*.

For RNA-seq analyses, quantification of gene expression was performed as indicated elsewhere (Gan *et al.*, 2016) and detailed as Supplemental information. From the lists of genes, we selected as differentially expressed those whose fold change was significantly (p adjusted < 0.05) and higher than 1.5 (Supplemental Data Sets 1 and 3) or lower than 0.67 (Supplemental Data Sets 2 and 4) in seedlings treated for 1 h with W+FR compared to those grown under W in either *C. hirsuta* (Supplemental Data Sets 1 and 2) or *A. thaliana* (Supplemental Data Sets 3 and 4).

Gene Ontology (GO) and MapMan analysis

A strict synteny based approach was used to identify conserved orthologs between the two species. The *A. thaliana* orthologs of the *C. hirsuta* genes were used for getting the GO term annotations and MapMan-Bins. The GO term annotations for *A. thaliana* genes, used as a reference, were obtained from The Gene Ontology Consortium (http://www.geneontology.org/) (Ashburner *et al.*,

2000). The results are presented as Supplemental Data Set 5. For the MapMan-Bin analyses, each list of genes were submitted to the “Mercator” gene function prediction pipeline (Lohse *et al.*, 2014), that annotates the query genes with the hierarchical ontology MapMan-Bins (Klie and Nikoloski, 2012; Thimm *et al.*, 2004). Based on these MMB annotations, exact Fischer tests for function enrichment within the six groups of differentially expressed genes were carried out and interpreted (Supplemental Data Set 6).

Protein extraction and immunoblot analysis

Methods for extracting and detecting phyA protein levels in *A. thaliana* or *C. hirsuta* seedlings (Gallemi *et al.*, 2017b; Martinez-Garcia *et al.*, 1999) are detailed as follows. Protein extracts from *C. hirsuta* seedlings analyzed in Figure 3 and Supplemental Figure 6 were prepared following the direct extract protocol (Martinez-Garcia *et al.*, 1999) with the modifications described below. Extracts were prepared from Ch^{WT}, *sis1* and RNAi-ChPHYA seedlings germinated and grown in the dark for 4 days. Ten seedlings per genotype were harvested in the dark and extracted in 1.5 mL microfuge tubes containing 300 µL of Laemmli buffer supplemented with protease inhibitors (10 µg/mL Aprotinin, 1 µg/mL E-64, 10 µg/mL Leupeptin, 1 µg/mL Pepstatin A, 100 µM PMSF). These extracts were prepared in duplicate and similar results were observed. Plant material was ground using disposable grinders in the Eppendorf tube at room temperature until the mixture was homogeneous (usually less than 15 s). Once all the samples were prepared, tubes were placed in boiling water for 3 minutes. Tubes were centrifuged in a microfuge at maximum speed (13000 g, 10 min) immediately before loading. Fifteen µL of each extract, equivalent to about 0.5 seedlings, were loaded per lane in an SDS - 8% PAGE.

Protein extracts analyzed in Figure 5 were prepared from At^{WT} and Ch^{WT} seedlings grown as indicated in the figure legend. Extracts were obtained from four biological replicates. Protein extracts analyzed in Figure 6 were prepared from At^{WT}, *phyA-501*, *phyA>AtPHYA* and *phyA>ChPHYA* seedlings germinated and grown in the dark for 4 days, as described (Gallemi *et al.*, 2017a). Extracts were obtained from three biological replicates. Each biological replicate was obtained from about 100 seedlings. Protein concentration in these extracts was

696 determined using the Pierce BCA Protein Assay kit (Cat no. 23225;
697 www.thermofisher.com). Five or 7.5 µg of each extract were loaded per lane in
698 an SDS - 8% PAGE.
699 Immunoblot analyses of PHYA and TUB were performed at the same
700 time with the antibodies (073D, commercial anti-TUB) and dilutions indicated
701 elsewhere (Martinez-Garcia *et al.*, 2014). Anti-mouse horseradish peroxidase-
702 conjugated antibody (www.promega.com) was used as a secondary antibody.
703 ECL or ECL-plus chemiluminescence kits (www3.gehealthcare.com) were used
704 for detection. Signal was visualized and quantified using the ChemiDoc Touch
705 Imaging System (www.bio-red.com).
706
707 **Hormone analyses**
708 Hormone extraction and analysis were carried out as described (Durgbanshi *et*
709 *al.*, 2005) with a few modifications. Briefly, 0.02 g of dry tissue (about 150 At^{WT}
710 seedlings and 100 Ch^{WT} seedlings) was extracted in 1 mL of ultrapure water
711 after spiking with 50 ng of [$^2H_2$]-IAA, in a ball mill (MillMix20, Domel, Železniki,
712 Slovenija). After centrifugation at 4000 g at 4ºC for 10 min, supernatants were
713 recovered and pH adjusted to 3 with 30% acetic acid. The water extract was
714 partitioned twice against 2 mL of diethyl ether and the organic layer recovered
715 and evaporated under vacuum in a centrifuge concentrator (Speed Vac, Jouan,
716 Saint Herblain Cedex, France). Once dried, the residue was resuspended in a
717 10:90 MeOH:H_2O solution by gentle sonication. The resulting solution was
718 filtered through 0.22 µm polytetrafluoroethylene membrane syringe filters (Albet
719 S.A., Barcelona, Spain) and directly injected into an ultra performance LC
720 system (Acquity SDS, Waters Corp., Milford, MA, USA). Chromatographic
721 separations were carried out on a reversed-phase C18 column (Gravity, 50 ×
722 2.1 mm, 1.8-µm particle size, Macherey-Nagel GmbH, Germany) using a
723 MeOH:H_2O (both supplemented with 0.1% acetic acid) gradient at a flow rate of
724 300 µL min^{-1}. IAA was quantified with a TQS triple quadrupole mass
725 spectrometer (Micromass, Manchester, UK) connected online to the output of
726 the column though an orthogonal Z-spray electrospray ion source.
727
728 **Data availability**

The Illumina RNA-seq reads are available from the website http://chi.mpipz.mpg.de/assembly. Source code of BAMLINK is available at http://chi.mpipz.mpg.de/software. The data that support the findings of this study are also available from the corresponding author on request.

Accession Numbers

Sequence data from this article can be found in the Arabidopsis Genome Initiative or the *C. hirsuta* (http://chi.mpipz.mpg.de/assembly) databases under the following accession numbers: *AtATHB2* (At4g16780), *ChATHB2* (CARHR223400), *AtPIL1* (At2g46970), *ChPIL1* (CARHR142340), *AtUBQ10* (At4g05320), *AtPHYA* (At1g09570), *ChPHYA* (CARHR009540), *ACS8* (At4g37770), *GH3.3* (At2g23170), *IAA19* (At3g15540), *IAA29* (At3g15540), *AtEF1α* (At5g60390), *ChEF1α* (CARHR274060 and CARHR274080), *SPC25* (At2g39960), *ChSPC25* (CARHR134880 and CARHR134890), *YLS8* (At5g08290) and *ChYLS8* (CARHR204840).

Supplemental Data

Supplemental Figure 1. Photosynthetic-related responses of *A. thaliana* and *C. hirsuta* seedlings to changing light conditions.

Supplemental Figure 2. Light spectra of the treatments used in this study.

Supplemental Figure 3. Longitudinal length of *A. thaliana* and *C. hirsuta* leaves respond differently to simulated shade.

Supplemental Figure 4. *A. thaliana* and *C. hirsuta* seedlings change gene expression differently in response to simulated shade.

Supplemental Figure 5. The expression of a set of shade-induced but auxin-dependent genes, identified in *A. thaliana*, is also shade-induced in *C. hirsuta*.

Supplemental Figure 6. Reduction of phyA activity in *C. hirsuta* seedlings results in a *sis* phenotype.

Supplemental Figure 7. Partial alignment of *ChPHYA/AtPHYA*, *ChEF1*α/*AtEF1*α, *ChSPC25*/*AtSPC25* and *ChYLS8*/*AtYLS8* sequences.

Supplemental Figure 8. Strategies to compare biological activity between AtphyA and ChphyA in transgenic lines.

Supplemental Figure 9. The expression of a set of shade-induced phyA-repressed genes, identified in *A. thaliana*, is attenuated in *C. hirsuta*.

Supplemental Figure 10. Alignment of *C. hirsuta* and *A. thaliana* phyA amino acid sequences.

Supplemental Table 1. RPKM of eight genes commonly used for normalizing in RT-qPCR analyses.

Supplemental Table 2. Primers used in this work.

Supplemental Data Set 1. Bioset of up-regulated genes in *C. hirsuta* seedlings in response to simulated shade.

Supplemental Data Set 2. Bioset of down-regulated genes in C. hirsuta seedlings in response to simulated shade.

Supplemental Data Set 3. Bioset of up-regulated genes in A. thaliana seedlings in response to simulated shade.

Supplemental Data Set 4. Bioset of down-regulated genes in A. thaliana seedlings in response to simulated shade.

Supplemental Data Set 5. Results of Venn Diagrams of the GO categorization.

Supplemental Data Set 6. Functional enrichment groups based on the MapMan-Bin analyses.

Supplemental Data Set 7. Bioset of shade-regulated OMCL groups in *Geranium pyrenaicum* petioles in response to simulated shade.

Supplemental Data Set 8. Bioset of shade-regulated OMCL groups in *Geranium robertianum* petioles in response to simulated shade.

Supplemental Data Set 9. Summary of statistical tests.

ACKNOWLEDGEMENTS

We thank Ester Botterweg and Mª Rosa Rodríguez (CRAG) for their technical support; Victor González and Martí Bernardo (Bioinformatics Core unit, CRAG) for help in statistical analyses; Peter Quail (PGEC, Albany, CA, USA) for providing the anti-phyA antibody; Fernando Valladares (National Museum of Natural History, Madrid, Spain) for discussions about the shade habit of *A. thaliana* and *C. hirsuta*; and to Charlotte Gommers (CRAG) for comments on the manuscript. MJM-C, SP and LC received predoctoral fellowships from the Spanish *Ministerio de Economía y Competitividad* (MINECO, FPI program), the *Agència d'Ajuts Universitaris i de Recerca* (AGAUR - Generalitat de Catalunya, FI program) and *La Caixa Foundation* (INPhINIT fellowship LCF/BQ/IN18/11660004), respectively. JM-R received a International CRAG "Severo Ochoa" postdoctoral program fellowship and a postdoctoral contract (H2020-MSCA-IF-2017 – Proposal 797473) funded by the European Commission. CT received a Marie Curie postdoctoral contract (FP7-PEOPLE-IEF-2008 – Proposal 237492) funded by the European Commission and a CRAG short-term fellowship. Our research is supported by grants from BBSRC (BB/H006974/1) and Max Planck Society (core grant) to MT, and from MINECO-FEDER (BIO2017-85316-R, and BIO2017-84041-P) and AGAUR (2017-SGR1211, 2017-SGR710 and Xarba) to JFM-G and MRC. We also acknowledge the support of the MINECO for the "Centro de Excelencia Severo Ochoa 2016-2019" award SEV-2015-0533 and by the CERCA Programme / Generalitat de Catalunya.

AUTHOR CONTRIBUTIONS

JFM-G conceived the original research plan, and directed and coordinated the study. AH, HJ, XG and MT performed RNAseq and analyzed the data. AG-C analyzed auxin levels; LM and MR-C measured and analyzed photosynthetic parameters and pigment levels; MJM-C, SP, CT, JM-R, PP-A and IR-V performed all the other experiments. All authors analyzed their data and discussed the results. JFM-G wrote the paper with revisions of MR-C and contributions or/and comments of all other authors.

COMPETING INTERESTS

The authors declare no competing interests.

