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# To grow or defend? Low red : far-red ratios reduce jasmonate sensitivity in Arabidopsis seedlings by promoting DELLA degradation and increasing JAZ10 stability

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## Summary

- How plants balance resource allocation between growth and defense under conditions of competitive stress is a key question in plant biology. Low red : far-red (R : FR) ratios, which signal a high risk of competition in plant canopies, repress jasmonate-induced defense responses. The mechanism of this repression is not well understood. We addressed this problem in Arabidopsis by investigating the role of DELLA and JASMONATE ZIM domain (JAZ) proteins.
- We showed that a quintuple *della* mutant and a *phyB* mutant were insensitive to jasmonate for several physiological readouts. Inactivation of the photoreceptor phyB by low R : FR ratios rapidly reduced DELLA protein abundance, and the inhibitory effect of FR on jasmonate signaling was missing in the *gai-1* mutant, which encodes a stable version of the GAI DELLA protein.
- We also demonstrated that low R : FR ratios and the *phyB* mutation stabilized the protein JAZ10. Furthermore, we demonstrated that *JAZ10* was required for the inhibitory effect of low R : FR on jasmonate responses, and that the *jaz10* mutation restored jasmonate sensitivity to the *phyB* mutant.
- We conclude that, under conditions of competition for light, plants redirect resource allocation from defense to rapid elongation by promoting DELLA degradation and enhancing JAZ10 stability.

## Introduction

Under natural conditions, plants must strike a balance in the allocation of limited resources to different physiological activities, including growth and defense. For plants, growth is essential not only to accumulate resources that can be transferred to offspring, but also to position new resource-harvesting structures in places within the canopy or the soil in which resources are less contested by competitors. In turn, investment of resources in defense is critical to fend off attacks from a wide variety of consumer organisms that feed on plants. The fact that resources are limited, but their uses are multiple, creates resource allocation tradeoffs. The 'growth vs defense' allocation dilemma has received considerable attention in the ecological literature (Herms & Mattson, 1992; Agrawal, 2000; Cipollini, 2004; Izaguirre *et al.*, 2006; Ballaré, 2009). However, the molecular mechanisms used by plants to solve this dilemma and to produce allocation decisions that are adaptive and appropriate for each particular environment are not well understood.

Recent work has demonstrated that part of the plant's solution to this dilemma is based on the regulation of jasmonate (JA) signaling by the photoreceptor phytochrome (Ballaré, 2011). It has been shown that low red to far-red (R : FR) ratios, which indicate a high risk of competition in plant canopies, antagonize JA responses (Moreno *et al.*, 2009; Cerrudo *et al.*, 2012; De Wit *et al.*, 2013; Izaguirre *et al.*, 2013; Kegge *et al.*, 2013; Cargnel *et al.*, 2014; Chico *et al.*, 2014). A down-regulation of JA responses reduces the expenditure of resources in defense, which presumably helps the plant to focus its fitness strategy on growth and morphological responses to overtop other plants. JAs are the principal hormones involved in the orchestration of plant defense against herbivores and necrotrophic pathogens (Howe & Jander, 2008; Browse, 2009; Wasternack & Hause, 2013). In turn, phytochrome, particularly phytochrome B (phyB), is the principal photoreceptor used by plants to detect the proximity of competitors (Ballaré, 1999; Smith, 2000; Casal, 2012).

phyB has two inter-convertible forms: Pfr, which is biologically active, and Pr, which is inactive. Pfr has an absorption maximum in the FR region of the spectrum, whereas Pr absorbs maximally in the R region. Light absorption leads to photoconversion between Pfr and Pr; therefore, under natural light conditions, the relative fraction of phyB molecules that are in the active Pfr form depends on the ratio of R to FR radiation (the R : FR ratio) (Smith, 1995). Green leaves strongly absorb R but

not FR radiation; therefore, in plant canopies, a reduction in R : FR from the characteristic value of 1.2 to values < 1 is a good signal of a heightened risk of competition (Ballaré *et al.*, 1990). Plants of shade-intolerant species respond to this signal with increased apical dominance and a rapid acceleration of stem and petiole elongation, which allows them to position new leaves in well-illuminated, upper canopy strata. This reconfiguration of plant morphology, triggered by low R : FR and other competition signals, is often referred to as the shade-avoidance syndrome (SAS) (Smith, 1995). At the molecular level, SAS involves increased activity of several growth-promoting hormones, including auxin (Morelli & Ruberti, 2000; Roig-Villanova *et al.*, 2007; Tao *et al.*, 2008; Keuskamp *et al.*, 2010; Hornitschek *et al.*, 2012; Li *et al.*, 2012) and gibberellins (GAs) (Garcia-Martinez & Gil, 2001; Djakovic-Petrovic *et al.*, 2007; Kurepin *et al.*, 2007). GAs act by promoting the proteasomal degradation of a family of growth-repressing proteins, known as DELLA proteins (Harberd *et al.*, 2009).

The JA pathway is activated in response to tissue damage caused by chewing insects and necrotrophic pathogens. This pathway begins with the release of α-linolenic acid from chloroplast membrane lipids, leading to the production of jasmonic acid (Wasternack & Hause, 2013). Jasmonic acid can then be conjugated to amino acids, such as isoleucine, to form the bioactive hormone, JA-Ile (Browse, 2009). Perception of the hormone is achieved by a co-receptor formed by the ubiquitin E3 ligase complex Skp1-Cul1-F-box protein CORONATINE INSENSITIVE 1 ($SCF^{COI1}$) and JA ZIM DOMAIN (JAZ) proteins. JA-Ile stimulates the specific binding of COI1 and JAZ proteins, which leads to ubiquitination of JAZs by $SCF^{COI1}$ and their subsequent proteasome-mediated degradation (Chini *et al.*, 2007; Thines *et al.*, 2007; Yan *et al.*, 2007, 2009; Melotto *et al.*, 2008; Pauwels *et al.*, 2010; Sheard *et al.*, 2010). JAZ proteins block JA responses by repressing the activity of: critical transcription factors that regulate resistance to insects (Fernández-Calvo *et al.*, 2011); anthocyanin biosynthesis and trichome initiation (Qi *et al.*, 2011); and JA–ethylene interactions and resistance to necrotrophic pathogens (Zhu *et al.*, 2011). Therefore, the degradation of JAZs triggers the activation of JA-induced defenses (Pauwels & Goossens, 2011; Kazan & Manners, 2012).

Repression of JA responses by phyB inactivation has been shown in Arabidopsis and other species and for several defenses that include phenolic compounds, volatile terpenes and extrafloral nectar (reviewed in Ballaré, 2014). This down-regulation is not a simple by-product of resource diversion to SAS (Moreno *et al.*, 2009; Cerrudo *et al.*, 2012; De Wit *et al.*, 2013), and the available genetic (Cerrudo *et al.*, 2012) and physiological (De Wit *et al.*, 2013) evidence demonstrates that it is not caused by increased salicylic acid (SA) signaling. In contrast with the effect of SA as a repressor of JA responses, the effect of low R : FR ratios appears to occur at the level of the COI1–JAZ co-receptor module (Cerrudo *et al.*, 2012).

