Exploring growth-defense tradeoffs in Arabidopsis. Phytochrome B inactivation requires JAZ10 to suppress plant immunity but not to trigger shade avoidance responses

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ABSTRACT

Under conditions that involve a high risk of competition for light among neighboring plants, shade-intolerant species often display increased shoot elongation and greater susceptibility to pathogens and herbivores. The functional links between morphological and defense responses to crowding are not well understood. In Arabidopsis, the protein JAZ10 is thought to play a key role connecting the inactivation of the photoreceptor phytochrome B (phyB), which takes place under competition for light, with the repression of jasmonate-mediated plant defenses. Here we show that a null mutation of the *JAZ10* gene in Arabidopsis did not affect plant growth nor did it suppress the shade-avoidance responses elicited by phyB inactivation. However, the *jaz10* mutation restored many of the defense traits that are missing in the *phyB* mutant, including the ability to express robust responses to jasmonate and to accumulate indolic glucosinolates. Furthermore, the *jaz10phyB* double mutant showed a significantly increased resistance to the pathogenic fungus *Botrytis cinerea* compared with the *phyB* parental line. Our results demonstrate that, by inactivating *JAZ10*, it is possible to partially uncouple shade avoidance from defense suppression in Arabidopsis. These findings may provide clues to improve plant resistance to pathogens in crops that are planted at high density.

Key words: Jasmonate; light quality; pathogens; plant immunity; red/far-red ratio; signaling.

SUMMARY STATEMENT

THE PHOTORECEPTOR PHYTOCHROME B (PHYB) IS A KEY MODULATOR OF ADAPTIVE PLASTICITY IN PLANT CANOPIES. LOW R:FR RATIOS, WHICH INDICATE PROXIMITY OF COMPETITORS, INACTIVATE PHYB AND PROMOTE SHOOT ELONGATION AND THE SHADE-AVOIDANCE SYNDROME. AT THE SAME TIME, LOW R:FR RATIOS DOWN-REGULATE PLANT DEFENSES AGAINST PATHOGENS AND PESTS, PRESUMABLY TO SAVE RESOURCES FOR RAPID GROWTH. HERE, WE ADDRESS THE FUNCTIONAL CONNECTIONS BETWEEN THESE TWO EFFECTS OF PHYB INACTIVATION. WE FOUND THAT JAZ10, ONE OF THE MEMBERS OF THE JAZ FAMILY OF REPRESSOR PROTEINS, IS REQUIRED FOR THE SUPPRESSION OF PLANT DEFENSES TRIGGERED BY PHYB INACTIVATION, BUT NOT FOR THE PROMOTION OF ELONGATION. OUR RESULTS DEMONSTRATE THAT IT IS POSSIBLE TO UNCOUPLE SHADE AVOIDANCE FROM DEFENSE SUPPRESSION IN ARABIDOPSIS VIA INACTIVATION OF JAZ10, AND MAY PROVIDE CLUES TO IMPROVE PLANT RESISTANCE TO PATHOGENS IN HIGH DENSITY CROPS.

INTRODUCTION

Defense responses in plants are frequently associated with reduced growth potential, presumably because these responses take up a significant amount of carbon and nutrients (Baldwin, 1998, Redman *et al.*, 2001, Zavala *et al.*, 2004, Zavala & Baldwin, 2006, Cipollini, 2007, Yan *et al.*, 2007, Ballhorn *et al.*, 2014). Conversely, fast growth is commonly associated with low levels of chemical defense and increased susceptibility to herbivory and pathogen attack (Cipollini, 1997, Kurashige & Agrawal, 2005, Donaldson *et al.*, 2006, Izaguirre *et al.*, 2006). In shade-intolerant species, conditions of high density or shading often result in increased disease incidence (Burdon & Chilvers, 1982, Augspurger & Kelly, 1984), and part of this effect of high density is thought to be mediated by reduced plant resistance to pathogen attack (reviewed in Roberts & Paul, 2006, Ballaré, 2014). Down-regulation of defense at high density may represent an evolved strategy that helps the plant to focus limited resources on those activities or plant organs that are more likely to increase the capture of new resources in a scenario of high competition. However, in agriculture, this repression of defense at high density may have negative impacts on crop health (Ballaré *et al.*, 2012, Anten & Vermeulen, 2016), and might be one of the factors that explains why modern crops, which are planted a very high density, require large inputs of pesticides (Oerke, 2006). In addition, accumulating evidence suggests that, during the course of plant domestication and crop improvement, there has been a gradual loss of defense-related traits (Rosenthal & Dirzo, 1997, Rasmann *et al.*, 2005, Rodriguez-Saona *et al.*, 2011, Dávila-Flores *et al.*, 2013), presumably because while focusing on selection for fast growth and yield, farmers and breeders have inadvertently selected against the expression of costly defenses.