FIGURE LEGENDS

Figure 1. *A. thaliana* and *C. hirsuta* differ in the hypocotyl elongation response to neighboring vegetation. **(A)** Phenotype of representative seedlings of wild-type *A. thaliana* (At^{WT}) and *C. hirsuta* (Ch^{WT}) after 3 days grown in W and retained in W (left panels) or transferred to W+FR (R:FR of 0.02; right panels) until day 7 (d7). Scale bar, 5 mm. **(B)** Hypocotyl length of d7 At^{WT} and Ch^{WT} seedlings grown for the last 4 days under the indicated R:FR. **(C)** Hypocotyl length of d4 At^{WT} and Ch^{WT} seedlings grown in darkness. **(D)** Hypocotyl length of d7 At^{WT} and Ch^{WT} seedlings grown under W in media supplemented with increasing concentrations of picloram (PIC). **(E)** Petiole and rachis length of 3-week-old leaves of At^{WT} and Ch^{WT} plants grown for the last 7 days under the indicated R:FR. **(F)** Carotenoid (CRT) and chlorophyll (CHL) levels of At^{WT} and Ch^{WT} seedlings grown in W and W+FR (as detailed in **A**). Values are means and s.e.m. of three to five independent samples. Asterisks indicate significant differences (**$p<0.01$) relative to W-grown plants.

Figure 2. *A. thaliana* and *C. hirsuta* seedlings respond to neighboring vegetation by altering gene expression. (A) RNA-seq was performed with RNA extracted from At^{WT} and Ch^{WT} seedlings that were grown in W for 7 days (d7) and then treated for 1 h with W+FR (R:FR = 0.02). White circles indicate the moment of harvesting for RNA extraction. Three independent biological replicates were used for each genotype and treatment. **(B)** Correlation between

log-transformed fold-change of 432 DEGs in At^{WT} and Ch^{WT}. The estimated regression equation is shown at the top of the graph. **(C)** IAA content in At^{WT} and Ch^{WT} seedlings grown and harvested as indicated in **A**. Whole seedlings were collected and lyophilized to measure IAA levels. Data are presented as the means and s.e.m. of three (At^{WT}) or four (Ch^{WT}) biological replicates. DW, dry weight. **(D)** Effect of W+FR treatment on *PIL1* and *ATHB2* expression in At^{WT} and Ch^{WT} seedlings (R:FR = 0.02). W-grown d7 seedlings of Col-0 and Ox were treated for 0, 1, 4 and 8 h with W+FR. Transcript abundance, normalized to EF1α is shown. Values are means and s.e.m. of three independent RT-qPCR biological replicates relative to values at 0 h for each species. In **C** and **D**, asterisks indicate significant differences (**p<0.01, *p<0.05) relative to 0 h samples.

Figure 3. Mutant *sis1* seedlings of *C. hirsuta* are deficient in phyA activity. **(A)** Phenotype of representative seedlings of Ch^{WT}, *sis1-1* and *sis1-2* after 3 days grown in W and retained in W (white panels) or transferred to W+FR (R:FR of 0.02; pink panels) until day 7 (d7). All panels are to the same scale. **(B)** Hypocotyl length of At^{WT}, *phyA-501* (*A. thaliana*), Ch^{WT}, *sis1-1* and *sis1-2* (*C. hirsuta*) lines grown for 4 days in darkness (Dark) or under monochromatic FR (2.6 $\mu mol \cdot m^{-2} \cdot s^{-1}$), R (38.9 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and blue (B, 1.9 $\mu mol \cdot m^{-2} \cdot s^{-1}$) light. **(C)** Hypocotyl length of *A. thaliana* L*er*, *phyB-4* and *phyB-1* seedlings grown for 4 days in darkness (Dark) or under monochromatic R (40.6 $\mu mol \cdot m^{-2} \cdot s^{-1}$) light. **(D)** Hypocotyl length of *A. thaliana* L*er*, *phyB-4* and *phyB-1* seedlings under the indicated R:FR. Seedlings were grown for 2 days in W (R:FR > 2.5) and then kept in W (R:FR > 2.5) or transferred to W+FR (R:FR of 0.06 or 0.02) until day 7 (d7). **(E)** Schematic diagram of the lesions found in the *ChPHYA* gene in the *sis1-1* and *sis1-2* alleles compared to the wild-type sequence (Ch^{WT}) and the predicted changes in the amino acid sequence. **(F)** Immunoblot detection of phyA and tubulin with mouse monoclonal anti-phyA (073D) and anti-TUB antibodies in extracts of etiolated seedlings of Ch^{WT}, *sis1-1* and *sis1-2* lines.

Figure 4. *C. hirsuta sis1* seedlings are impaired in their tolerance to plant proximity. **(A)** Effect of W+FR treatment on *PIL1* and *ATHB2* expression in Ch^{WT} *sis1-1*- and *sis1-2* seedlings. Seedlings were grown as in Figure 2D.

Transcript abundance, normalized to EF1α is shown. Values are means and s.e.m. of three independent RT-qPCR biological replicates relative to values at 0 h for each genotype. Asterisks indicate significant differences (**p<0.01) relative to 0 h samples. **(B)** Carotenoid (CRT) and chlorophyll (CHL) levels of At^{WT} and phyA-501 *A. thaliana* and Ch^{WT}, *sis1-1* and *sis1-2 C. hirsuta* seedlings grown in W and W+FR (as detailed in Figure 1A). Values are means and s.e.m. of five independent samples. Asterisks indicate significant differences (**p<0.01) relative to W-grown plants. **(C,D)** Hypocotyl length of d7 At^{WT}, *phyA-501* (*A. thaliana*) **(C)** and Ch^{WT}, *sis1-1*, *sis1-2* (*C. hirsuta*) **(D)** seedlings grown for the last 4 days under the indicated R:FR. Asterisks indicate significant differences (*p<0.05, **p<0.01) relative to the corresponding wild-type plant grown under the same R:FR. In **D**, asterisks apply for both *sis1* mutants.

Figure 5. *C. hirsuta* seedlings have higher phyA levels than those of *A. thaliana*. (A) Cartoon showing the design of the experiment. Wild-type seedlings of *A. thaliana* (At^{WT}) and *C. hirsuta* (Ch^{WT}), grown as in Figure 1A, were harvested at the indicated times of W or W+FR treatments (asterisks) for RNA extraction. **(B)** Evolution of *PHYA* transcript levels in *A. thaliana* and *C. hirsuta* wild-type seedlings grown as detailed in **A**. Primers used (Supplemental Figure 8A) allow quantifying and comparing expression levels by RT-qPCR between both species. *PHYA* transcript abundance was normalized to three reference genes (*EF1α*, *SPC25* and *YLS8*). Values are means and s.e.m. of three independent RT-qPCR biological replicates relative to *PHYA* transcript levels of d3 *A. thaliana* seedlings. Two-way ANOVA showed that *PHYA* levels are significantly different (**p<0.01) between species under either W or W+FR. **(C)** Immunoblot detection of phyA and tubulin with the antibodies indicated in Figure 3C in extracts of At^{WT} and Ch^{WT} seedlings grown as detailed at the top of the section: 5-day-old etiolated seedlings were exposed to W light and material was harvested before and after 6 h of W-exposure (arrows). **(D)** Evolution of relative phyA protein levels (PHYA:TUB) in At^{WT} and Ch^{WT} seedlings exposed to simulated shade, as detailed at the top of the section: 5-day-old etiolated seedlings were exposed to W+FR light and material was harvested before and after 6, 8 and 10 h of simulated shade exposure (arrows). Values are means and s.e.m. of four independent biological replicates relative to PHYA:TUB levels

of etiolated At^{WT} seedlings. Two way ANOVA showed that relative PHYA levels under W+FR are significantly increased (**p<0.01) in *C. hirsuta* than *A. thaliana*.

Figure 6. ChphyA has a stronger activity than AtphyA in repressing shade-induced hypocotyl elongation. (A) Cartoon detailing the constructs used to complement *A. thaliana phyA-501* mutant plants. **(B)** Relative PHYA:TUB in etiolated seedlings of At^{WT}, *phyA-501*, and selected *phyA>AtPHYA* (blue bars) and *phyA>ChPHYA* (red bars) complementation lines. Seedlings were grown as indicated in Supplemental Figure 8. Values are means and s.e.m. of four independent biological replicates relative to PHYA:TUB levels of etiolated At^{WT} seedlings. **(C)** Cartoon illustrating how phyA activity in simulated shade was established as differences in hypocotyl length between simulated shade- and the W-grown seedlings (Hyp_{W+FR}-Hyp_W). Seedlings were grown for 2 days under W then for 5 additional days under W or W+FR (R:FR = 0.02), when hypocotyls were measured. **(D)** Hyp_{W+FR}-Hyp_W in seedlings of At^{WT}, *phyA-501*, and selected *phyA>AtPHYA* (blue bars) and *phyA>ChPHYA* (red bars) complementation lines. **(E)** Cartoon illustrating how phyA activity in de-etiolation was established as differences in hypocotyl length between dark- and FR-grown seedlings (Hyp_D-Hyp_{FR}). Seedlings were grown as indicated in Figure 3B. **(F)** Hyp_D-Hyp_{FR} in seedlings of At^{WT}, *phyA-501*, and selected *phyA>AtPHYA* (blue bars) and *phyA>ChPHYA* (red bars) complementation lines. In **C** and **E**, mutant *phyA-501* seedlings have no phyA activity.

Figure 7. ChphyA has a stronger activity than AtphyA in repressing shade-induced expression of *ACS8*, *GH3.3*, *IAA19* and *IAA29* genes. (A) Effect of phyA in the shade-induced expression of *ACS8*, *GH3.3*, *IAA19* and *IAA29*. W-grown d5 seedlings of At^{WT} and *phyA-501* were treated for 0, 1, 8 and 24 h with W+FR (R:FR = 0.02), when material was harvested for RNA extraction, as indicated at the top of the panel. Transcript abundance, normalized to EF1α is shown. Values are means and s.e.m. of three independent RT-qPCR biological replicates relative to values at 0 h for At^{WT}. Asterisks indicate significant differences (**p<0.01, *p<0.05) between *phyA-501* and At^{WT} seedlings exposed for the same time to W+FR. **(B)** Correlation

between *ACS8*, *GH3.3*, *IAA19* and *IAA29* expression and relative levels of PHYA protein in the seedlings of At^{WT}, *phyA-501*, *phyA>AtPHYA* (blue lines and dots) and *phyA>ChPHYA* (red lines and dots) complementation lines. Gene expression was quantified in W-grown d5 seedlings exposed to W+FR (R:FR=0.02) during 24 h, as indicated at the top of the panel. Transcript abundance was normalized to *EF1*α. Relative phyA protein levels (PHYA:TUB, data already shown in Supplemental Figure 8) were estimated in etiolated seedlings. Values are means and s.e.m. of three independent RT-qPCR biological replicates relative to values of At^{WT}. The estimated regression lines for the *phyA>AtPHYA* (blue line) and *phyA>ChPHYA* (red line) complementation lines are shown for each correlation.

Figure 8. Model of how increased phyA activity in *C. hirsuta* might implement the shade tolerance of hypocotyl elongation. Increases in phyA activity caused by the constitutive overexpression of *PHYA* also attenuate the shade-induced hypocotyl elongation in transgenic plants, and it results in partially tolerant *A. thaliana* seedlings.

REFERENCES

Ashburner, M., Ball, C.A., Blake, J.A., Botstein, D., Butler, H., Cherry, J.M., Davis, A.P., Dolinski, K., Dwight, S.S., Eppig, J.T., Harris, M.A., Hill, D.P., Issel-Tarver, L., Kasarskis, A., Lewis, S., Matese, J.C., Richardson, J.E., Ringwald, M., Rubin, G.M. and Sherlock, G. (2000) Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat Genet*, **25**, 25-29.

Barkoulas, M., Hay, A., Kougioumoutzi, E. and Tsiantis, M. (2008) A developmental framework for dissected leaf formation in the Arabidopsis relative Cardamine hirsuta. *Nat Genet*, **40**, 1136-1141.

Bealey, C.E. and Robertson, P.A. (1992) Coppice management for pheasants. In *Ecology and Management of Coppice Woodlands* (Buckley, G.P. ed: Springer-Science+Business Media, B.V., pp. 193-210.