The mechanisms by which low R : FR ratios antagonize JA responses are unclear. One possibility is that phyB inactivation leads to a shift in the balance between JAZ and DELLA proteins (Ballaré, 2014). Low R : FR ratios may result in increased GA signaling (Garcia-Martinez & Gil, 2001; Kurepin *et al.*, 2007), and prolonged treatments of low R : FR ratio have been shown to reduce the abundance of DELLA fusion proteins (RGA-GFP) in rapidly elongating Arabidopsis hypocotyls and petioles (Djakovic-Petrovic *et al.*, 2007). DELLAs are positive regulators of JA responses (Navarro *et al.*, 2008), because DELLAs bind to JAZs and prevent them from repressing their target transcription factors, such as MYCs (Hou *et al.*, 2010; Yang *et al.*, 2012). Therefore, increased DELLA turnover could make more JAZ proteins available for interaction with MYCs, and hence for the repression of JA-mediated defense responses (Hou *et al.*, 2010; Yang *et al.*, 2012; Huot *et al.*, 2014). Although this model is attractive because of its simplicity, it is unclear whether DELLA turnover induced by low R : FR is sufficiently fast to explain the rapid effects of light quality on JA-induced gene expression (Moreno *et al.*, 2009; Cerrudo *et al.*, 2012; De Wit *et al.*, 2013), and whether the degradation patterns detected in hypocotyls and petioles are representative of those that take place in organs in which the bulk of the defense responses are expressed (e.g. leaf laminas). Recent work has also indicated that supplemental FR radiation can increase the stability of JAZ proteins (Chico *et al.*, 2014), but the evidence that this stabilization plays a functional role in dampening JA signaling is limited to the observation that the *JAZ10* gene appears to be necessary for the effect of low R : FR ratios increasing plant susceptibility to the necrotrophic fungus *Botrytis cinerea* (Cerrudo *et al.*, 2012). Supplemental FR radiation has been shown recently to accelerate the degradation of the transcription factor MYC2 (Chico *et al.*, 2014). However, clear effects of low R : FR ratios reducing JA responses have also been demonstrated for genes that are not targets of MYCs (such as *PDF1.2*) (Moreno *et al.*, 2009; Cerrudo *et al.*, 2012; De Wit *et al.*, 2013), suggesting that multiple mechanisms could connect phyB with JA signaling (Moreno & Ballaré, 2014).

In the experiments reported here, we investigate the mechanisms of repression of JA signaling by low R : FR ratios in Arabidopsis seedlings using two sets of readouts for the JA response, one connected with growth inhibition (hypocotyl elongation and biomass accumulation) and one related to defense (accumulation of leaf phenolics). We found that a quintuple *della* mutant and a *phyB* mutant are insensitive to JA for the physiological responses characterized in our study. We show that phyB inactivation triggers rapid DELLA degradation, and that this effect is required to suppress JA responses, because the inhibitory effect of FR is missing in the *gai-1* mutant, which produces a version of the GAI1 DELLA protein that is resistant to GA-induced degradation. We further demonstrate that low R : FR ratios and the *phyB* mutation reduce the turnover of the JAZ10 protein, and that the *JAZ10* gene is required for the inhibitory effect of low R : FR ratios or the *phyB* mutation on JA signaling. We conclude that, under conditions of high competition for light, shade-intolerant species, such as Arabidopsis, rapidly change the balance between DELLA and JAZ proteins, in favor of the latter, and that this change plays an important role in the reconfiguration of their resource allocation strategy, from defense to rapid elongation.

## Materials and Methods

### Plant material and growth conditions

Surface-sterilized seeds of Arabidopsis (*Arabidopsis thaliana* (L.) Heynh) were germinated on 0.7% agar, 1.5% sucrose and Murashige and Skoog (MS) medium in glass jars with filtered vents that allowed gas exchange but prevented contamination at 22°C in a growth chamber (Supporting Information Fig. S1). White light (WL; photosynthetic photon flux density, 150 μmol $m^{-2}$ $s^{-1}$; R : FR ratio = 4.5) was provided by fluorescent lamps. Two photoperiods were used in the experiments, as indicated in the relevant figure legends: long days (LD), 14 h WL : 10 h darkness; short days (SD), 8 h WL : 16 h darkness. The *phyB-9* (Reed *et al.*, 1994), *sav3-2* (Tao *et al.*, 2008), RNAi7 (Yan *et al.*, 2007), *jaz8* (CS849856) (Jiang *et al.*, 2014), *jaz9-1* (SALK_004872C) (Yang *et al.*, 2012), *jaz10.1* (SAIL_92_D08), *35S::JAZ1-GUS* (Thines *et al.*, 2007), and the *35S::JAZ10-GUS* (Chico *et al.*, 2014) lines were all in the Col-0 background. The GA-insensitive gain-of-function *gai-1* mutant (Koorneef *et al.*, 1985) and the *gai-t6 rga-t2 rgl1-1 rgl3-1 rgl2-1* quintuple *della* (*5× della*, CS 16298-ABRC) (Keller *et al.*, 2011) and *gai-t6 rga-t2 rgl1-1 rgl2-1* quadruple *della* (*4× della*) (Achard *et al.*, 2006) knockout mutants were all in the Ler background. In most experiments, 12-d-old plants were used for measurements of hypocotyl length, fresh weight, phenolic compounds and protein stability. The double *jaz10phyB* mutant was obtained by crossing the *phyB-9* and *jaz10.1* mutants. Long-hypocotyl seedlings from the F2 generation were selected, and tested for resistance to glufosinate-ammonium (Basta, Bayer) and for *JAZ10* genomic sequence integrity by PCR using the primers 5′-ATGTCGAAAGCTACCATAGAA-3′ and 5′-TTAGGCCGATGTCGGATAGTA-3′ (Chung *et al.*, 2008). PCR products were resolved on a 2.5% agarose gel and plants without a product for this reaction were selfed until the F4 generation. The presence of the *phyB-9* mutation was tested in the successive generations based on the long-hypocotyl phenotype and photomorphogenic behavior under R light. The transgenic line *35S::JAZ10-GUS* was crossed with the *phyB-9* mutant and long-hypocotyl F2 seedlings selected for hygromycin resistance and positive GUS staining. Selected plants were selfed and phenotypically tested until the F4, which used to perform the experiments.

### Light treatments

Arabidopsis plants receiving 150 μmol $m^{-2}$ $s^{-1}$ WL were placed in front of banks of water-cooled incandescent lamps covered with either opaque screens (ambient 'Amb' light treatment) or FR-transmitting filters ('FR' treatment), as described by Moreno *et al.* (2009) (Fig. S1). The FR treatment reduced the R : FR ratio of the integrated horizontal radiation to *c.* 0.55. Previous studies in canopies of mustard (*Sinapis alba*) and chamico (*Datura ferox*) seedlings have indicated that this R : FR ratio in the horizontal light flux corresponds to a leaf area index of *c.* 0.5, in which mutual shading among neighboring plants is negligible (Ballaré *et al.*, 1991). Neither air temperature nor the level of WL received by the plants was affected by the FR treatment.

### JA treatments

Hormone treatments were performed by adding solutions of methyl-JA (MeJA) (Sigma) at the indicated concentrations on top of the medium in which plants were grown, following the protocols described in the relevant figures. Plants not assigned to the hormone treatment were treated with distilled water, which was supplemented with ethanol in the same proportion as that used to dissolve MeJA. Wounding treatments were carried out by applying pressure with a small forceps for 5 s on two leaves of the rosette.

### Measurements of growth, secondary metabolites and gene expression

Hypocotyl growth was measured at the end of the experiment with a caliper; fresh weight was determined with an analytical balance. For measurement of soluble phenolic compounds, the seedlings were weighed and placed in 400 μl of acidified methanol (99 : 1, v/v) at 4°C for 48 h (Rabino & Mancinelli, 1986; Demkura *et al.*, 2010) After the addition of 300 μl of deionized water, soluble phenolics were separated from chlorophylls by the addition of 700 μl of chloroform. The absorbance of the extracts was determined using a spectrophotometer (UV1700; Shimadzu, Kyoto, Japan), and $A_{320}$ values were referred to fresh weight. These absorbance values correlated well with anthocyanin content and were less variable than the absorbance data at 530 nm. Total RNA was extracted from 100 mg of frozen tissue using the LiCl–phenol/chloroform method (Izaguirre *et al.*, 2003). Purified fractions of total RNA were subjected to RQ1 (RNase-free) DNase treatment (Promega) to avoid contamination with genomic DNA. For cDNA synthesis, fractions of 2 μg of RNA were reverse transcribed using oligo(dT) as primer and M-MLV reverse transcriptase (Invitrogen) according to the manufacturer's instructions. Quantitative real-time PCR (qPCR) was performed in a 7500 Real Time PCR System (Applied Biosystems, Foster City, CA, USA) following the manufacturer's standard method for absolute quantification using FastStart Universal SYBR Green Master Mix (Roche Applied Science, Indianapolis, IN, USA) and primers at a final concentration of 500 nM. The *A. thaliana UBIQUITIN* (*UBC*) gene was used to normalize for differences in the concentrations of cDNA samples. Primer sequences were as follows: *UBC*, CTGCGACTCAGGGAATCTTCTAA (forward primer); TTGTGCCATTGAATTGAACCC (reverse primer); *PDF1.2*, TTGCTGCTTTCGACGCA (forward primer), TGTCCCACTTGGCTTCTCG (reverse primer); *ERF1*, CCTCGGCGATTCTCAATTTTT (forward primer), CCGAAAGCGACTCTTGAACTCT (reverse primer).