Many plant responses to changes in population density are mediated by the photoreceptor phytochrome B (phyB). This photoreceptor continuously monitors the red (R) to far-red (FR) ratio (R:FR ratio) of the light received by the plant. Under conditions of leaf shading or high planting density, preferential absorption of R light by chlorophyll reduces the R:FR ratio, which causes a reduction in the proportion of phyB molecules that are in their active (Pfr) form. This depletion of active phyB is used by the plant as a reliable signal of actual or potential competition, and activates an escape strategy known as the shade-avoidance syndrome, or SAS (Smith, 1995, Ballaré, 1999, Casal, 2012, Pierik & de Wit, 2014, Fraser *et al.*, 2016). SAS is characterized by increased stem and petiole elongation and changes in leaf angles that, in a crowded stand, tend to maximize the likelihood of light interception for the individual plant. In response to phyB inactivation plants also down-regulate defenses and become more susceptible to pathogens and herbivores (reviewed in Ballaré, 2014).

An important question is whether or not the reduction of plant resistance to herbivores and pathogens is a consequence of SAS (for example an unavoidable byproduct of redirecting resources to rapid growth) or whether shoot elongation responses and defense repression are correlated but triggered through at least partially independent pathways. Evidence for the latter idea is provided by the observations of Moreno *et al.* (2009), who showed that the suppression of Arabidopsis defenses against *Spodoptera frugiperda* can also be demonstrated in a mutant that fails to induce the morphological component of SAS. In addition, Cerrudo *et al.* (2012) showed that treatment of Arabidopsis plants with light depleted in the blue region of the spectrum induced a strong SAS phenotype, which is similar to the phenotype of plants grown under low R:FR ratios; however, in contrast with the effect of low R:FR, low blue failed to make the plants more susceptible to the necrotrophic fungus *Botrytis cinerea*. If the effect of low R:FR ratios reducing defense is not a simple consequence of the promotion of shoot growth, it may be possible to deliberately manipulate defense responses to competition without affecting growth by targeting defense-specific signaling elements.

Repression of plant defense under conditions in which phyB is inactivated correlate with a simultaneous suppression of jasmonic acid (JA) and salicylic acid signaling (reviewed in Ballaré, 2014). JA signaling, which is critical for defense against insects and necrotrophs (Browse, 2009, Goossens *et al.*, 2016), is regulated by the interaction of two families of transcriptional repressors: the DELLA and JASMONATE ZIM-DOMAIN (JAZ) proteins. JAZs (a family of 13 members in Arabidopsis) (Kazan & Manners, 2012, Thireault *et al.*, 2015) are repressors of JA signaling, because they interfere with key transcription factors that are responsible for activating JA responses (Chini *et al.*, 2007, Thines *et al.*, 2007, Yan *et al.*, 2007). In turn, DELLA proteins, which are repressors of gibberellin (GA) responses can physically interact with JAZ proteins, making them less available to repress JA-dependent transcription (Hou *et al.*, 2010, Yang *et al.*, 2012). GA promotes growth and repress defense by promoting the degradation of DELLAs via the proteasome pathway and, similarly, JA represses growth and activates defense by triggering the degradation of JAZs (Ballaré, 2014, Huot *et al.*, 2014, Havko *et al.*, 2016). The available evidence suggests that low R:FR ratios tip the DELLA-JAZ balance in favor of the JAZs, by promoting DELLA degradation and increasing JAZ stability (Leone *et al.*, 2014). This shift in the DELLA-JAZ balance, presumably accompanied by more specific effects of low R:FR ratios on the stability of MYC transcription factors (which are essential for activating JA-induced responses) (Chico *et al.*, 2014), result in a redirection of resources toward rapid elongation and away from defense (Ballaré, 2014, Mazza & Ballaré, 2015).