Benkov, M.A., Yatsenko, A.M. and Tikhonov, A.N. (2019) Light acclimation of shade-tolerant and sun-resistant Tradescantia species: photochemical activity of PSII and its sensitivity to heat treatment. *Photosynth Res*, **139**, 203-214.

Bou-Torrent, J., Galstyan, A., Gallemi, M., Cifuentes-Esquivel, N., Molina-Contreras, M.J., Salla-Martret, M., Jikumaru, Y., Yamaguchi, S., Kamiya, Y. and Martinez-Garcia, J.F. (2014) Plant proximity perception

dynamically modulates hormone levels and sensitivity in Arabidopsis. *J Exp Bot*, **65**, 2937-2947.

Bou-Torrent, J., Toledo-Ortiz, G., Ortiz-Alcaide, M., Cifuentes-Esquivel, N., Halliday, K.J., Martinez-Garcia, J.F. and Rodriguez-Concepcion, M. (2015) Regulation of Carotenoid Biosynthesis by Shade Relies on Specific Subsets of Antagonistic Transcription Factors and Cofactors. *Plant Physiol*, **169**, 1584-1594.

Cagnola, J.I., Ploschuk, E., Benech-Arnold, T., Finlayson, S.A. and Casal, J.J. (2012) Stem transcriptome reveals mechanisms to reduce the energetic cost of shade-avoidance responses in tomato. *Plant Physiol*, **160**, 1110-1119.

Callahan, H.S., Pigliucci, M. and Schlichting, C.D. (1997) Developmental phenotypic plasticity: where ecology and evolution meet molecular biology. *Bioessays*, **19**, 519-525.

Canton, F.R. and Quail, P.H. (1999) Both phyA and phyB mediate light-imposed repression of PHYA gene expression in Arabidopsis. *Plant Physiol*, **121**, 1207-1216.

Carroll, S.B. (2008) Evo-devo and an expanding evolutionary synthesis: a genetic theory of morphological evolution. *Cell*, **134**, 25-36.

Casal, J.J. (2012) Shade avoidance. *Arabidopsis Book*, **10**, e0157.

Casal, J.J., Candia, A.N. and Sellaro, R. (2014) Light perception and signalling by phytochrome A. *J Exp Bot*, **65**, 2835-2845.

Cifuentes-Esquivel, N., Bou-Torrent, J., Galstyan, A., Gallemi, M., Sessa, G., Salla Martret, M., Roig-Villanova, I., Ruberti, I. and Martinez-Garcia, J.F. (2013) The bHLH proteins BEE and BIM positively modulate the shade avoidance syndrome in Arabidopsis seedlings. *The Plant Journal*, **75**, 989-1002.

Ciolfi, A., Sessa, G., Sassi, M., Possenti, M., Salvucci, S., Carabelli, M., Morelli, G. and Ruberti, I. (2013) Dynamics of the shade-avoidance response in Arabidopsis. *Plant Physiol*, **163**, 331-353.

de Wit, M., Keuskamp, D.H., Bongers, F.J., Hornitschek, P., Gommers, C.M.M., Reinen, E., Martinez-Ceron, C., Fankhauser, C. and Pierik, R. (2016) Integration of Phytochrome and Cryptochrome Signals Determines Plant Growth during Competition for Light. *Curr Biol*, **26**, 3320-3326.

de Wit, M., Ljung, K. and 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.

Dieterle, M., Bauer, D., Buche, C., Krenz, M., Schafer, E. and Kretsch, T. (2005) A new type of mutation in phytochrome A causes enhanced light sensitivity and alters the degradation and subcellular partitioning of the photoreceptor. *Plant J*, **41**, 146-161.

Durgbanshi, A., Arbona, V., Pozo, O., Miersch, O., Sancho, J.V. and Gomez-Cadenas, A. (2005) Simultaneous determination of multiple phytohormones in plant extracts by liquid chromatography-electrospray tandem mass spectrometry. *J Agric Food Chem*, **53**, 8437-8442.

Gallemi, M., Galstyan, A., Paulisic, S., Then, C., Ferrandez-Ayela, A., Lorenzo-Orts, L., Roig-Villanova, I., Wang, X., Micol, J.L., Ponce, M.R., Devlin, P.F. and Martinez-Garcia, J.F. (2016) DRACULA2 is a

dynamic nucleoporin with a role in regulating the shade avoidance syndrome in Arabidopsis. *Development*, **143**, 1623-1631.

Gallemi, M., Molina-Contreras, M.J., Paulisic, S., Salla-Martret, M., Sorin, C., Godoy, M., Franco-Zorrilla, J.M., Solano, R. and Martinez-Garcia, J.F. (2017a) A non-DNA-binding activity for the ATHB4 transcription factor in the control of vegetation proximity. *New Phytol*.

Gallemi, M., Molina-Contreras, M.J., Paulisic, S., Salla-Martret, M., Sorin, C., Godoy, M., Franco-Zorrilla, J.M., Solano, R. and Martinez-Garcia, J.F. (2017b) A non-DNA-binding activity for the ATHB4 transcription factor in the control of vegetation proximity. *New Phytol*, **216**, 798-813.

Gan, X., Hay, A., Kwantes, M., Haberer, G., Hallab, A., Ioio, R.D., Hofhuis, H., Pieper, B., Cartolano, M., Neumann, U., Nikolov, L.A., Song, B., Hajheidari, M., Briskine, R., Kougioumoutzi, E., Vlad, D., Broholm, S., Hein, J., Meksem, K., Lightfoot, D., Shimizu, K.K., Shimizu-Inatsugi, R., Imprialou, M., Kudrna, D., Wing, R., Sato, S., Huijser, P., Filatov, D., Mayer, K.F., Mott, R. and Tsiantis, M. (2016) The Cardamine hirsuta genome offers insight into the evolution of morphological diversity. *Nat Plants*, **2**, 16167.

Genoud, T., Schweizer, F., Tscheuschler, A., Debrieux, D., Casal, J.J., Schafer, E., Hiltbrunner, A. and Fankhauser, C. (2008) FHY1 mediates nuclear import of the light-activated phytochrome A photoreceptor. *PLoS Genet*, **4**, e1000143.

Gommers, C.M., Keuskamp, D.H., Buti, S., van Veen, H., Koevoets, I.T., Reinen, E., Voesenek, L.A. and Pierik, R. (2017) Molecular Profiles of Contrasting Shade Response Strategies in Wild Plants: Differential Control of Immunity and Shoot Elongation. *Plant Cell*, **29**, 331-344.

Gommers, C.M., Visser, E.J., St Onge, K.R., Voesenek, L.A. and Pierik, R. (2013) Shade tolerance: when growing tall is not an option. *Trends Plant Sci*, **18**, 65-71.

Gommers, C.M.M., Buti, S., Tarkowska, D., Pencik, A., Banda, J.P., Arricastres, V. and Pierik, R. (2018) Organ-specific phytohormone synthesis in two Geranium species with antithetical responses to far-red light enrichment. *Plant Direct*, **2**, e00066.

Han, S., Jiang, J., Li, H., Song, A., Chen, S. and Chen, F. (2015) The differential response of two Chrysanthemum cultivars to shading: photosynthesis, chloroplast, and Sieve Element-companion cell ultrastructure. *HortScience*, **50**, 1192-1195.

Hay, A. and Tsiantis, M. (2016) Cardamine hirsuta: a comparative view. *Curr Opin Genet Dev*, **39**, 1-7.

Hay, A.S., Pieper, B., Cooke, E., Mandakova, T., Cartolano, M., Tattersall, A.D., Ioio, R.D., McGowan, S.J., Barkoulas, M., Galinha, C., Rast, M.I., Hofhuis, H., Then, C., Plieske, J., Ganal, M., Mott, R., Martinez-Garcia, J.F., Carine, M.A., Scotland, R.W., Gan, X., Filatov, D.A., Lysak, M.A. and Tsiantis, M. (2014) Cardamine hirsuta: a versatile genetic system for comparative studies. *Plant J*, **78**, 1-15.

Hersch, M., Lorrain, S., de Wit, M., Trevisan, M., Ljung, K., Bergmann, S. and Fankhauser, C. (2014) Light intensity modulates the regulatory network of the shade avoidance response in Arabidopsis. *Proc Natl Acad Sci U S A*, **111**, 6515-6520.

Heyer, A.G., Mozley, D., Landschutze, V., Thomas, B. and Gatz, C. (1995) Function of phytochrome A in potato plants as revealed through the study of transgenic plants. *Plant Physiol*, **109**, 53-61.

Hofhuis, H., Moulton, D., Lessinnes, T., Routier-Kierzkowska, A.L., Bomphrey, R.J., Mosca, G., Reinhardt, H., Sarchet, P., Gan, X., Tsiantis, M., Ventikos, Y., Walker, S., Goriely, A., Smith, R. and Hay, A. (2016) Morphomechanical Innovation Drives Explosive Seed Dispersal. *Cell*, **166**, 222-233.

Hornitschek, P., Kohnen, M.V., Lorrain, S., Rougemont, J., Ljung, K., Lopez-Vidriero, I., Franco-Zorrilla, J.M., Solano, R., Trevisan, M., Pradervand, S., Xenarios, I. and 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.

Hornitschek, P., Lorrain, S., Zoete, V., Michielin, O. and Fankhauser, C. (2009) Inhibition of the shade avoidance response by formation of non-DNA binding bHLH heterodimers. *EMBO J*, **28**, 3893-3902.

Karve, A.A., Jawdy, S.S., Gunter, L.E., Allen, S.M., Yang, X., Tuskan, G.A., Wullschleger, S.D. and Weston, D.J. (2012) Initial characterization of shade avoidance response suggests functional diversity between Populus phytochrome B genes. *New Phytol*, **196**, 726-737.

Kim, J.I., Shen, Y., Han, Y.J., Park, J.E., Kirchenbauer, D., Soh, M.S., Nagy, F., Schafer, E. and Song, P.S. (2004) Phytochrome phosphorylation modulates light signaling by influencing the protein-protein interaction. *Plant Cell*, **16**, 2629-2640.

Klie, S. and Nikoloski, Z. (2012) The Choice between MapMan and Gene Ontology for Automated Gene Function Prediction in Plant Science. *Front Genet*, **3**, 115.

Kohnen, M.V., Schmid-Siegert, E., Trevisan, M., Petrolati, L.A., Senechal, F., Muller-Moule, P., Maloof, J., Xenarios, I. and Fankhauser, C. (2016) Neighbor Detection Induces Organ-Specific Transcriptomes, Revealing Patterns Underlying Hypocotyl-Specific Growth. *Plant Cell*, **28**, 2889-2904.

Kozuka, T., Kobayashi, J., Horiguchi, G., Demura, T., Sakakibara, H., Tsukaya, H. and Nagatani, A. (2010) Involvement of auxin and brassinosteroid in the regulation of petiole elongation under the shade. *Plant Physiol*, **153**, 1608-1618.

Li, J., Li, G., Wang, H. and Deng, X.W. (2011) Phytochrome signaling mechanism. *Arabidopsis Book*, **9**, e0148.

Li, L., Ljung, K., Breton, G., Schmitz, R.J., Pruneda-Paz, J., Cowing-Zitron, C., Cole, B.J., Ivans, L.J., Pedmale, U.V., Jung, H.S., Ecker, J.R., Kay, S.A. and Chory, J. (2012) Linking photoreceptor excitation to changes in plant architecture. *Genes Dev*, **26**, 785-790.

Lohse, M., Nagel, A., Herter, T., May, P., Schroda, M., Zrenner, R., Tohge, T., Fernie, A.R., Stitt, M. and Usadel, B. (2014) Mercator: a fast and simple web server for genome scale functional annotation of plant sequence data. *Plant Cell Environ*, **37**, 1250-1258.

Martinez-Garcia, J.F., Galstyan, A., Salla-Martret, M., Cifuentes-Esquivel, N., Gallemí, M. and Bou-Torrent, J. (2010) Regulatory components of shade avoidance syndrome. *Advances in Botanical Research*, **53**, 65-116.