### β-Glucuronidase (GUS) histochemical staining and activity

Seedlings were incubated in ice-cold 90% acetone for 20 min. The solution was changed to GUS staining solution (1 mM X-Gluc (5-bromo-4-chloro-3-indolyl-beta-D-glucuronic acid), 100 mM NaPi (sodium phosphate) buffer, pH 7.0, 10 mM EDTA, 0.1% (v/v) Triton X-100). The seedlings were incubated overnight at 37°C in the dark, followed by de-staining and

clearing by several changes of 70% ethanol. Seedlings were inspected under a dissection microscope for staining intensity, and photographed. For the quantification of GUS activity, 12-d-old seedlings were harvested, frozen in liquid nitrogen and ground in 50 µl of extraction buffer (phosphate buffer 50 mM, pH 7, $Na_2EDTA$ 10 mM, pH 8, sodium dodecylsulfate (SDS) 0.1%, Triton X100 0.1% and β-Me (2-mercaptoethanol) 4.32 mM). The tissue was stored at −80°C. GUS activity was measured by monitoring the cleavage of the β-glucuronidase 4-methylumbelliferyl β-D-glucuronide (MUG) substrate (Jefferson *et al.*, 1987) using a Beckman Coulter DTX 800/880 fluorometer (Pasadena, CA, USA). Total protein content (Lowry *et al.*, 1951) was used to normalize GUS activity.

### Immunoblots

Total proteins were extracted using plant protein extraction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10% glycerol, 0.1% NP-40, 1 mM phenylmethylsulfonylfluoride (PMSF) and 1 × protease inhibitor cocktail (Roche)). Protein content was quantified by the Bradford assay (Bradford, 1976). Equal amounts of protein were subjected to sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by Western blotting analysis. Immunodetection of RGA was performed using anti-RGA (Agrisera, AS11 1630, Vännäs, Sweden). Rabbit anti-goat immunoglobulin G horseradish peroxidase (IgG HRP) conjugate secondary antibody (BioRad) was used for detection.

### Statistics and data analysis

Statistical analyses were carried out using INFOSTAT software (InfoStat/Professional version 1.1, Universidad Nacional de Córdoba, Córdoba, Argentina). Data were analyzed using factorial ANOVA, with light treatment, MeJA or genotype as factors. Differences between means were tested using a *post-hoc* Tukey test only when the relevant interaction terms were significant in the ANOVA. Appropriate transformations of the primary data were used when needed to meet the assumptions of the analysis. The number of independent experiments ($n$) used to calculate the treatment means are indicated in the relevant figure legends. In each experiment, 20 (growth and pigment analyses) or four to five (protein stability) individual seedlings were pooled to obtain the response value for each treatment/genotype combination. Therefore, the physiological results are based on measurements of between 100 and 200 seedlings per treatment/genotype combination, and the protein stability data (Western blot and GUS activity data) on tissue obtained from 15–25 seedlings per treatment/genotype combination.

## Results

### JA inhibits seedling growth and promotes the accumulation of phenolic compounds, and these effects are eliminated by low R : FR ratios acting through phyB

In de-etiolating 7-d-old Arabidopsis seedlings, JA treatment (50 µM MeJA) inhibited hypocotyl elongation and seedling growth (biomass accumulation), and induced the accumulation of soluble leaf phenolics (putative defenses). These responses to JA required a functional COI1–JAZ co-receptor module (Fig. S2). For all growth and defense readouts, the JA effect was absent in plants grown under low R : FR (WL + FR) or in *phyB* mutants (Fig. 1b–d). The hypocotyl elongation results are in contrast with those reported in another study, where increased JA-induced growth inhibition was apparently stronger in *phyB* than in Col-0 (see Fig. S5 in Hou *et al.*, 2010), but they agree well with those reported by Chen *et al.* (2013), and are consistent with the majority of the evidence indicating that phyB Pfr is a positive regulator of JA sensitivity (Ballaré, 2011). The interactive effects of the R : FR ratio and MeJA treatments on growth and metabolite readouts (Fig. 1) were qualitatively similar to those obtained for responses at the gene expression level under our *in vitro* system (Fig. S3), and were therefore used throughout this study to evaluate phyB-induced changes in JA sensitivity.

### Growth and phenolic responses to JA require DELLA

DELLA proteins are positive regulators of JA responses (Navarro *et al.*, 2008; Hou *et al.*, 2010; Yang *et al.*, 2012). We tested the role of DELLAs in our system by comparing JA responses between wild-type (Ler-0) and quintuple *della* seedlings (*5× della*).The Ler-0 wild-type responded to JA and low R : FR ratio in the same way as the Col-0 seedlings used as controls in previous experiments: treatment with 50 µM MeJA inhibited growth and promoted the accumulation of soluble phenolics, and low R : FR canceled the effect of JA on growth and defense (Fig 2a,c,e). Under the conditions used in our experiments, *5× della* mutant seedlings were not taller than Ler-0 seedlings. This is probably an effect of the LD photoperiod, because, under SD, *5× della* hypocotyls were *c.* 25% longer than those of Ler-0 seedlings (Fig. 2b). Regardless of photoperiod, MeJA failed to inhibit hypocotyl elongation (Fig. 2a,b) and biomass accumulation (Fig. 2c,d) in the *5× della* mutant. The mutant also failed to respond to MeJA with increased accumulation of leaf phenolics (Fig. 2e,f). The lack of growth inhibition in *5× della* is consistent with previous observations (Yang *et al.*, 2012).The lack of a phenolic response to JA in *5× della* has not been reported previously. A quadruple *della* mutant (*4× della*: *gai-t6 rga-t2 rgl1-1 rgl2-1*), showed a similar lack of sensitivity to JA for the physiological responses measured in this study (Fig. S4), suggesting that this lack of response to JA in *5× della* is not exclusively caused by the lack of RGA-LIKE3 (RGL3), which is known to be a positive modulator of JA signaling (Wild *et al.*, 2012).

### Low R : FR ratios trigger rapid DELLA turnover

Previous work using *pRGA::GFP–RGA* (Djakovic-Petrovic *et al.*, 2007) and *pUBQ10::mCITRINE-RGA* or *pUBQ10::mCITRINE-GAI* (Y. Jaillais & J. Chory, unpublished) *Arabidopsis* plants demonstrated that low R : FR ratios reduce DELLA abundance in petioles and hypocotyls, presumably as a consequence of increased GA signaling. These previous experiments generally used prolonged irradiation treatments, and it is unclear whether