Previous work has suggested that JAZ10, one of the members of the JAZ family, is required for the effects of FR radiation repressing JA-dependent defenses (Cerrudo *et al.*, 2012, Leone *et al.*, 2014). JAZ10 could therefore be an interesting target for manipulating the effects of phyB on resource allocation in response to competition. However, the role of JAZ10 in the reconfiguration of plant form and function during shade avoidance is not fully understood. Because JA is known to repress growth and elongation (e.g., Cipollini, 2005), and JAZ10 is an important player in the growth repression branch of the JA pathway (Yan *et al.*, 2007), it is unclear how the presence or absence of JAZ10 could affect morphological responses to phyB inactivation and the balance between growth and defense. To gain a better understanding of the molecular mechanisms that regulate growth and defense responses to phyB inactivation, we compared the phenotypes of the Arabidopsis single *phyB* and double *jaz10phyB* mutants at the levels of gene expression, accumulation of defense metabolites, plant morphology, and biotic defense. We found that JAZ10 was completely dispensable for morphological responses to phyB inactivation; however, JAZ10 was required for the full expression of the low defense phenotype in the *phyB* mutant. The *jaz10phyB* double mutant had a robust SAS phenotype, which was almost identical to that of *phyB* but, in contrast with *phyB*, it had relatively high levels of induced defenses and nearly wild type resistance to infection by *B. cinerea*. These results suggest that genetic inactivation of JAZ10 in Arabidopsis partially uncouples the effects of phyB on plant morphology from the effects on plant immunity.

MATERIALS AND METHODS

Plant material and growth conditions

Arabidopsis thaliana (L.) Heynh seeds were germinated as described previously (Moreno *et al.*, 2009). Seven days after germination, seedlings were transferred to individual pots (0.11 l) with a vermiculite : perlite : peat (1 : 1 : 1) mixture. Seedlings were watered every 2 days with tap water to keep the soil near field capacity, and supplemented every 7 days with a 0.75 g l^{-1} Hakaphos Rojo solution 18-18-18 (Compo). Plants were grown in a growth chamber under short-day conditions (8 h/16 h, light/dark cycles) at 18 - 22°C and under 150 µmol m^{-2} s^{-1} of photosynthetically active radiation (PAR) provided by fluorescent bulbs. Rosette-stage plants of similar age (typically between 18 and 28 days old) and size were selected for the experiments and randomly assigned to the treatments. For FR irradiation treatments, plants were kept under the PAR source and supplemented from one of the sides with FR radiation (Moreno *et al.*, 2009). In some experiments, after one week of growth in the growth chamber, the plants were transferred to an unheated glasshouse, where they were grown for three additional weeks until used in infection bioassays. In the glasshouse,

plants were exposed to natural short day conditions (≈10 h/14 h light/dark cycles); temperature during the experimental period varied between 9 and 19 °C, and peak levels of natural PAR at plant level were ≈900 µmol m^{-2} s^{-1}. The Columbia (Col-0) ecotype of *A. thaliana* was used as the wild type control in all experiments. Seeds of the *phyB-9* mutant (Reed *et al.*, 1993), the *jaz10.1* null mutant (SAIL_92_D08; ABRC, www.arabidopsis.org), and the *jaz10.1 phyB9* double mutant (*jaz10phyB*) (Leone *et al.*, 2014), and Col-0 wild type were obtained from plants grown at the same time and under identical conditions.