Martinez-Garcia, J.F., Gallemi, M., Molina-Contreras, M.J., Llorente, B., Bevilaqua, M.R. and Quail, P.H. (2014) The shade avoidance syndrome in Arabidopsis: the antagonistic role of phytochrome a and B differentiates vegetation proximity and canopy shade. *PLoS One*, **9**, e109275.

Martinez-Garcia, J.F., Monte, E. and Quail, P.H. (1999) A simple, rapid and quantitative method for preparing Arabidopsis protein extracts for immunoblot analysis. *Plant J*, **20**, 251-257.

Mathews, S. (2010) Evolutionary studies illuminate the structural-functional model of plant phytochromes. *Plant Cell*, **22**, 4-16.

Oka, Y., Ono, Y., Toledo-Ortiz, G., Kokaji, K., Matsui, M., Mochizuki, N. and Nagatani, A. (2012) Arabidopsis phytochrome a is modularly structured to integrate the multiple features that are required for a highly sensitized phytochrome. *Plant Cell*, **24**, 2949-2962.

Ortiz-Alcaide, M., Llamas, E., Gomez-Cadenas, A., Nagatani, A., Martinez-Garcia, J.F. and Rodriguez-Concepcion, M. (2019) Chloroplasts modulate elongation responses to canopy shade by retrograde pathways involving HY5 and ABA. *Plant Cell*.

Procko, C., Crenshaw, C.M., Ljung, K., Noel, J.P. and Chory, J. (2014) Cotyledon-Generated Auxin Is Required for Shade-Induced Hypocotyl Growth in Brassica rapa. *Plant Physiol*, **165**, 1285-1301.

Reed, J.W., Nagpal, P., Poole, D.S., Furuya, M. and Chory, J. (1993) Mutations in the gene for the red/far-red light receptor phytochrome B alter cell elongation and physiological responses throughout Arabidopsis development. *Plant Cell*, **5**, 147-157.

Robson, P.R., McCormac, A.C., Irvine, A.S. and Smith, H. (1996) Genetic engineering of harvest index in tobacco through overexpression of a phytochrome gene. *Nat Biotechnol*, **14**, 995-998.

Roig-Villanova, I., Bou-Torrent, J., Galstyan, A., Carretero-Paulet, L., Portoles, S., Rodriguez-Concepcion, M. and Martinez-Garcia, J.F. (2007) Interaction of shade avoidance and auxin responses: a role for two novel atypical bHLH proteins. *The EMBO Journal*, **26**, 4756-4767.

Roig-Villanova, I., Bou, J., Sorin, C., Devlin, P.F. and Martinez-Garcia, J.F. (2006) Identification of primary target genes of phytochrome signaling. Early transcriptional control during shade avoidance responses in Arabidopsis. *Plant Physiology*, **141**, 85-96.

Roig-Villanova, I. and Martinez-Garcia, J.F. (2016) Plant Responses to Vegetation Proximity: A Whole Life Avoiding Shade. *Front Plant Sci*, **7**, 236.

Sasidharan, R., Chinnappa, C.C., Staal, M., Elzenga, J.T., Yokoyama, R., Nishitani, K., Voesenek, L.A. and Pierik, R. (2010) Light quality-mediated petiole elongation in Arabidopsis during shade avoidance involves cell wall modification by xyloglucan endotransglucosylase/hydrolases. *Plant Physiol*, **154**, 978-990.

Seo, H.S., Watanabe, E., Tokutomi, S., Nagatani, A. and Chua, N.H. (2004) Photoreceptor ubiquitination by COP1 E3 ligase desensitizes phytochrome A signaling. *Genes Dev*, **18**, 617-622.

Sheerin, D.J., Menon, C., zur Oven-Krockhaus, S., Enderle, B., Zhu, L., Johnen, P., Schleifenbaum, F., Stierhof, Y.D., Huq, E. and Hiltbrunner, A. (2015) Light-activated phytochrome A and B interact with

members of the SPA family to promote photomorphogenesis in Arabidopsis by reorganizing the COP1/SPA complex. *Plant Cell*, **27**, 189-201.

Smith, H. (1982) Light quality, photoperception, and plant strategy. *Annual Review of Plant Physiology*, **33**, 481-518.

Tao, Y., Ferrer, J.L., Ljung, K., Pojer, F., Hong, F., Long, J.A., Li, L., Moreno, J.E., Bowman, M.E., Ivans, L.J., Cheng, Y., Lim, J., Zhao, Y., Ballare, C.L., Sandberg, G., Noel, J.P. and Chory, J. (2008) Rapid synthesis of auxin via a new tryptophan-dependent pathway is required for shade avoidance in plants. *Cell*, **133**, 164-176.

Thimm, O., Blasing, O., Gibon, Y., Nagel, A., Meyer, S., Kruger, P., Selbig, J., Muller, L.A., Rhee, S.Y. and Stitt, M. (2004) MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. *Plant J*, **37**, 914-939.

Ueoka-Nakanishi, H., Hori, N., Ishida, K., Ono, N., Yamashino, T., Nakamichi, N. and Mizuno, T. (2011) Characterization of shade avoidance responses in Lotus japonicus. *Biosci Biotechnol Biochem*, **75**, 2148-2154.

Valladares, F. and Niinemets, U. (2008) Shade Tolerance, a Key Plant Feature of Complex Nature and Consequences. *Annual Review of Ecology, Evolution, and Systematics*, **39**, 237-257.

Vlad, D., Kierzkowski, D., Rast, M.I., Vuolo, F., Dello Ioio, R., Galinha, C., Gan, X., Hajheidari, M., Hay, A., Smith, R.S., Huijser, P., Bailey, C.D. and Tsiantis, M. (2014) Leaf shape evolution through duplication, regulatory diversification, and loss of a homeobox gene. *Science*, **343**, 780-783.

Vuolo, F., Mentink, R.A., Hajheidari, M., Bailey, C.D., Filatov, D.A. and Tsiantis, M. (2016) Coupled enhancer and coding sequence evolution of a homeobox gene shaped leaf diversity. *Genes Dev*, **30**, 2370-2375.

Yang, C., Xie, F., Jiang, Y., Li, Z., Huang, X. and Li, L. (2018) Phytochrome A Negatively Regulates the Shade Avoidance Response by Increasing Auxin/Indole Acidic Acid Protein Stability. *Dev Cell*, **44**, 29-41 e24.

Yanovsky, M.J., Casal, J.J. and Whitelam, G.C. (1995) Phytochrome A, phytochrome B and HY4 are invovled in hypocotyl growth responses to natural radiation in Arabidopsis: Weak de-etiolation of the phyA mutant under dense canopies. *Plant Cell Environ*, **18**, 788-794.

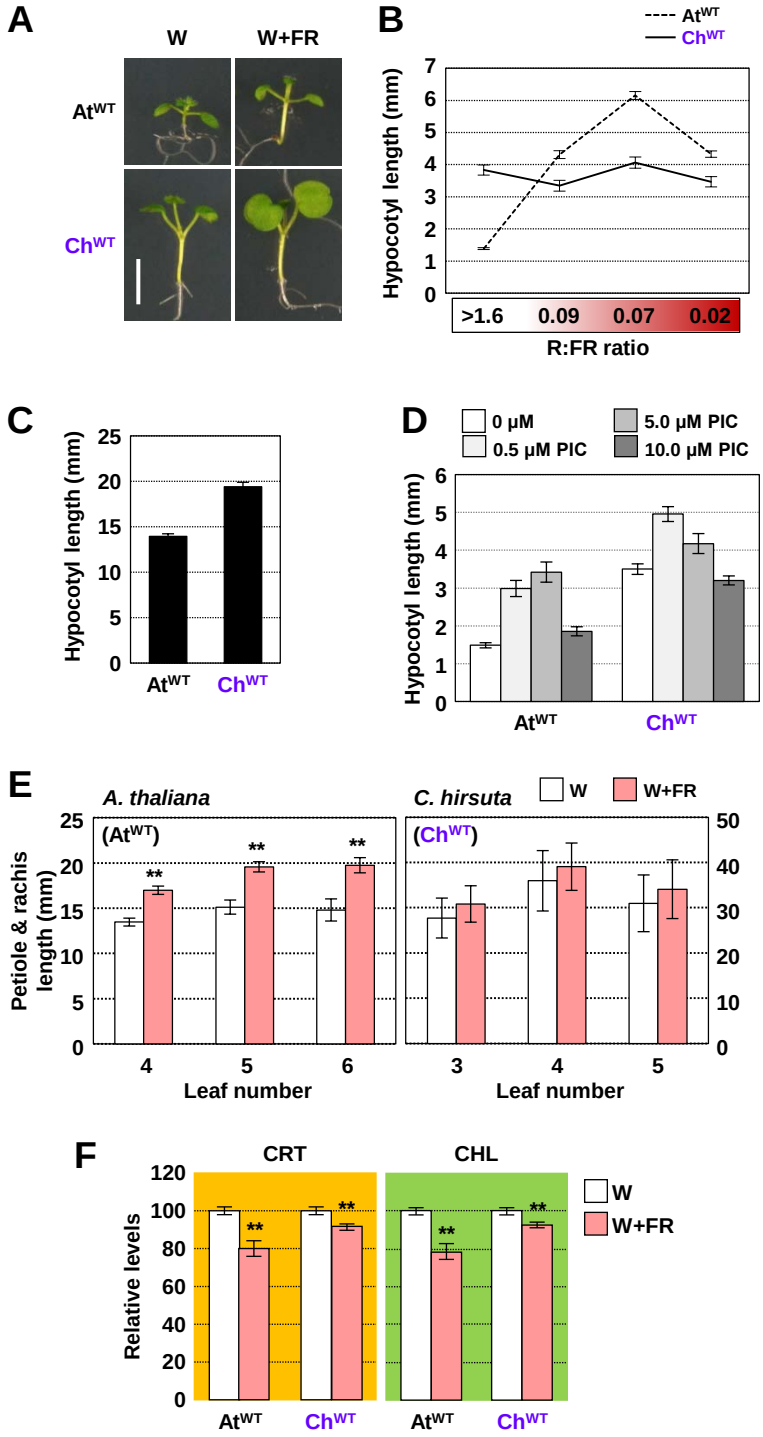

Figure 1. *A. thaliana* and *C. hirsuta* differ in the hypocotyl elongation response to neighboring vegetation. **(A)** Phenotype of representative seedlings of wild-type *A. thaliana* (At^{WT}) and *C. hirsuta* (Ch^{WT}) after 3 days grown in W and retained in W (left panels) or transferred to W+FR (R:FR of 0.02; right panels) until day 7 (d7). Scale bar, 5 mm. **(B)** Hypocotyl length of d7 At^{WT} and Ch^{WT} seedlings grown for the last 4 days under the indicated R:FR. **(C)** Hypocotyl length of d4 At^{WT} and Ch^{WT} seedlings grown in darkness. **(D)** Hypocotyl length of d7 At^{WT} and Ch^{WT} seedlings grown under W in media supplemented with increasing concentrations of picloram (PIC). **(E)** Petiole and rachis length of 3-week-old leaves of At^{WT} and Ch^{WT} plants grown for the last 7 days under the indicated R:FR. **(F)** Carotenoid (CRT) and chlorophyll (CHL) levels of At^{WT} and Ch^{WT} seedlings grown in W and W+FR (as detailed in **A**). Values are means and s.e.m. of three to five independent samples. Asterisks indicate significant differences (**p<0.01) relative to W-grown plants.