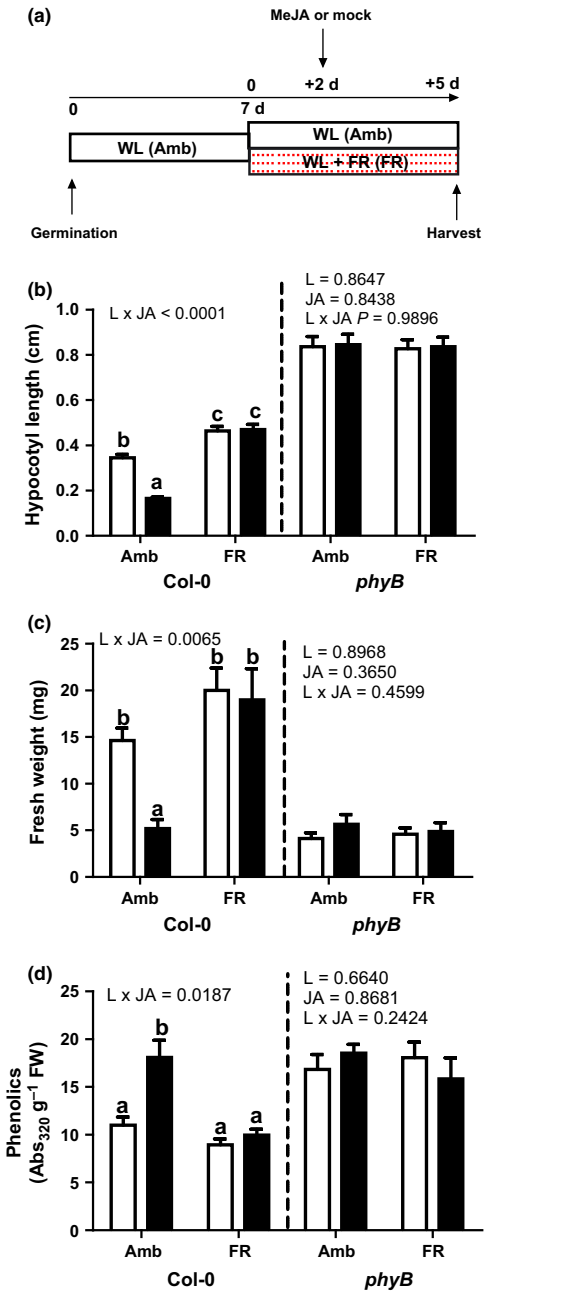


**Fig. 1** Experimental protocol and interactive effects of jasmonate (JA) and phytochrome B (phyB) inactivation on Arabidopsis growth and defense. (a) Schematic representation of the experimental protocol. Seedlings were germinated and grown for 7 d under white light (WL), and then transferred to the test light conditions: WL (Amb) or far-red (FR) (WL supplemented with FR radiation, WL + FR). Two days after transfer, seedlings were treated with methyl jasmonate (MeJA) (50 μM) or a mock solution, and left for three additional days under the light treatments before being harvested for the quantification of growth and defense responses. (b) Hypocotyl length. (c) Seedling biomass (fresh weight). (d) Accumulation of soluble phenolic compounds. Seedlings were grown under long days (14 h WL: 10 h darkness) unless indicated otherwise. Open bars, control; closed bars, JA. Error bars, +1 SE; $n = 8$–10. Significant terms in the factorial analysis of variance are indicated for each response variable with their associated $P$ value: L, light treatment (Amb vs FR); JA (MeJA vs mock). When the L × JA interaction term was statistically significant, means were separated using the Tukey test, and different letters indicate significant differences between treatment means.

the effects of low R : FR on DELLA degradation are sufficiently fast to account for the rapid effects of phyB manipulations on JA sensitivity. We used an anti-RGA antibody to monitor DELLA stability in whole seedlings under the experimental conditions described in Fig. 1a. Immunoblot analysis demonstrated that the low R : FR ratios caused a rapid (within minutes) reduction in RGA protein abundance, and that the *phyB* mutant had constitutively low RGA levels (Fig. 3).

## The effect of low R : FR ratios repressing JA-mediated responses is missing in a DELLA gain-of-function mutant, *gai-1*

Having determined that phyB inactivation causes a rapid decline in DELLA levels, we reasoned that DELLA degradation could mediate the effect of low R : FR canceling JA responses, as DELLAs can interfere with the ability of JAZ proteins to interact with their target transcription factors (Hou *et al.*, 2010). We tested this hypothesis with the GA-insensitive, gain-of-function *gai-1* mutant (Koorneef *et al.*, 1985), which encodes a version (gai) of the GAI DELLA protein that is resistant to GA-induced degradation (Peng & Harberd, 1997). The effects of low R : FR ratio, canceling JA-induced growth inhibition (Fig. 4a,b) and the accumulation of leaf phenolics (Fig. 4c), were clearly missing in *gai-1*.

## JAZ10 links phyB inactivation with repression of JA sensitivity

It has been shown recently that the effect of simulated shadelight decreasing *Arabidopsis* resistance to the necrotrophic pathogen *Botrytis cinerea* is missing in lines disrupted in the expression of the *JAZ10* gene (Cerrudo *et al.*, 2012; see also Cargnel *et al.*, 2014). Because plant defenses against necrotrophs are orchestrated by JA, we investigated the role of JAZ10 in the cross-talk between phyB and JA signaling. Previous work in de-etiolating Arabidopsis seedlings has demonstrated a requirement for phyA, a member of the phy family not involved in R : FR responses, in the degradation of another JAZ protein: JAZ1 (Robson *et al.*, 2010). More recently, FR supplementation has been shown to reduce the turnover of several JAZ proteins (including JAZ1 and JAZ10) (Chico *et al.*, 2014). We tested the effects of the R : FR treatment used in our physiological experiments on JAZ10 turnover in a transgenic line in which JAZ10–GUS was expressed under the 35S constitutive promoter (*35S::JAZ10-GUS*) (Chico *et al.*, 2014). In preliminary experiments, we found that JAZ10–GUS levels were significantly higher in plants receiving light with a low R : FR ratio than in plants grown under WL. The effect of low R : FR increasing JAZ10 levels persisted even after 30 min of leaf wounding (Fig. 5). Seedlings treated with MeJA (20 μM) displayed a rapid turnover of JAZ10–GUS, as expected (Fig. 6). When the kinetics of JAZ10–GUS levels were measured after transferring Arabidopsis seedlings from high to low R : FR ratio (ambient light → ambient light supplemented with FR), we found that GUS activity tended to accumulate if the seedlings were not exposed to MeJA. Moreover, FR supplementation counteracted the negative effect of MeJA (20 μM) on JAZ10–GUS

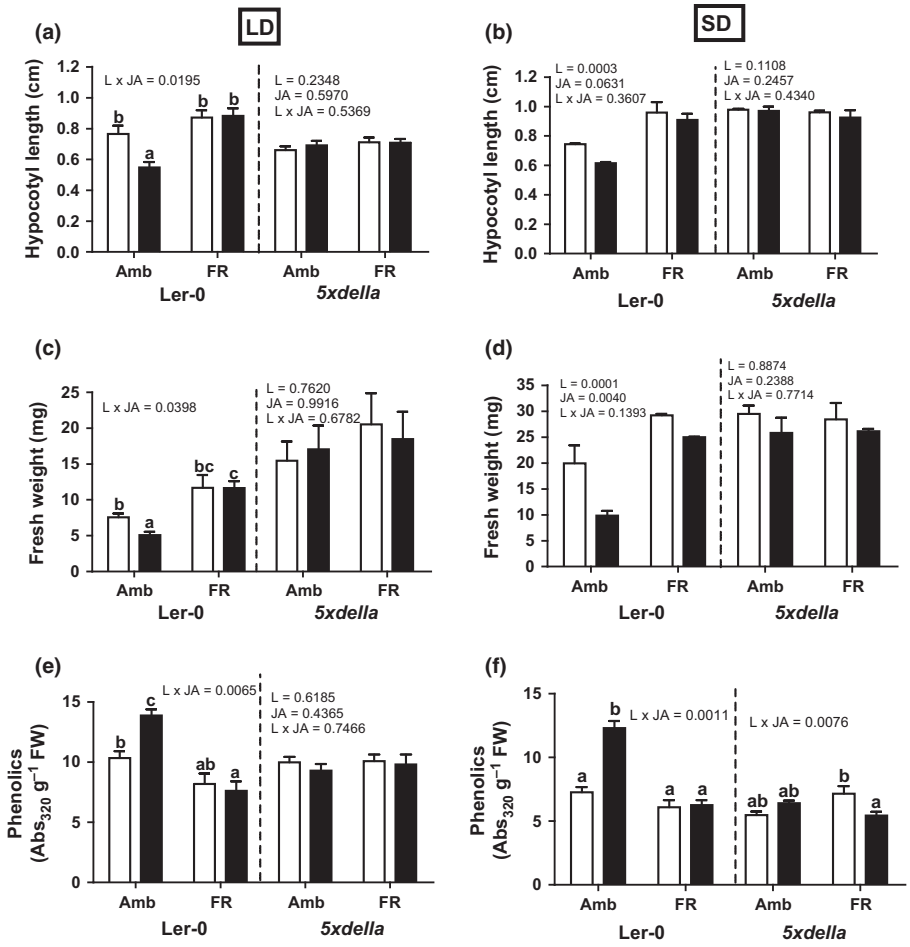


**Fig. 2** DELLAs are required for Arabidopsis growth and defense responses to jasmonate (JA) under long day (LD; 14 h white light (WL) : 10 h darkness) and short day (SD; 8 h WL : 16 h darkness) conditions. Open bars, control; closed bars, JA. (a, b) Hypocotyl length. (c, d) Seedling biomass (fresh weight). (e, f) Accumulation of soluble phenolic compounds. Irradiation and methyl jasmonate (MeJA) treatment protocol as in Fig. 1(a). Error bars, +1 SE; $n = 8$. Significant terms in the factorial analysis of variance are indicated for each response variable with their associated $P$ value: L, light treatment (Amb vs far-red (FR)); JA (MeJA vs mock). When the L × JA interaction term was statistically significant, means were separated using the Tukey test, and different letters indicate significant differences between treatment means.