MeJA treatments

Plant responses to JA were assessed by spraying soil-grown Arabidopsis rosettes with a methyl-jasmonate (MeJA) (Sigma-Aldrich) solution, at the concentration indicated in the relevan figure legends. Plants not assigned to the JA treatment were sprayed with distilled water, which was supplemented with ethanol in the same proportion (0.04%) as that used to dissolve MeJA in the solution used for the JA treatment. Rosettes were harvested at different time points after MeJA treatment and immediately frozen in liquid nitrogen.

Gene expression

Samples for analyses of leaf phenolics were taken 3 h after treatment with MeJA. 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 polymerase chain reaction (qPCR) was performed in a 7500 Real-Time PCR System (Applied Biosystems) following the manufacturer's standard method for absolute quantification using FastStart Universal SYBR Green Master Mix (Roche Applied Science) and primers at a final concentration of 500 nM (annealing temperature 60 °C). The *A. thaliana UBC* (*UBIQUITIN-CONJUGATING EMZYME*) gene was used to normalize for differences in concentrations of cDNA samples. *UBC* is very suitable for normalization of gene expression in Arabidopsis (Czechowski *et al.*, 2005), and we found that the mean cycle threshold values (C_T) for *UBC* in our samples were not affected by MeJA treatment, and did not vary among genotypes. Primer sequences are listed in Supporting Information Table S1.

Morphological responses

The effects of FR radiation and the *phyB* and *jaz10* mutations on plant morphology were characterized at the seedling and rosette stage using classic markers of SAS, including hypocotyl length, leaf angles and lamina:petiole ratios, as described previously (Moreno et al., 2009, Keller et al., 2011).

Leaf phenolics and glucosinolates (GS)

Accumulation of soluble phenolic compounds was measured spectrophotometrically in leaf extracts (Mazza *et al.*, 2000). We used six (21-day-old) plants per genotype and from each plant we collected two leaf samples. Each leaf sample consisted of the lamina of a fully expanded leaf, which was weighed and placed in 1.5 mL of 99:1 methanol:HCL and allowed to extract for 48 h at -20°C. Absorbance was read a 320 nm, and the results of the two samples from the same plant were averaged.

GS were extracted from freeze-dried tissue without the midvein and quantified using established protocols (Brown *et al.*, 2003), as described in Cargnel *et al.* (2014). In each experiment we used four biological replicates, and each replicate consisted of a pool of three individual plants. The experiment was repeated four times with similar results. We focused on indolic glucosinolates, particularly indol 3-ylmethyl glucosinolate (I3M), because although this glucosinolate is not directly involved in pathogen defense in Arabidopsis, it serves as a precursor for the generation of toxic hydrolysis products by endogenous thioglucosidases (Bednarek *et al.*, 2009, Buxdorf *et al.*, 2013), and it is known to be up-regulated by MeJA treatment and down-regulated by low R:FR ratios (Cargnel *et al.*, 2014). Aliphatic glucosinolates, such as 4-methylsulfinylbutyl (4MSOB), which are abundant in Arabidopsis tissue, are generally not induced by MeJA (Brader *et al.*, 2001, Mewis *et al.*, 2005, Guo *et al.*, 2013), and only slightly affected by light quality under our growth conditions (Cargnel *et al.*, 2014).