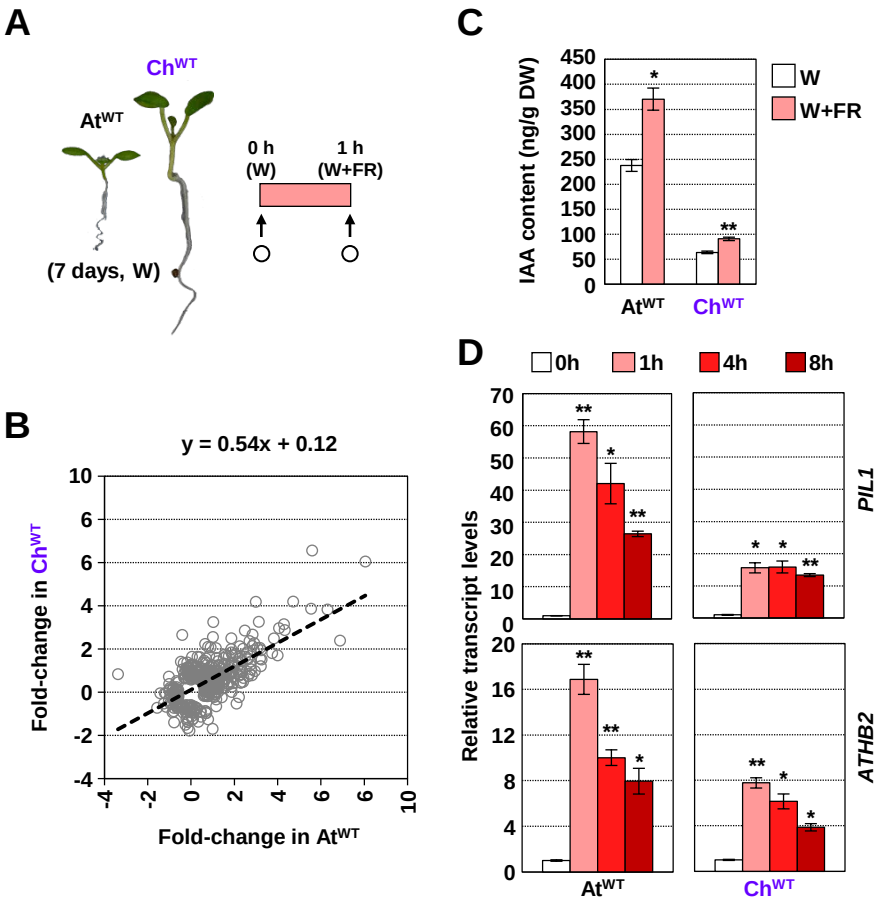

Figure 2. *A. thaliana* and *C. hirsuta* seedlings respond to neighboring vegetation by altering gene expression. (A) RNA-seq was performed with RNA extracted from At^{WT} and Ch^{WT} seedlings that were grown in W for 7 days (d7) and then treated for 1 h with W+FR (R:FR = 0.02). White circles indicate the moment of harvesting for RNA extraction. Three independent biological replicates were used for each genotype and treatment. **(B)** Correlation between log-transformed fold-change of 432 DEGs in At^{WT} and Ch^{WT}. The estimated regression equation is shown at the top of the graph. **(C)** IAA content in At^{WT} and Ch^{WT} seedlings grown and harvested as indicated in **A**. Whole seedlings were collected and lyophilized to measure IAA levels. Data are presented as the means and s.e.m. of three (At^{WT}) or four (Ch^{WT}) biological replicates. DW, dry weight. **(D)** Effect of W+FR treatment on *PIL1* and *ATHB2* expression in At^{WT} and Ch^{WT} seedlings (R:FR = 0.02). W-grown d7 seedlings of Col-0 and Ox were treated for 0, 1, 4 and 8 h with W+FR. Transcript abundance, normalized to EF1α is shown. Values are means and s.e.m. of three independent RT-qPCR biological replicates relative to values at 0 h for each species. In **C** and **D**, asterisks indicate significant differences (**p<0.01, *p<0.05) relative to 0 h samples.

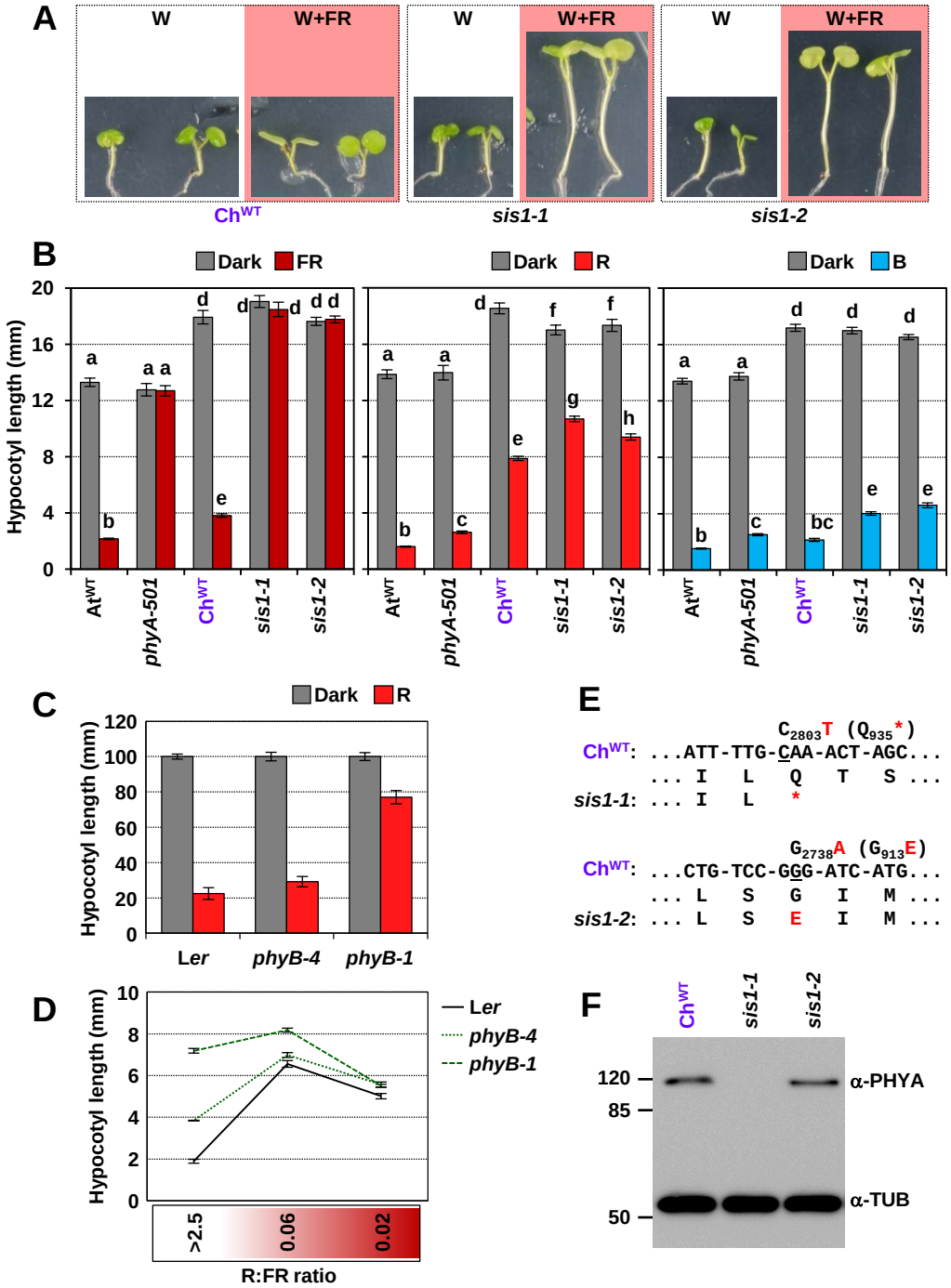

Figure 3. Mutant *sis1* seedlings of *C. hirsuta* are deficient in phyA activity. (A) Phenotype of representative seedlings of Ch^{WT}, *sis1-1* and *sis1-2* after 3 days grown in W and retained in W (white panels) or transferred to W+FR (R:FR of 0.02; pink panels) until day 7 (d7). All panels are to the same scale. **(B)** Hypocotyl length of At^{WT}, *phyA-501* (*A. thaliana*), Ch^{WT}, *sis1-1* and *sis1-2* (*C. hirsuta*) lines grown for 4 days in darkness (Dark) or under monochromatic FR (2.6 µmol·m^{-2}·s^{-1}), R (38.9 µmol·m^{-2}·s^{-1}) and blue (B, 1.9 µmol·m^{-2}·s^{-1}) light. **(C)** Hypocotyl length of *A. thaliana* Ler, *phyB-4* and *phyB-1* seedlings grown for 4 days in darkness (Dark) or under monochromatic R (40.6 µmol·m^{-2}·s^{-1}) light. **(D)** Hypocotyl length of *A. thaliana* Ler, *phyB-4* and *phyB-1* seedlings under the indicated R:FR. Seedlings were grown for 2 days in W (R:FR > 2.5) and then kept in W (R:FR > 2.5) or transferred to W+FR (R:FR of 0.06 or 0.02) until day 7 (d7). **(E)** Schematic diagram of the lesions found in the *ChPHYA* gene in the *sis1-1* and *sis1-2* alleles compared to the wild-type sequence (Ch^{WT}) and the predicted changes in the amino acid sequence. **(F)** Immunoblot detection of phyA and tubulin with mouse monoclonal anti-phyA (073D) and anti-TUB antibodies in extracts of etiolated seedlings of Ch^{WT}, *sis1-1* and *sis1-2* lines.