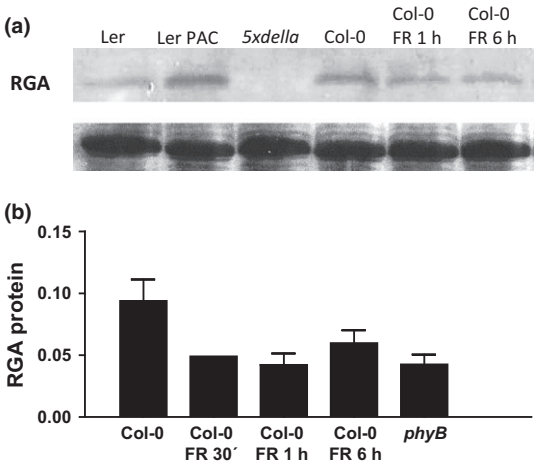


**Fig. 3** Far-red (FR) supplementation of white light triggers DELLA (RGA) protein degradation. (a) Turnover of the RGA protein in 12-d-old Arabidopsis seedlings treated with supplemental FR radiation for 1 or 6 h. The *5×della* mutant (Ler background) was used as a negative control; Ler + paclobutrazol (PAC), which is a gibberellin (GA) biosynthesis inhibitor, was used as a positive control. RGA protein was detected with an anti-RGA antibody. Image is from a representative experiment; Coomassie blue staining of the gel was used as loading control. (b) Quantification of RGA levels relative to the relevant loading controls at different times after treatment with supplemental FR and in the *phyB* mutant. Error bars, +1SE; values are means of three independent experiments ($n = 3$).

stability (Fig. 6). Finally, JA-induced degradation of JAZ10–GUS was slower in the *phyB* background that in the Col-0 background (Fig. 7). We found no evidence of an effect of alteration of R : FR on JAZ1-GUS protein stability (Fig. S5), suggesting that JAZ1 turnover is not affected by reductions in R : FR ratio that simulate the proximity of non-shading neighbors.

We next used a genetic approach to test whether *JAZ10* is required for the effect of low R : FR ratios decreasing JA sensitivity. We used a *jaz10* mutant and an independent line in which *JAZ10* expression was down-regulated by RNAi (Yan *et al.*, 2007). In both genotypes (*jaz10* and RNAi7), the effect of low R : FR ratio depressing JA responses was significantly attenuated or missing, and this was true for JA-induced growth inhibition and accumulation of phenolic compounds (Figs 8, S6). Interestingly, exploration of the phenotype of other *jaz* single mutants (*jaz8*, *jaz9*, see the Materials and Methods section) did not reveal FR-insensitive phenotypes (Figs S7, S8).

These experiments demonstrate that the effect of low R : FR ratios reducing JA sensitivity requires *JAZ10*, which could provide a functional explanation for the bioassay results reported by Cerrudo *et al.* (2012). To further test the role of *JAZ10*, we introgressed the *jaz10* mutation in the *phyB* background and tested the double mutant for JA response phenotypes. The *phyB jaz10* double mutant displayed an elongated (SAS) phenotype that was essentially

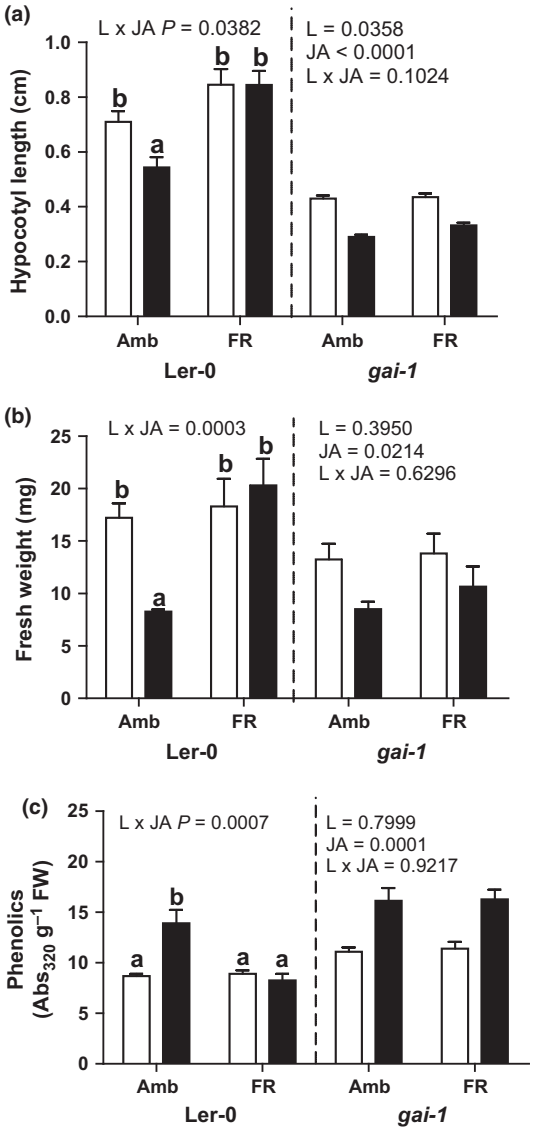


**Fig. 4** DELLA (GAI) turnover is required for the inhibitory effect of low red : far-red (R : FR) ratios on jasmonate (JA) response in Arabidopsis seedlings. Open bars, control; closed bars, JA. (a) Hypocotyl length. (b) Seedling biomass (fresh weight). (c) Accumulation of soluble phenolic compounds. Irradiation and methyl jasmonate (MeJA) treatment protocol as in Fig. 1(a). Error bars, +1 SE; $n = 6$–8. Significant terms in the factorial analysis of variance are indicated for each response variable with their associated $P$ value: L, light treatment (Amb vs FR); JA (MeJA vs mock). When the L × JA interaction term was statistically significant, means were separated using the Tukey test, and different letters indicate significant differences between treatment means.