***Botrytis cinerea* culture and infection bioassays**

B. cinerea (strain B05) was grown and maintained on potato dextrose agar (1.5 % agar, 2 % potato extract, 2 % dextrose). Spores were collected from agar plates with distilled water and a glass rod, filtered and resuspended in a 0.1 M sucrose/0.07 M KH_2PO_4 solution to induce germination (Elad, 1991). We used two experimental approaches to evaluate the susceptibility of Arabidopsis plants to the fungus. In one of them, inoculation was carried out in a growth chamber using spore suspension droplets (Cargnel *et al.*, 2014). Briefly, droplets of 5 µL of spore suspension ($3.5x10^5$ spores ml^{-1}) were placed on the adaxial surface of three young leaves (one droplet per leaf) of 4-week-old plants (Supporting Information Fig. S1). Each individual pot, containing a single plant, was placed in a clear polyester chamber to prevent desiccation of the inoculation droplets. After 48 h, infected leaves were collected and photographed. Lesion areas were measured using Adobe Photoshop software (Adobe Systems). The lesion areas from the three infected leaves belonging to the same plant were averaged, and each plant was used as a replicate for the statistical analysis. The second approach was designed to more closely mimic fungal infection under natural conditions. Plants (4-week-old; 12 true leaves), contained in individual pots, were arranged in 30 x 50 cm plastic trays to form a canopy matrix that included all 4 genotypes (Col-0, *phyB*, *jaz10* and *jaz10phyB*) distributed at random within the tray (16-20 plants per tray; 4-5 plants of each genotype) (Supporting Information Fig. S1). These mixed canopies were sprayed with a *B. cinerea* spore suspension ($2x10^5$ spores ml^{-1}) and the trays were covered with clear plastic film (Rolopac, Buenos Aires) to maintain a high relative humidity. The film had 10 small holes to allow ventilation and was taken out four days after spraying; plant survival was evaluated 4 d later. The experiment was repeated 4 times in consecutive weeks with independent sets of plants, and the mortality rates were calculated as the average of four experiments.

Statistical analyses

Statistical analyses were carried out using INFOSTAT software (professional version 1.1) (Di Rienzo *et al.*, 2011). Data on gene expression, morphology, metabolites and lesion area were analyzed using factorial analysis of variance (ANOVA). When the interaction terms in the factorial analyses were statistically significant ($p < 0.05$), differences between means were assessed using Duncan comparisons. Appropriate transformations of the primary data were used when needed to meet the assumptions of the analysis.

RESULTS

JAZ10 is dispensable for the expression of growth responses triggered by phyB inactivation

The *phyB* mutant displayed a well-characterized SAS phenotype, which included elongated hypocotyls and petioles, hyponastic leaves, and reduced expansion of the leaf lamina (Fig. 1). The morphology of *jaz10* plants was very similar to that of Col-0 plants under our growth conditions, and the introduction of the *jaz10* mutation into the *phyB* background had virtually no effect on the SAS morphology displayed by the *phyB* single mutant (Fig. 1). Moreover, the *jaz10* mutant showed normal elongation and leaf angle responses to supplemental FR radiation (Supporting Information Fig. S2). The expression of classic SAS marker genes, such as *PIL1* (Salter *et al.*, 2003) and *ATHB2* (Carabelli *et al.*, 1993), was clearly up-regulated in *phyB* compared with Col-0 plants, and this enhanced expression of shade markers was totally conserved in the *jaz10phyB* double mutant (Fig. 2). Under MeJA treatment, *phyB* plants still displayed a characteristic SAS morphology (erect leaves, low lamina:petiole ratios and long petioles), and this elongated phenotype was conserved in the *jaz10phyB* double mutant (Supporting Information Fig. S3). These results suggest that, for plants at the rosette stage, repression of JA signaling by the JAZ10 protein is not required for the expression of shade avoidance responses.

Inactivation of *JAZ10* increases defense levels in the *phyB* mutant

In experiments in which plants were treated with MeJA, to induce plant defense, the *phyB* mutant expressed low levels of defense-related genes, including genes that encode for the transcription factors MYC2, MYB34, and ERF1, and the plant defensin PDF1.2 (Fig. 3). The *jaz10* mutant tended to have slightly increased expression of some of the JA response markers compared to Col-0 under MeJA treatment, which would be consistent with previous reports of enhanced JA sensitivity in this mutant (Yan *et al.*, 2007, Demianski *et al.*, 2012), although these trends were not always significant. Interestingly, introduction of the *jaz10* mutation into the *phyB* background tended to restore the expression of JA marker genes to levels that were comparable with those of Col-0 plants (Fig. 3).