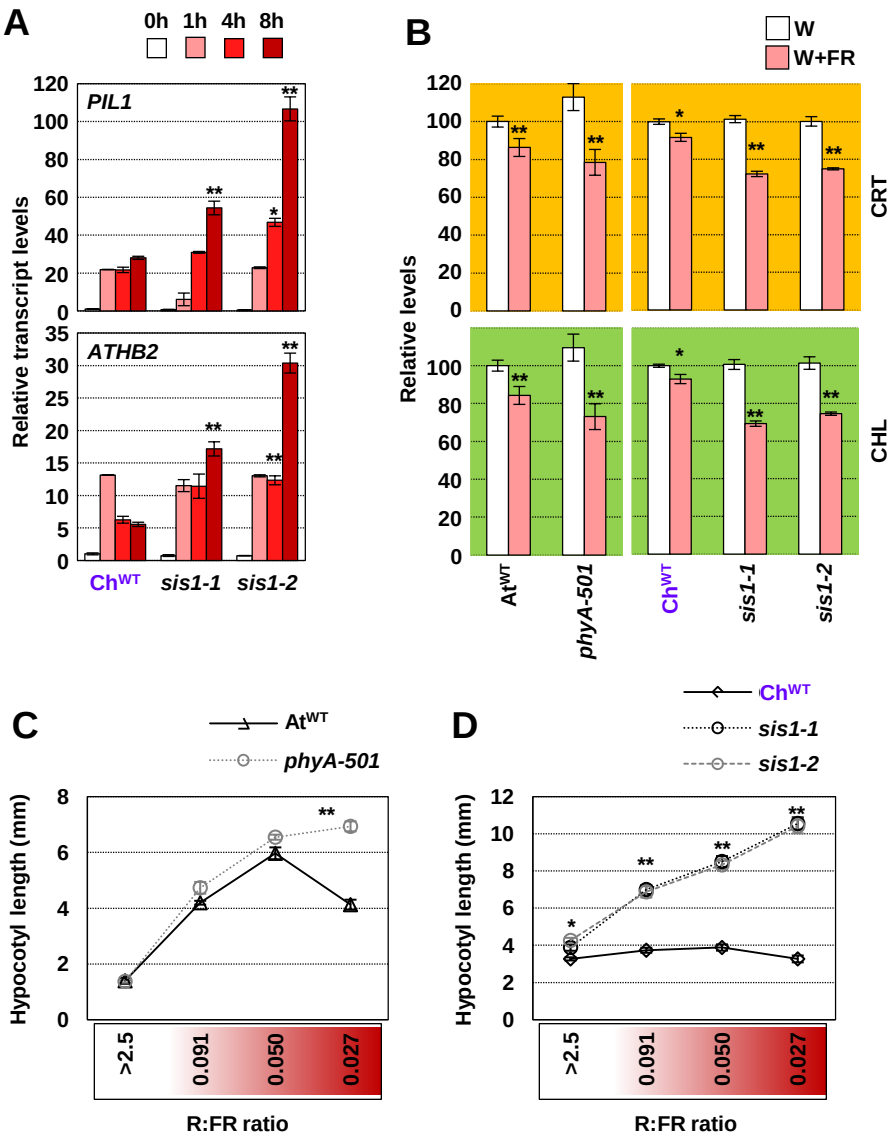

Figure 4. *C. hirsuta sis1* seedlings are impaired in their tolerance to plant proximity. (A) Effect of W+FR treatment on *PIL1* and *ATHB2* expression in Ch^{WT} *sis1-1*- and *sis1-2* seedlings. Seedlings were grown as in Figure 2D. Transcript abundance, normalized to EF1α is shown. Values are means and s.e.m. of three independent RT-qPCR biological replicates relative to values at 0 h for each genotype. Asterisks indicate significant differences (**p<0.01) relative to 0 h samples. **(B)** Carotenoid (CRT) and chlorophyll (CHL) levels of At^{WT} and phyA-501 *A. thaliana* and Ch^{WT}, *sis1-1* and *sis1-2 C. hirsuta* seedlings grown in W and W+FR (as detailed in Figure 1A). Values are means and s.e.m. of five independent samples. Asterisks indicate significant differences (**p<0.01) relative to W-grown plants. **(C,D)** Hypocotyl length of d7 At^{WT}, *phyA-501* (*A. thaliana*) **(C)** and Ch^{WT}, *sis1-1*, *sis1-2* (*C. hirsuta*) **(D)** seedlings grown for the last 4 days under the indicated R:FR. Asterisks indicate significant differences (*p<0.05, **p<0.01) relative to the corresponding wild-type plant grown under the same R:FR. In **D**, asterisks apply for both *sis1* mutants.

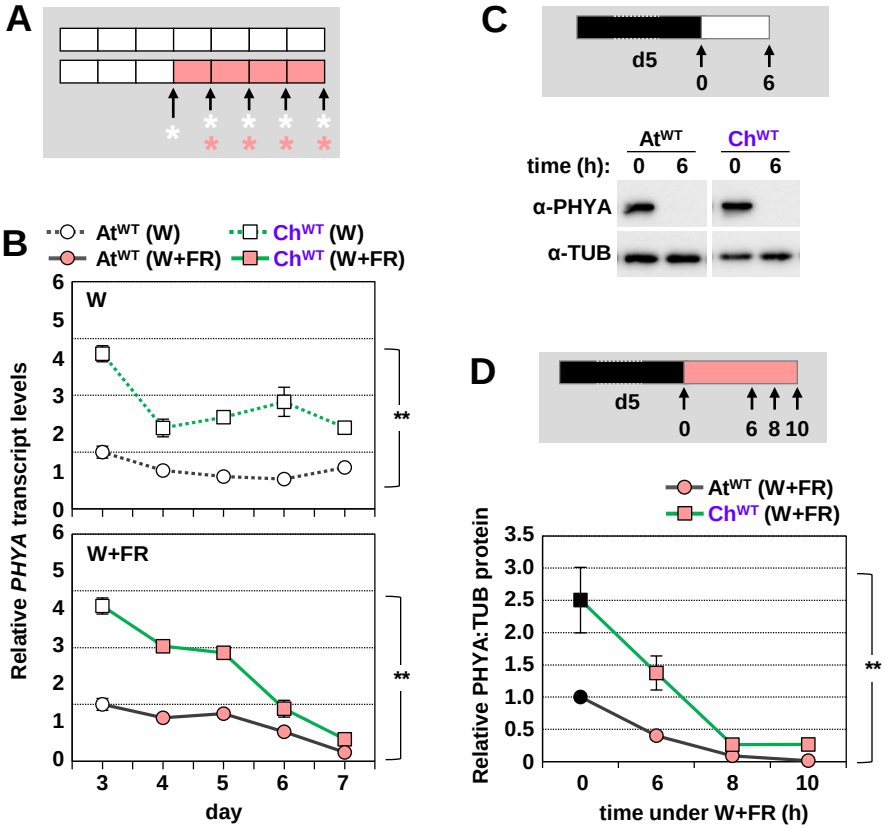

Figure 5. *C. hirsuta* seedlings have higher phyA levels than those of *A. thaliana*. (A) Cartoon showing the design of the experiment. Wild-type seedlings of *A. thaliana* (At^{WT}) and *C. hirsuta* (Ch^{WT}), grown as in Figure 1A, were harvested at the indicated times of W or W+FR treatments (asterisks) for RNA extraction. **(B)** Evolution of *PHYA* transcript levels in *A. thaliana* and *C. hirsuta* wild-type seedlings grown as detailed in **A**. Primers used (Supplemental Figure 6A) allow quantifying and comparing expression levels by RT-qPCR between both species. *PHYA* transcript abundance was normalized to three reference genes (*EF1 ,DSPC25* and *YLS8*). Values are means and s.e.m. of three independent RT-qPCR biological replicates relative to *PHYA* transcript levels of d3 *A. thaliana* seedlings. Two-way ANOVA showed that *PHYA* levels are significantly different (**p<0.01) between species under either W or W+FR. **(C)** Immunoblot detection of phyA and tubulin with the antibodies indicated in Figure 3C in extracts of At^{WT} and Ch^{WT} seedlings grown as detailed at the top of the section: 5-day-old etiolated seedlings were exposed to W light and material was harvested before and after 6 h of W-exposure (arrows). **(D)** Evolution of relative phyA protein levels (PHYA:TUB) in At^{WT} and Ch^{WT} seedlings exposed to simulated shade, as detailed at the top of the section: 5-day-old etiolated seedlings were exposed to W+FR light and material was harvested before and after 6, 8 and 10 h of simulated shade exposure (arrows). Values are means and s.e.m. of four independent biological replicates relative to PHYA:TUB levels of etiolated At^{WT} seedlings. Two way ANOVA showed that relative PHYA levels under W+FR are significantly increased (**p<0.01) in *C. hirsuta* than *A. thaliana*.

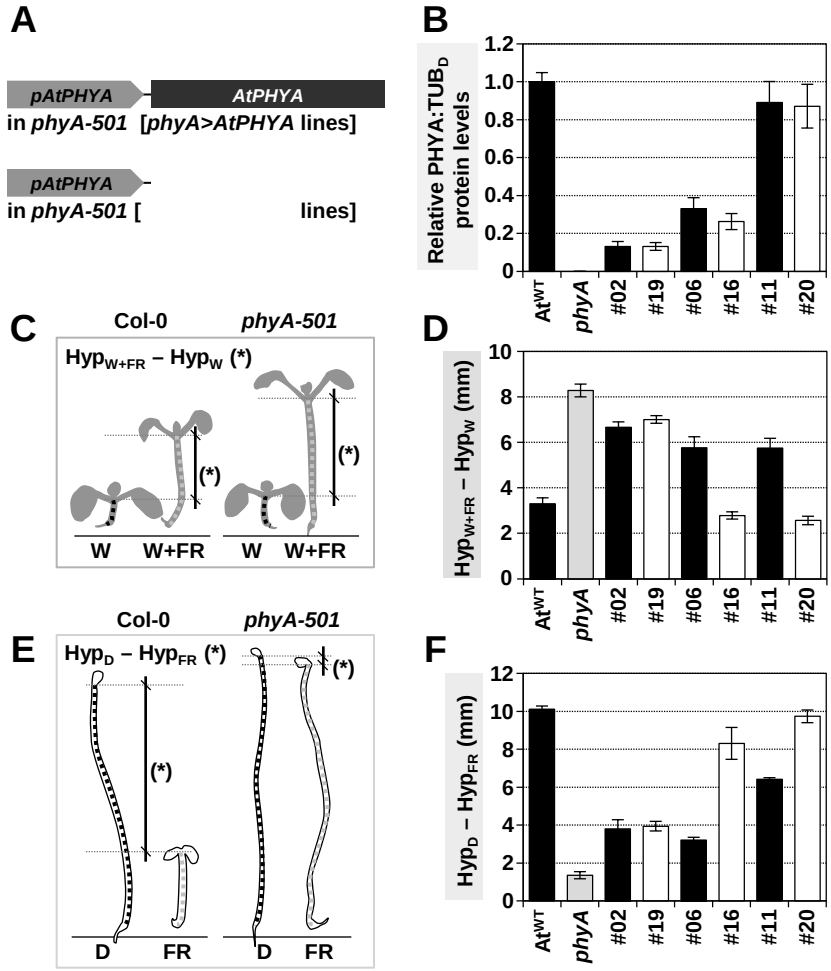

Figure 6. ChphyA has a stronger activity than AtphyA in repressing shade-induced hypocotyl elongation. (A) Cartoon detailing the constructs used to complement *A. thaliana phyA-501* mutant plants. **(B)** Relative PHYA:TUB in etiolated seedlings of At^{WT}, *phyA-501*, and selected *phyA>AtPHYA* (blue bars) and *phyA>ChPHYA* (red bars) complementation lines. Seedlings ere grown as indicated in Supplemental Figure 8. Values are means and s.e.m. of four independent biological replicates relative to PHYA:TUB levels of etiolated At^{WT} seedlings. **(C)** Cartoon illustrating how phyA activity in simulated shade was established as differences in hypocotyl length between simulated shade- and the W-grown seedlings (Hyp_{W+FR}-Hyp_W). Seedlings were grown for 2 days under W then for 5 additional days under W or W+FR (R:FR = 0.02), when hypocotyls were measured. **(D)** Hyp_{W+FR}-Hyp_W in seedlings of At^{WT}, *phyA-501*, and selected *phyA>AtPHYA* (blue bars) and *phyA>ChPHYA* (red bars) complementation lines. **(E)** Cartoon illustrating how phyA activity in de-etiolation was established as differences in hypocotyl length between dark- and FR-grown seedlings (Hyp_D-Hyp_{FR}). Seedlings were grown as indicated in Figure 3B. **(F)** Hyp_D-Hyp_{FR} in seedlings of At^{WT}, *phyA-501*, and selected *phyA>AtPHYA* (blue bars) and *phyA>ChPHYA* (red bars) complementation lines. In **C** and **E**, mutant *phyA-501* seedlings have no phyA activity.