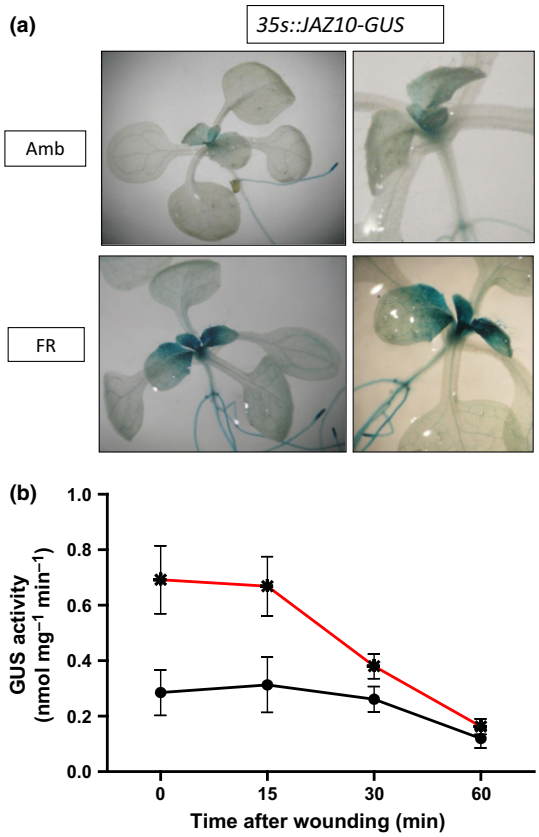


**Fig. 5** Low red : far-red (R : FR) ratios increase the levels of JAZ10-GUS and retard JAZ10-GUS degradation triggered by leaf wounding in Arabidopsis seedlings. (a) Photographs of *35S::JAZ10-GUS* 2-wk-old plants taken 4 h after transfer to the white light (WL) + FR treatment (FR); seedlings that remained under WL (Amb) are shown for comparison. Plants were cultivated in Murashige and Skoog (MS) medium with 1% sucrose in clear plastic boxes under WL and short-day (SD: 8 h WL : 16 h darkness) conditions. Entire plants were carefully harvested and subjected to histochemical β-glucuronidase (GUS) staining (see the Materials and Methods section) and photographed. (b) Kinetics of JAZ10-GUS degradation after wounding seedlings of the FR (red) and Amb (black) treatments with a small forceps, as indicated in the Materials and Methods section. Bars, ±1SE; $n = 11$–14.

identical to that of the *phyB* simple mutant (Fig. 9a). Remarkably, however, the double mutant regained sensitivity towards JA for the markers analyzed (hypocotyl, growth inhibition and accumulation of soluble phenolics) (Fig. 9). These results demonstrate that *jaz10* is epistatic to *phyB* for attenuation of JA sensitivity.

## Discussion

Down-regulation of JA responses by low R : FR ratios is a central mechanism by which shade-intolerant plants redirect resources from defense into rapid growth when they face an increased risk of competition, which has important implications for crop health in modern agricultural settings (Ballaré *et al.*, 2012). Our experiments suggest that the molecular basis of this reduced JA sensitivity in plants exposed to low R : FR ratios involves a shift in the balance between DELLA and JAZ proteins, which results in repression of the JA pathway. The principal lines of experimental evidence supporting this model (Fig. 10) are discussed below.

The rapid turnover of DELLA proteins, as demonstrated in this study for RGA (Fig. 3), could provide a mechanism by which phyB inactivation at high canopy density suppresses JA-induced defenses. Our data support this mechanism by showing that: (1) for several physiological outputs connected with growth (inhibition of hypocotyl elongation and fresh weight accumulation) and putative defense (accumulation of phenolic compounds), a *5× della* mutant was indistinguishable from the *phyB* mutant in terms of its lack of responses to MeJA (Fig. 2); and (2), the *gai-1*

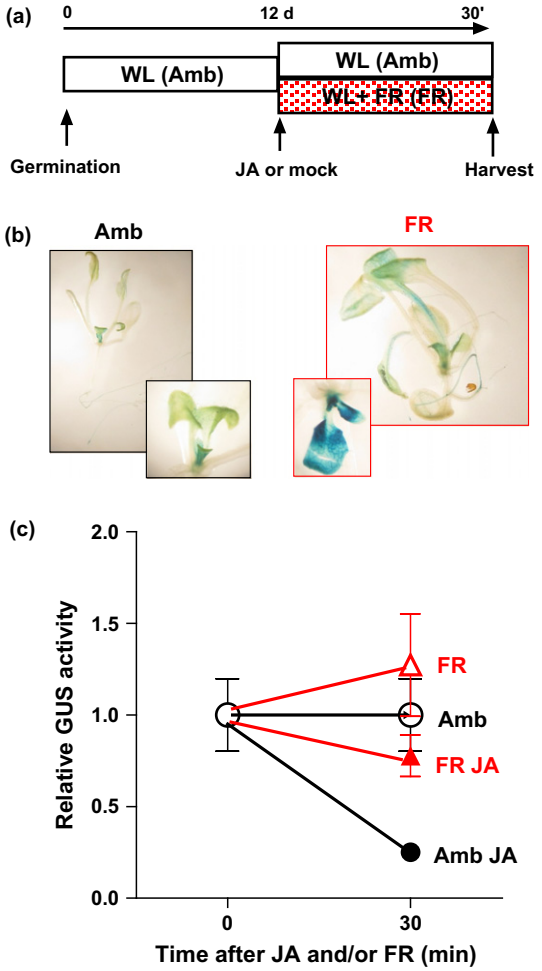


**Fig. 6** Low red : far-red (R : FR) ratios increase JAZ10 protein stability in Arabidopsis seedlings. (a) Protocol used to evaluate the effects of light quality on JAZ10 kinetics. Seedlings were germinated and grown for 12 d under white light (WL) (14 h WL : 10 h darkness). Seedlings were treated with methyl jasmonate (MeJA; 20 μM) or a mock solution and transferred to the test light conditions: WL (Amb) or WL + FR (FR). After 30 min, the seedlings were harvested for measurement of β-glucuronidase (GUS) activity. (b) Representative photographs of *35S::JAZ10-GUS* seedlings after 4 h under Amb or FR light conditions (no JA treatment). (c) Kinetics of JAZ10-GUS under the four combinations of light and hormone treatment. Values are given relative to the GUS activity value at time zero. Bars, ±1 SE; $n = 7$.

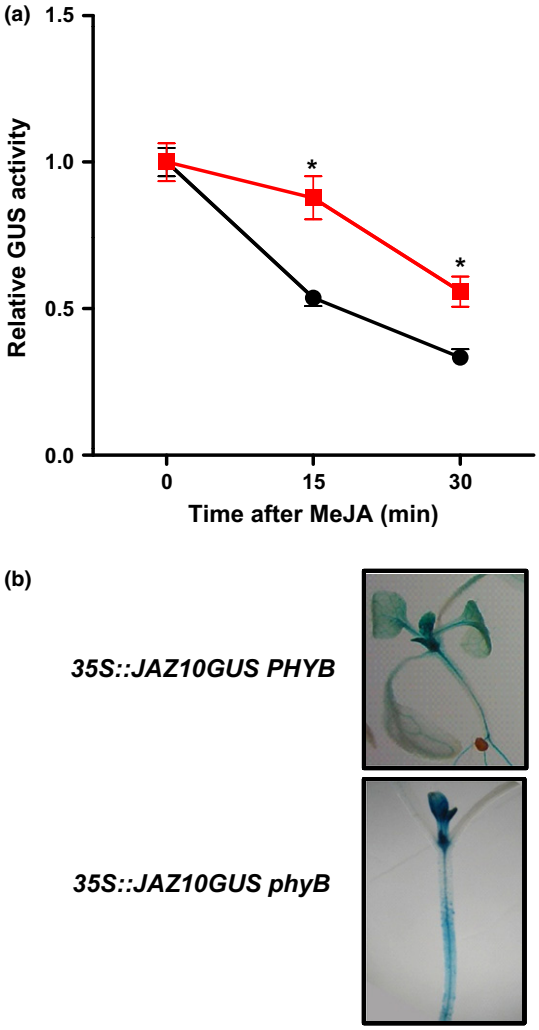


**Fig. 7** JAZ10 protein stability increases in the *phyB* background. (a) Dynamics of β-glucuronidase (GUS) activity in response to methyl jasmonate (MeJA). Arabidopsis seedlings were germinated and grown for 2 wk on Murashige and Skoog (MS) medium with 1% sucrose under white light (WL) (8 h WL : 16 h darkness). Seedlings were treated with MeJA (20 μM) or a mock solution and harvested at the indicated times for measurement of enzymatic activity. Activity values are given relative to the initial value. Black circles, *35S::JAZ10-GUS PHYB*; red squares *35S::JAZ10-GUS phyB*. Bars, ±1 SE; asterisks indicate significant differences between treatment means ($P < 0.05$; $n = 14$–18). (b) Representative images of *35S::JAZ10-GUS PHYB* (top) and *35S::JAZ10-GUS phyB* (bottom) seedlings (note the elongated phenotype of the *35S::JAZ10-GUS phyB* line).

mutant, which encodes a stable version of the GAI DELLA protein (Peng & Harberd, 1997), failed to attenuate JA responses when exposed to low R : FR ratios (Fig. 4). The latter result suggests that, at least for the physiological outputs measured in this study, stabilization of GAI is sufficient to eliminate the low R : FR effect. It should be noted that, in older plants (4-wk-old), grown in soil, MeJA has measurable inhibitory effects on the growth of both *phyB* and *5×della* mutants (I. Cerrudo *et al.*, unpublished). These results suggest that ontogeny or stress conditions may change the relative importance of DELLAs in the model proposed in Fig. 10 for the modulation of JA signaling.