In accordance with the low levels of expression of defense-related genes, the *phyB* mutant had reduced levels of indolic glucosinolates, characterized by reduced concentrations of indol-3-ylmethyl glucosinolate (I3M) both under control and MeJA-induced conditions (Fig. 4). In contrast, the *jaz10phyB* double mutant had I3M concentrations that were comparable with those of Col-0 and *jaz10* plants (Fig. 4). The low I3M concentration in *phyB* leaves, and partial recovery of I3M concentrations in *jaz10phyB* was broadly consistent with the gene expression data for *MYC2* and

MYB34 (Fig. 3), which encode transcription factors that play a key role regulating indolic glucosinolate biosynthesis (Celenza *et al.*, 2005, Frerigmann, 2016).

phyB plants had low levels of soluble phenolic compounds, and were clearly hyposensitive to MeJA compared with Col-0 plants (Fig. 5). In the double mutant, the levels of soluble leaf phenolics were still low compared with Col-0 plants, but in contrast to the *phyB* single mutant, leaf phenolics increased under MeJA treatment. These results suggest that the *jaz10* mutation, which did not promote accumulation of leaf phenolics in *PHYB* plants, can partially rescue the ability of the *phyB* mutant to respond to exogenous MeJA (Fig. 5).

Inactivation of *JAZ10* increases the resistance of *phyB* plants to *B. cinerea*

In droplet-inoculation bioassays carried out in a growth chamber, *phyB* was highly susceptible to *B. cinerea* (Fig. 6), which correlated with the reduced levels of secondary metabolites and expression of JA-related genes. In contrast, the *jaz10phyB* double mutant showed resistance levels that were comparable to the wild type (Fig. 6), even though, as shown previously (Fig. 1 & Supporting Information Fig. S3), its overall morphology was almost identical to that of *phyB* plants.

In complementary greenhouse experiments, we attempted to more closely simulate natural infections by spraying the canopies with *B. cinerea* spore suspensions, and keeping the plants under conditions of high illumination and natural photoperiods. Under these conditions, plants were severely affected by the fungus, and even Col-0 plants, which usually survive to the droplet inoculation tests, displayed mortality rates of approximately 30 % when sprayed with suspensions that contained 2×10^5 spores per ml. In these greenhouse bioassays, *phyB* was extremely susceptible to the fungus, with mortality rates >75 % 8 d after infection (Fig. 7). In contrast, *jaz10phyB* plants were relatively resistant and displayed survival rates that were comparable to those of Col-0 plants (Fig. 7).

DISCUSSION

Repression of plant defense and JA markers under shade or in response to phyB inactivation has been documented in many previous studies (McGuire & Agrawal, 2005, Izaguirre *et al.*, 2006, Moreno *et al.*, 2009, Agrawal *et al.*, 2012, Cerrudo *et al.*, 2012, de Wit *et al.*, 2013, Izaguirre *et al.*, 2013, Cargnel *et al.*, 2014, Chico *et al.*, 2014, Leone *et al.*, 2014). Our results demonstrate that although the increase in Arabidopsis susceptibility to *B. cinerea* is concomitantly expressed with the promotion of classic SAS markers, the suppression of plant defense is not simply an unavoidable

consequence of the elongated phenotype. These two effects of phyB inactivation are mediated by at least partially independent pathways, with different requirements of the JAZ10 protein (Supporting Information Fig. S4). The role of JAZ10 in the mechanisms of defense repression and the implications of our findings are discussed below.