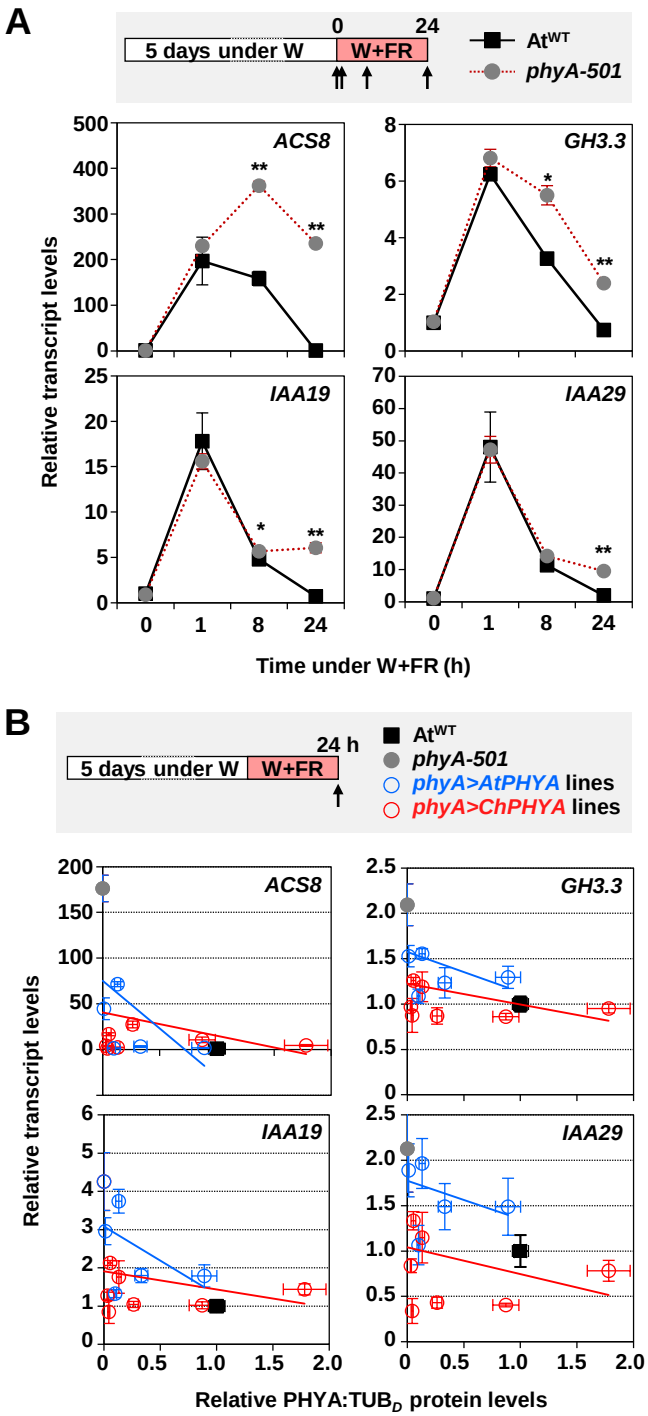

Figure 7. ChphyA has a stronger activity than AtphyA in repressing shade-induced expression of *ACS8*, *GH3.3*, *IAA19* and *IAA29* genes. (A) Effect of phyA in the shade-induced expression of *ACS8*, *GH3.3*, *IAA19* and *IAA29*. W-grown d5 seedlings of At^{WT} and *phyA-501* were treated for 0, 1, 8 and 24 h with W+FR (R:FR = 0.02), when material was harvested for RNA extraction, as indicated at the top of the panel. Transcript abundance, normalized to EF1α is shown. Values are means and s.e.m. of three independent RT-qPCR biological replicates relative to values at 0 h for At^{WT}. Asterisks indicate significant differences (**p<0.01, *p<0.05) between *phyA-501* and At^{WT} seedlings exposed for the same time to W+FR. **(B)** Correlation between *ACS8*, *GH3.3*, *IAA19* and *IAA29* expression and relative levels of PHYA protein in the seedlings of At^{WT}, *phyA-501*, *phyA>AtPHYA* (blue lines and dots) and *phyA>ChPHYA* (red lines and dots) complementation lines. Gene expression was quantified in W-grown d5 seedlings exposed to W+FR (R:FR=0.02) during 24 h, as indicated at the top of the panel. Transcript abundance was normalized to *EF1α*. Relative phyA protein levels (PHYA:TUB, data already shown in Supplemental Figure 8) were estimated in etiolated seedlings. Values are means and s.e.m. of three independent RT-qPCR biological replicates relative to values of At^{WT}. The estimated regression lines for the *phyA>AtPHYA* (blue line) and *phyA>ChPHYA* (red line) complementation lines are shown for each correlation.

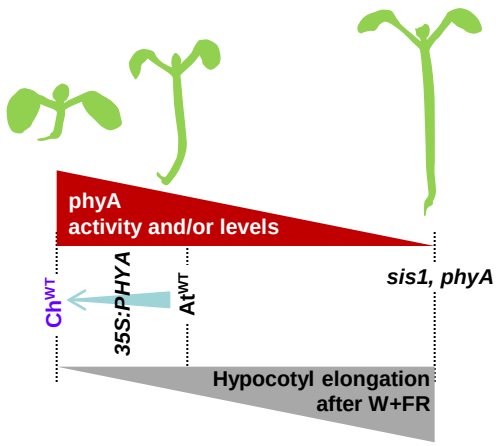

Figure 8. Model of how an increased phyA activity in *C. hirsuta* might implement the shade tolerance of hypocotyl elongation. Increases in phyA activity caused by the constitutive overexpression of *PHYA* also attenuate the shade-induced hypocotyl elongation in transgenic plants, and it results in partially tolerant *A. thaliana* seedlings.

Parsed Citations

Ashburner, M., Ball, C.A., Blake, J.A., Botstein, D., Butler, H., Cherry, J.M., Davis, A.P., Dolinski, K., Dwight, S.S., Eppig, J.T., Harris, M.A., Hill, D.P., Issel-Tarver, L., Kasarskis, A., Lewis, S., Matese, J.C., Richardson, J.E., Ringwald, M., Rubin, G.M. and Sherlock, G. (2000) Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat Genet, 25, 25-29.

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Barkoulas, M., Hay, A., Kougioumoutzi, E. and Tsiantis, M. (2008) A developmental framework for dissected leaf formation in the Arabidopsis relative Cardamine hirsuta. Nat Genet, 40, 1136-1141.

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Bealey, C.E. and Robertson, P.A. (1992) Coppice management for pheasants. In Ecology and Management of Coppice Woodlands (Buckley, G.P. ed: Springer-Science+Business Media, B.V., pp. 193-210.

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Benkov, M.A., Yatsenko, A.M. and Tikhonov, A.N. (2019) Light acclimation of shade-tolerant and sun-resistant Tradescantia species: photochemical activity of PSII and its sensitivity to heat treatment. Photosynth Res, 139, 203-214.

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Bou-Torrent, J., Galstyan, A., Gallemi, M., Cifuentes-Esquivel, N., Molina-Contreras, M.J., Salla-Martret, M., Jikumaru, Y., Yamaguchi, S., Kamiya, Y. and Martinez-Garcia, J.F. (2014) Plant proximity perception dynamically modulates hormone levels and sensitivity in Arabidopsis. J Exp Bot, 65, 2937-2947.

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Bou-Torrent, J., Toledo-Ortiz, G., Ortiz-Alcaide, M., Cifuentes-Esquivel, N., Halliday, K.J., Martinez-Garcia, J.F. and Rodriguez-Concepcion, M. (2015) Regulation of Carotenoid Biosynthesis by Shade Relies on Specific Subsets of Antagonistic Transcription Factors and Cofactors. Plant Physiol, 169, 1584-1594.

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Cagnola, J.I., Ploschuk, E., Benech-Arnold, T., Finlayson, S.A. and Casal, J.J. (2012) Stem transcriptome reveals mechanisms to reduce the energetic cost of shade-avoidance responses in tomato. Plant Physiol, 160, 1110-1119.

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Callahan, H.S., Pigliucci, M. and Schlichting, C.D. (1997) Developmental phenotypic plasticity: where ecology and evolution meet molecular biology. Bioessays, 19, 519-525.

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Canton, F.R. and Quail, P.H. (1999) Both phyA and phyB mediate light-imposed repression of PHYA gene expression in Arabidopsis. Plant Physiol, 121, 1207-1216.

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Carroll, S.B. (2008) Evo-devo and an expanding evolutionary synthesis: a genetic theory of morphological evolution. Cell, 134, 25-36.

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Casal, J.J. (2012) Shade avoidance. Arabidopsis Book, 10, e0157.

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Casal, J.J., Candia, A.N. and Sellaro, R. (2014) Light perception and signalling by phytochrome A. J Exp Bot, 65, 2835-2845.

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Cifuentes-Esquivel, N., Bou-Torrent, J., Galstyan, A., Gallemi, M., Sessa, G., Salla Martret, M., Roig-Villanova, I., Ruberti, I. and Martinez-Garcia, J.F. (2013) The bHLH proteins BEE and BIM positively modulate the shade avoidance syndrome in Arabidopsis seedlings. The Plant Journal, 75, 989-1002.

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Ciolfi, A., Sessa, G., Sassi, M., Possenti, M., Salvucci, S., Carabelli, M., Morelli, G. and Ruberti, I. (2013) Dynamics of the shade-avoidance response in Arabidopsis. Plant Physiol, 163, 331-353.

Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

de Wit, M., Keuskamp, D.H., Bongers, F.J., Hornitschek, P., Gommers, C.M.M., Reinen, E., Martinez-Ceron, C., Fankhauser, C. and

Pierik, R. (2016) Integration of Phytochrome and Cryptochrome Signals Determines Plant Growth during Competition for Light. Curr Biol, 26, 3320-3326.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