DELLA proteins positively regulate JA defense signaling (Navarro *et al.*, 2008) by interfering with the ability of JAZ proteins to interact with their target transcription factors (Hou *et al.*, 2010). Therefore, the rapid DELLA turnover triggered by low R : FR ratios documented here (Fig. 3) is predicted to release JAZ proteins from JAZ–DELLA complexes, thereby facilitating the repression of JA responses (Fig. 10). In addition to reducing DELLA abundance, low R : FR ratios will increase the availability of (growth-promoting) bHLH transcription factors, known as PHYTOCHROME INTERACTING FACTORS (PIF) (Lorrain *et al.*, 2008; Hornitschek *et al.*, 2012; Li *et al.*, 2012). As PIFs interact with (and are repressed by) DELLAs (de Lucas *et al.*, 2008; Feng *et al.*, 2008), an increase in PIF protein levels under low R : FR ratios is predicted to decrease the number of DELLA molecules available for

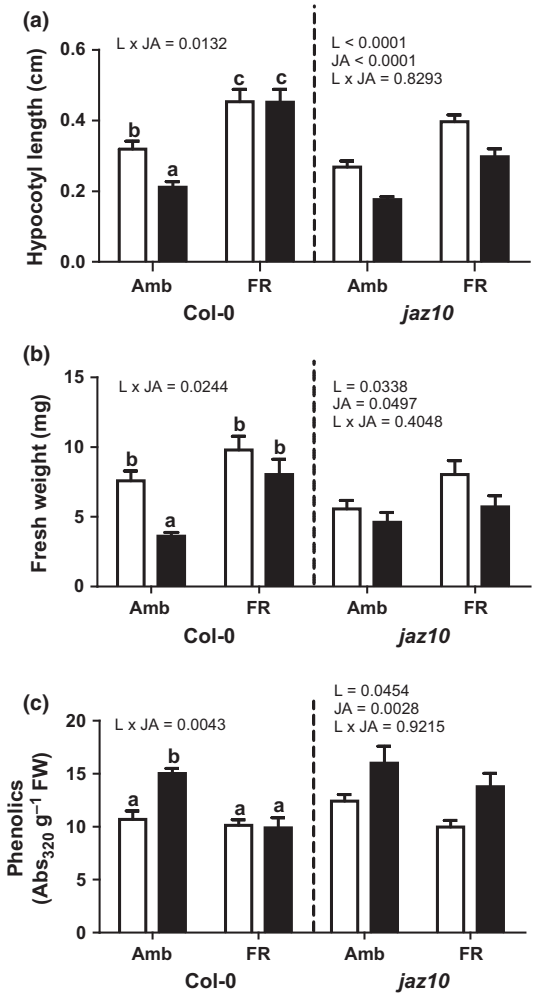


**Fig. 8** Low red : far-red (R : FR) ratios fail to inhibit jasmonate (JA) responses in a *jaz10* mutant. (a) Hypocotyl length. (b) Seedling biomass (fresh weight). (c) Accumulation of soluble phenolic compounds. Irradiation of Arabidopsis seedlings and methyl jasmonate (MeJA) treatment protocol as in Fig. 1(a). Open bars, control; closed bars, JA. Error bars, +1 SE; $n = 6$–8. Significant terms in the factorial analysis of variance are indicated for each response variable with their associated *P* value. L, light treatment (Amb vs FR); JA (MeJA vs mock). When the L × JA interaction term was statistically significant, means were separated using the Tukey test, and different letters indicate significant differences between treatment means.

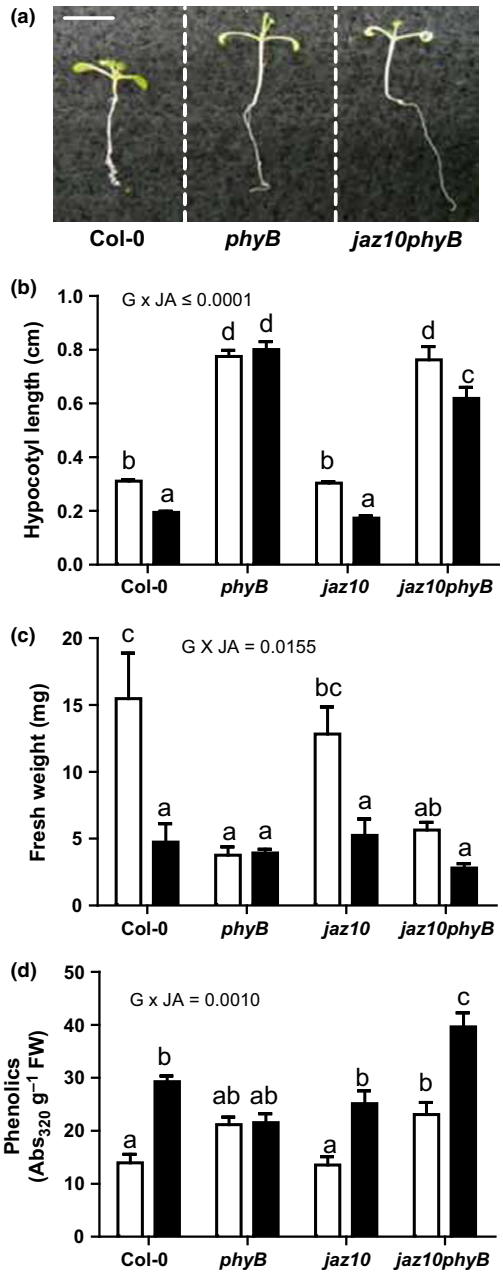


**Fig. 9** The *jaz10* mutation rescues the jasmonate (JA)-insensitive phenotype of a *phyB* mutant. (a) Photographs of 10-d-old Arabidopsis seedlings showing that the constitutive expression of the shade-avoidance syndrome (SAS) phenotype is similar in the simple *phyB* mutant and in the double *jaz10 phyB* mutant. Bar, 1 cm. (b) Hypocotyl length. (c) Seedling biomass (fresh weight). (d) Accumulation of soluble phenolic compounds. Irradiation and methyl jasmonate (MeJA) treatment protocol as in Fig. 1(a). Open bars, control; closed bars, JA. Error bars, +1 SE; $n = 5$. Significant terms in the factorial analysis of variance are indicated for each response variable with their associated *P* value: G, genotype; JA (MeJA vs mock). Primary data for fresh weight were log-transformed to meet the assumptions of the ANOVA. When the G × JA interaction term was statistically significant, means were separated using the Tukey test, and different letters indicate significant differences between treatment means.

interaction with JAZ, which will result in further suppression of JA responses (Fig. 10).

The repression of JA responses by low R : FR ratios is unlikely to be solely dependent on changes in DELLA availability. We found that the low R : FR treatment that is effective in suppressing JA responses (e.g. Fig. 1) produces an increase in JAZ10–GUS protein levels (Figs 5, 6), which is consistent with a recent report (Chico *et al.*, 2014), and we further demonstrated that low R : FR ratios reduce the rate of JAZ10–GUS degradation elicited by wounding and MeJA treatment (Figs 5, 6). In the light of our physiological experiments (Figs 8, 9), this increased JAZ stability is predicted to have a significant effect, attenuating JA responses. It is unclear why we did not find stabilization of JAZ1-GUS by low R : FR, as reported by Chico *et al.* (2014), but the difference could be related to the use of different *35S:JAZ1-GUS* lines, or to the fact that the FR treatment used by Chico *et al.* (2014) resulted in a much lower R : FR ratio (0.2) than that used in our

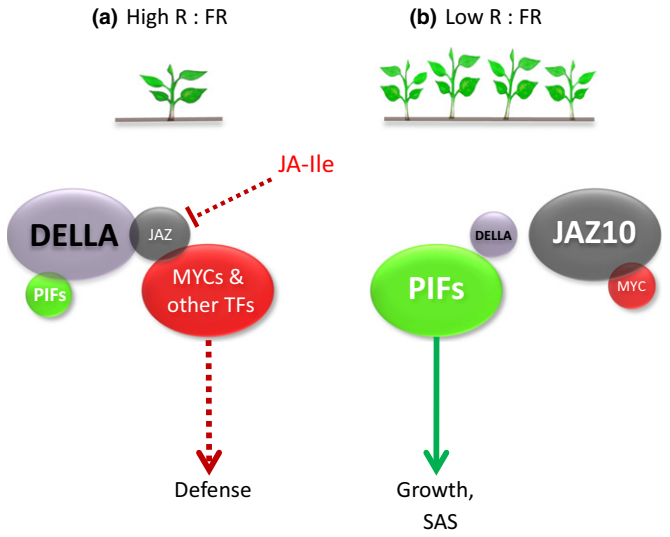


**Fig. 10** A regulatory hub for growth and defense? Current model of the mechanism by which low red : far-red (R : FR) ratios affect the activity of key jasmonate (JA) signaling players and antagonize JA responses in Arabidopsis seedlings. (a) Under high R : FR, phyB Pfr levels are high, which reduce the levels and activity of PHYTOCHROME INTERACTING FACTORS (PIFs) (Lorrain *et al.*, 2008; Hornitschek *et al.*, 2012; Li *et al.*, 2012; Park *et al.*, 2012). PIFs are also inhibited by abundant DELLA proteins (Feng *et al.*, 2008; de Lucas *et al.*, 2008), which, in addition, keep JAZ proteins from repressing their target transcription factors, such as MYCs (Hou *et al.*, 2010). Under these conditions, elongation growth is repressed and JA-mediated defense responses can be readily activated following JA-induced JAZ protein degradation. (b) Under low R : FR ratios, PIF levels and activity increase, and DELLAs are rapidly degraded (Fig. 3), thereby promoting the shade-avoidance syndrome (SAS). DELLA degradation frees up JAZ proteins, which are also stabilized (Fig. 6, see also Chico *et al.*, 2014) and are therefore present and available to repress defense-activating transcription factors. Under these conditions, MYC levels can also be reduced (Chico *et al.*, 2014), which further suppress defense responses. TF, transcription factor.

experiments (0.55 in the radiation coming from the sides of the plant). Importantly, an additional layer of regulation uncovered by Chico *et al.* (2014) is the de-stabilization of MYC transcription factors under their low R : FR treatment, which should further contribute to the attenuation of JA responses (Fig. 10).

There are 12 *JAZ* genes in the Arabidopsis genome, and genetic studies have revealed a great deal of functional redundancy among the various JAZ proteins. Single knockouts in the *JAZ1*, *JAZ2*, *JAZ5*, *JAZ7* and *JAZ9* loci failed to produce JA-related phenotypes in Arabidopsis (Thines *et al.*, 2007; Demianski *et al.*, 2012), although, in *Populus*, stabilization of a single JAZ protein (PtJAZ6) appeared to be sufficient to block JA signaling during the interaction between the plant and the mutualistic fungus *Laccaria bicolor* (Plett *et al.*, 2014). In terms of interactions with DELLAs, several Arabidopsis JAZ proteins, including JAZ1, JAZ3, JAZ4, JAZ9, JAZ10 and JAZ11, have been shown to interact with different strengths with the conserved GRAS domain of DELLA proteins (Hou *et al.*, 2010; Yang *et al.*, 2012). We focused on JAZ10 because previous work had shown that the FR-induced increase in Arabidopsis sensitivity to the necrotrophic fungal pathogen *B. cinerea* was missing in *jaz10* and two RNAi lines disrupted for the expression of *JAZ10* (Cerrudo *et al.*, 2012; see also Cargnel *et al.*, 2014). The present data clearly demonstrate that *JAZ10* is required to mediate the repressive action of low R : FR on several JA responses (Figs 8, S6), and further show that the low sensitivity of *phyB* mutant seedlings to MeJA can be rescued by the *jaz10* mutation, although the presence of the *jaz10* mutation does not reverse the constitutive SAS phenotype of the *phyB* mutant (Fig. 9). Other *jaz* mutations tested in our experiments (*jaz8* and *jaz9*) failed to produce an FR-insensitive phenotype (Figs S7, S8). Collectively the genetic evidence obtained in this study indicates that the protein JAZ10 plays a key role linking phyB inactivation with the attenuation of JA signaling in Arabidopsis seedlings.

We do not know whether the apparently distinct function of JAZ10 in linking phyB and JA signaling is related to the fact that alternative splicing within the Jas domain results in two JAZ10 isoforms (JAZ10.3 and JAZ10.4) that are relatively stable in the presence of bioactive JA (Chung & Howe, 2009; Chung *et al.*, 2010). If supplemental FR results in increased levels of these isoforms, this could hint at a plausible mechanism for JA de-sensitization. The availability of Arabidopsis lines in which the various splice variants of JAZ10 are expressed from the native *JAZ10* promoter in the *jaz10* mutant background (Moreno *et al.*, 2013) provides tools to understand the potential role of these proteins in attenuating JA signaling under low R : FR ratios.

## Conclusion

Down-regulation of JA signaling under light conditions that indicate a high risk of competition has been documented for a wide range of physiological responses in several species. Using a highly simplified setup to test for JA responses in young Arabidopsis seedlings, we conclude that DELLA turnover and increased JAZ10 stability play an important role in the molecular mechanism by which JA signaling is repressed in seedlings exposed to low R : FR ratios. The evidence obtained in this and other recent studies suggests that the regulation of JA responses by competition signals has multiple levels of control, which include: a reduced DELLA pool available for interactions with JAZ proteins; increased JAZ stability; and increased turnover of MYC transcription factors (Fig. 10). In addition, the effects of phyB inactivation have been shown to be local (i.e. restricted to the plant parts that experience a low R : FR ratio; Izaguirre *et al.*, 2013). This regulation at multiple molecular levels and at a (local) modular scale may provide a powerful mechanism to fine tune the strength of the JA-mediated defense response as a function of the intensity and spatial distribution of the light signals that indicate a threat of competition.

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## Supporting Information

Additional supporting information may be found in the online version of this article.

**Fig. S1** Experimental setup used to test the interactive effects of the red : far-red (R : FR) ratio and jasmonate (JA) on growth and defense in Arabidopsis seedlings.

**Fig. S2** Methyl jasmonate (MeJA)-induced growth inhibition and accumulation of soluble phenolic compounds are missing in the *coi1-1* mutant and in a transgenic line that constitutively expresses the JAZ10.4 splice variant of the JAZ10 protein (Chung & Howe, 2009).

**Fig. S3** Interactive effects of red : far-red (R : FR) ratio and jasmonate (JA) treatment on expression of JA marker genes in the experimental setup depicted in Fig. S1.

**Fig. S4** A quadruple *della* mutant (*4× della, gai-t6 rga-t2 rgl1-1 rgl2-1*) has a jasmonate (JA)-insensitive phenotype that resembles that of the *5× della* (*gai-t6 rga-t2 rgl1-1 rgl3-1 rgl2-1*) mutant.

**Fig. S5** Low red : far-red (R : FR) ratios do not increase JAZ1 protein stability.

**Fig. S6** Low red : far-red (R : FR) ratios fail to inhibit jasmonate (JA) responses in a RNAi line disrupted for the expression of the *JAZ10* gene.

**Fig. S7** The effect of low red : far-red (R : FR) ratios antagonizing jasmonate (JA) responses was fully conserved in a *jaz8* mutant.

**Fig. S8** The effect of low red : far-red (R : FR) ratios antagonizing jasmonate (JA) responses was fully conserved in the *jaz9-1* mutant.

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