de Wit, M., Ljung, K. and 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.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Dieterle, M., Bauer, D., Buche, C., Krenz, M., Schafer, E. and Kretsch, T. (2005) A new type of mutation in phytochrome A causes enhanced light sensitivity and alters the degradation and subcellular partitioning of the photoreceptor. Plant J, 41, 146-161.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Durgbanshi, A., Arbona, V., Pozo, O., Miersch, O., Sancho, J.V. and Gomez-Cadenas, A. (2005) Simultaneous determination of multiple phytohormones in plant extracts by liquid chromatography-electrospray tandem mass spectrometry. J Agric Food Chem, 53, 8437-8442.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Gallemi, M., Galstyan, A., Paulisic, S., Then, C., Ferrandez-Ayela, A., Lorenzo-Orts, L., Roig-Villanova, I., Wang, X., Micol, J.L., Ponce, M.R., Devlin, P.F. and Martinez-Garcia, J.F. (2016) DRACULA2 is a dynamic nucleoporin with a role in regulating the shade avoidance syndrome in Arabidopsis. Development, 143, 1623-1631.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Gallemi, M., Molina-Contreras, M.J., Paulisic, S., Salla-Martret, M., Sorin, C., Godoy, M., Franco-Zorrilla, J.M., Solano, R. and Martinez-Garcia, J.F. (2017a) A non-DNA-binding activity for the ATHB4 transcription factor in the control of vegetation proximity. New Phytol.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Gallemi, M., Molina-Contreras, M.J., Paulisic, S., Salla-Martret, M., Sorin, C., Godoy, M., Franco-Zorrilla, J.M., Solano, R. and Martinez-Garcia, J.F. (2017b) A non-DNA-binding activity for the ATHB4 transcription factor in the control of vegetation proximity. New Phytol, 216, 798-813.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Gan, X., Hay, A., Kwantes, M., Haberer, G., Hallab, A., Ioio, R.D., Hofhuis, H., Pieper, B., Cartolano, M., Neumann, U., Nikolov, L.A., Song, B., Hajheidari, M., Briskine, R., Kougioumoutzi, E., Vlad, D., Broholm, S., Hein, J., Meksem, K., Lightfoot, D., Shimizu, K.K., Shimizu-Inatsugi, R., Imprialou, M., Kudrna, D., Wing, R., Sato, S., Huijser, P., Filatov, D., Mayer, K.F., Mott, R. and Tsiantis, M. (2016) The Cardamine hirsuta genome offers insight into the evolution of morphological diversity. Nat Plants, 2, 16167.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Genoud, T., Schweizer, F., Tscheuschler, A., Debrieux, D., Casal, J.J., Schafer, E., Hiltbrunner, A. and Fankhauser, C. (2008) FHY1 mediates nuclear import of the light-activated phytochrome A photoreceptor. PLoS Genet, 4, e1000143.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Gommers, C.M., Keuskamp, D.H., Buti, S., van Veen, H., Koevoets, I.T., Reinen, E., Voesenek, L.A. and Pierik, R. (2017) Molecular Profiles of Contrasting Shade Response Strategies in Wild Plants: Differential Control of Immunity and Shoot Elongation. Plant Cell, 29, 331-344.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Gommers, C.M., Visser, E.J., St Onge, K.R., Voesenek, L.A. and Pierik, R. (2013) Shade tolerance: when growing tall is not an option. Trends Plant Sci, 18, 65-71.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Gommers, C.M.M., Buti, S., Tarkowska, D., Pencik, A., Banda, J.P., Arricastres, V. and Pierik, R. (2018) Organ-specific phytohormone synthesis in two Geranium species with antithetical responses to far-red light enrichment. Plant Direct, 2, e00066.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Han, S., Jiang, J., Li, H., Song, A., Chen, S. and Chen, F. (2015) The differential response of two Chrysanthemum cultivars to shading: photosynthesis, chloroplast, and Sieve Element-companion cell ultrastructure. HortScience, 50, 1192-1195.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Hay, A. and Tsiantis, M. (2016) Cardamine hirsuta: a comparative view. Curr Opin Genet Dev, 39, 1-7.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Hay, A.S., Pieper, B., Cooke, E., Mandakova, T., Cartolano, M., Tattersall, A.D., Ioio, R.D., McGowan, S.J., Barkoulas, M., Galinha, C., Rast, M.I., Hofhuis, H., Then, C., Plieske, J., Ganal, M., Mott, R., Martinez-Garcia, J.F., Carine, M.A., Scotland, R.W., Gan, X., Filatov, D.A., Lysak, M.A. and Tsiantis, M. (2014) Cardamine hirsuta: a versatile genetic system for comparative studies. Plant J, 78, 1-15.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Hersch, M., Lorrain, S., de Wit, M., Trevisan, M., Ljung, K., Bergmann, S. and Fankhauser, C. (2014) Light intensity modulates the regulatory network of the shade avoidance response in Arabidopsis. Proc Natl Acad Sci U S A, 111, 6515-6520.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Heyer, A.G., Mozley, D., Landschutze, V., Thomas, B. and Gatz, C. (1995) Function of phytochrome A in potato plants as revealed through the study of transgenic plants. Plant Physiol, 109, 53-61.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Hofhuis, H., Moulton, D., Lessinnes, T., Routier-Kierzkowska, A.L., Bomphrey, R.J., Mosca, G., Reinhardt, H., Sarchet, P., Gan, X., Tsiantis, M., Ventikos, Y., Walker, S., Goriely, A., Smith, R. and Hay, A. (2016) Morphomechanical Innovation Drives Explosive Seed Dispersal. Cell, 166, 222-233.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Hornitschek, P., Kohnen, M.V., Lorrain, S., Rougemont, J., Ljung, K., Lopez-Vidriero, I., Franco-Zorrilla, J.M., Solano, R., Trevisan, M., Pradervand, S., Xenarios, I. and 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.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Hornitschek, P., Lorrain, S., Zoete, V., Michielin, O. and Fankhauser, C. (2009) Inhibition of the shade avoidance response by formation of non-DNA binding bHLH heterodimers. EMBO J, 28, 3893-3902.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Karve, A.A., Jawdy, S.S., Gunter, L.E., Allen, S.M., Yang, X., Tuskan, G.A., Wullschleger, S.D. and Weston, D.J. (2012) Initial characterization of shade avoidance response suggests functional diversity between Populus phytochrome B genes. New Phytol, 196, 726-737.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Kim, J.I., Shen, Y., Han, Y.J., Park, J.E., Kirchenbauer, D., Soh, M.S., Nagy, F., Schafer, E. and Song, P.S. (2004) Phytochrome phosphorylation modulates light signaling by influencing the protein-protein interaction. Plant Cell, 16, 2629-2640.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Klie, S. and Nikoloski, Z. (2012) The Choice between MapMan and Gene Ontology for Automated Gene Function Prediction in Plant Science. Front Genet, 3, 115.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Kohnen, M.V., Schmid-Siegert, E., Trevisan, M., Petrolati, L.A., Senechal, F., Muller-Moule, P., Maloof, J., Xenarios, I. and Fankhauser, C. (2016) Neighbor Detection Induces Organ-Specific Transcriptomes, Revealing Patterns Underlying Hypocotyl-Specific Growth. Plant Cell, 28, 2889-2904.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Kozuka, T., Kobayashi, J., Horiguchi, G., Demura, T., Sakakibara, H., Tsukaya, H. and Nagatani, A. (2010) Involvement of auxin and brassinosteroid in the regulation of petiole elongation under the shade. Plant Physiol, 153, 1608-1618.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Li, J., Li, G., Wang, H. and Deng, X.W. (2011) Phytochrome signaling mechanisms. Arabidopsis Book, 9, e0148.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Li, L., Ljung, K., Breton, G., Schmitz, R.J., Pruneda-Paz, J., Cowing-Zitron, C., Cole, B.J., Ivans, L.J., Pedmale, U.V., Jung, H.S., Ecker, J.R., Kay, S.A. and Chory, J. (2012) Linking photoreceptor excitation to changes in plant architecture. Genes Dev, 26, 785-790.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Lohse, M., Nagel, A., Herter, T., May, P., Schroda, M., Zrenner, R., Tohge, T., Fernie, A.R., Stitt, M. and Usadel, B. (2014) Mercator: a fast and simple web server for genome scale functional annotation of plant sequence data. Plant Cell Environ, 37, 1250-1258.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Martinez-Garcia, J.F., Galstyan, A., Salla-Martret, M., Cifuentes-Esquivel, N., Gallemí, M. and Bou-Torrent, J. (2010) Regulatory components of shade avoidance syndrome. Advances in Botanical Research, 53, 65-116.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Martinez-Garcia, J.F., Gallemi, M., Molina-Contreras, M.J., Llorente, B., Bevilaqua, M.R. and Quail, P.H. (2014) The shade avoidance syndrome in Arabidopsis: the antagonistic role of phytochrome a and B differentiates vegetation proximity and canopy shade. PLoS One, 9, e109275.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Martinez-Garcia, J.F., Monte, E. and Quail, P.H. (1999) A simple, rapid and quantitative method for preparing Arabidopsis protein extracts for immunoblot analysis. Plant J, 20, 251-257.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Mathews, S. (2010) Evolutionary studies illuminate the structural-functional model of plant phytochromes. Plant Cell, 22, 4-16.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Oka, Y., Ono, Y., Toledo-Ortiz, G., Kokaji, K., Matsui, M., Mochizuki, N. and Nagatani, A. (2012) Arabidopsis phytochrome a is modularly structured to integrate the multiple features that are required for a highly sensitized phytochrome. Plant Cell, 24, 2949-2962.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Ortiz-Alcaide, M., Llamas, E., Gomez-Cadenas, A., Nagatani, A., Martinez-Garcia, J.F. and Rodriguez-Concepcion, M. (2019) Chloroplasts modulate elongation responses to canopy shade by retrograde pathways involving HY5 and ABA. Plant Cell.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Procko, C., Crenshaw, C.M., Ljung, K., Noel, J.P. and Chory, J. (2014) Cotyledon-Generated Auxin Is Required for Shade-Induced Hypocotyl Growth in Brassica rapa. Plant Physiol, 165, 1285-1301.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Reed, J.W., Nagpal, P., Poole, D.S., Furuya, M. and Chory, J. (1993) Mutations in the gene for the red/far-red light receptor phytochrome B alter cell elongation and physiological responses throughout Arabidopsis development. Plant Cell, 5, 147-157.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Robson, P.R., McCormac, A.C., Irvine, A.S. and Smith, H. (1996) Genetic engineering of harvest index in tobacco through overexpression of a phytochrome gene. Nat Biotechnol, 14, 995-998.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Roig-Villanova, I., Bou-Torrent, J., Galstyan, A., Carretero-Paulet, L., Portoles, S., Rodriguez-Concepcion, M. and Martinez-Garcia, J.F. (2007) Interaction of shade avoidance and auxin responses: a role for two novel atypical bHLH proteins. The EMBO Journal, 26, 4756-4767.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Roig-Villanova, I., Bou, J., Sorin, C., Devlin, P.F. and Martinez-Garcia, J.F. (2006) Identification of primary target genes of phytochrome signaling. Early transcriptional control during shade avoidance responses in Arabidopsis. Plant Physiology, 141, 85-96.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Roig-Villanova, I. and Martinez-Garcia, J.F. (2016) Plant Responses to Vegetation Proximity: A Whole Life Avoiding Shade. Front Plant Sci, 7, 236.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Sasidharan, R., Chinnappa, C.C., Staal, M., Elzenga, J.T., Yokoyama, R., Nishitani, K., Voesenek, L.A. and Pierik, R. (2010) Light quality-mediated petiole elongation in Arabidopsis during shade avoidance involves cell wall modification by xyloglucan endotransglucosylase/hydrolases. Plant Physiol, 154, 978-990.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Seo, H.S., Watanabe, E., Tokutomi, S., Nagatani, A. and Chua, N.H. (2004) Photoreceptor ubiquitination by COP1 E3 ligase desensitizes phytochrome A signaling. Genes Dev, 18, 617-622.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Sheerin, D.J., Menon, C., zur Oven-Krockhaus, S., Enderle, B., Zhu, L., Johnen, P., Schleifenbaum, F., Stierhof, Y.D., Huq, E. and Hiltbrunner, A. (2015) Light-activated phytochrome A and B interact with members of the SPA family to promote photomorphogenesis in

Arabidopsis by reorganizing the COP1/SPA complex. Plant Cell, 27, 189-201.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Smith, H. (1982) Light quality, photoperception, and plant strategy. Annual Review of Plant Physiology, 33, 481-518.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Tao, Y., Ferrer, J.L., Ljung, K., Pojer, F., Hong, F., Long, J.A., Li, L., Moreno, J.E., Bowman, M.E., Ivans, L.J., Cheng, Y., Lim, J., Zhao, Y., Ballare, C.L., Sandberg, G., Noel, J.P. and Chory, J. (2008) Rapid synthesis of auxin via a new tryptophan-dependent pathway is required for shade avoidance in plants. Cell, 133, 164-176.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Thimm, O., Blasing, O., Gibon, Y., Nagel, A., Meyer, S., Kruger, P., Selbig, J., Muller, L.A., Rhee, S.Y. and Stitt, M. (2004) MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. Plant J, 37, 914-939.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Ueoka-Nakanishi, H., Hori, N., Ishida, K., Ono, N., Yamashino, T., Nakamichi, N. and Mizuno, T. (2011) Characterization of shade avoidance responses in Lotus japonicus. Biosci Biotechnol Biochem, 75, 2148-2154.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Valladares, F. and Niinemets, U. (2008) Shade Tolerance, a Key Plant Feature of Complex Nature and Consequences. Annual Review of Ecology, Evolution, and Systematics, 39, 237-257.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Vlad, D., Kierzkowski, D., Rast, M.I., Vuolo, F., Dello Ioio, R., Galinha, C., Gan, X., Hajheidari, M., Hay, A., Smith, R.S., Huijser, P., Bailey, C.D. and Tsiantis, M. (2014) Leaf shape evolution through duplication, regulatory diversification, and loss of a homeobox gene. Science, 343, 780-783.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Vuolo, F., Mentink, R.A., Hajheidari, M., Bailey, C.D., Filatov, D.A. and Tsiantis, M. (2016) Coupled enhancer and coding sequence evolution of a homeobox gene shaped leaf diversity. Genes Dev, 30, 2370-2375.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Yang, C., Xie, F., Jiang, Y., Li, Z., Huang, X. and Li, L. (2018) Phytochrome A Negatively Regulates the Shade Avoidance Response by Increasing Auxin/Indole Acidic Acid Protein Stability. Dev Cell, 44, 29-41 e24.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Yanovsky, M.J., Casal, J.J. and Whitelam, G.C. (1995) Phytochrome A, phytochrome B and HY4 are involved in hypocotyl growth responses to natural radiation in Arabidopsis: Weak de-etiolation of the phyA mutant under dense canopies. Plant Cell Environ, 18, 788-794.
Pubmed: Author and Title
Google Scholar: Author Only Title Only Author and Title

Photoreceptor Activity Contributes to Contrasting Responses to Shade in Cardamine and Arabidopsis Seedlings

Maria Jose Molina-Contreras, Sandi Paulisic, Christiane Then, Jordi Moreno-Romero, Pedro Pastor-Andreu, Luca Morelli, Irma Roig-Villanova, Huw Jenkins, Asis Hallab, Xiangchao Gan, Aurelio Gómez-Cadenas, Miltos Tsiantis, Manuel Rodriguez-Concepcion and Jaime F. Martinez-Garcia

Plant Cell; originally published online September 17, 2019;
DOI 10.1105/tpc.19.00275