JAZ10 is an important link between phyB and JA responses

Previous studies have shown a high degree of redundancy among members of the JAZ family (reviewed in Pauwels & Goossens, 2011). However, under certain environmental conditions and for certain responses, apparently specific roles of some JAZs are beginning to emerge (Kazan & Manners, 2012). Null mutants of *jaz9* (Yang *et al.*, 2012), *jaz10* (Cerrudo *et al.*, 2012, Demianski *et al.*, 2012, Leone *et al.*, 2014) and *jaz7* (Thatcher *et al.*, 2016, Yu *et al.*, 2016) have been shown to have mutant-specific phenotypes under certain assay conditions. JAZ10 has been implicated in disease responses, and genetic lines in which the expression of *JAZ10* has been disrupted show increased resistance to *B. cinerea* under simulated shadelight (Cerrudo *et al.*, 2012) and increased susceptibility to the biotrophic pathogen *Pseudomonas syringae* DC3000 (Demianski *et al.*, 2012). Null *jaz10* mutants and *JAZ10* RNAi lines have also been shown to have increased sensitivity to the growth inhibitory effects of JA under ambient light (Yan *et al.*, 2007, Demianski *et al.*, 2012) or simulated shadelight (Leone *et al.*, 2014). JAZ10 can physically interact with DELLA proteins (Yang *et al.*, 2012) and part of the previously reported effects of the *jaz10* mutation on growth and defense might be mediated by changes in the balance between JAZ10 and DELLA proteins (Yang *et al.*, 2012, Leone *et al.*, 2014). Our results with seedlings at the rosette stage demonstrate that JAZ10 is required for the repression of JA-mediated defense caused by phyB inactivation, but not for the effects of *phyB* promoting the SAS morphology or attenuating the growth-inhibitory effects of JA. Thus, whereas the *jaz10* mutation significantly increases fungal resistance in the *phyB* mutant (Figs. 5 & 6), it is clear that this mutation does have any detectable impact on the expression of classic SAS markers and the reconfiguration of shoot morphology that are triggered by *phyB* or supplemental FR radiation (Figs. 1 and 2 & Supporting Information Fig. S2 & S3). Assuming that elimination of JAZ10 would make DELLA proteins are more available to repress PIF transcription factors (as could be inferred from the studies on JAZ9 reported by Yang *et al.*, 2012), it would appear that the degradation of DELLA proteins caused by phyB inactivation (Djakovic-Petrovic *et al.*, 2007, Leone *et al.*, 2014) is sufficient to allow normal SAS responses in plants carrying the *jaz10* mutation. Previous work has shown that constitutive expression of JA responses (in the *cev1* mutant) can reduce petiole elongation responses to low R:FR ratios (de Wit *et al.*, 2013). This suggests that activation of the JA pathway, and consequent degradation of JAZ proteins, can attenuate growth responses triggered by

competition signals (which is confirmed by our petiole length data in plants exposed to MeJA, Supporting Information Fig. S3). Given that the absence of *JAZ10* does not compromise the SAS phenotype of the *phyB* mutant (Fig. 1 & Supporting Information Fig. S3), it remains to be determined which are the JAZs (or combinations of JAZs) whose inactivation is required for the effect of MeJA attenuating shade-avoidance responses.

Why do plants repress their defenses when they face a high risk of competition?

This and previous studies (Moreno *et al.*, 2009, Cerrudo *et al.*, 2012, de Wit *et al.*, 2013) demonstrate that the repression of Arabidopsis resistance to biotic stress caused by low R:FR ratios is not a simple consequence of the reconfiguration of plant architecture that is elicited by phyB inactivation. The *jaz10phyB* double mutant provides compelling evidence that a plant expressing a full "shade-avoidance" morphology can still mount an efficient defense against *B. cinerea*, at least within the reference framework of our bioassays. Why, then, do plants normally repress their defenses when they face a high risk of competition? Plants might have evolved to use a conservative resource investing strategy. A rapid shift in resource allocation, maximizing SAS in response to neighbor proximity cues, may allow the plant to anticipate conditions of more intense competition. It is worth noting, in addition, that plants appear to have mechanisms to limit the negative effects on fitness of the repression of defense under competition. One of them takes advantage of the modular nature of the plant itself. It has been shown that the suppression of wound- and JA-induced responses is restricted to those plant parts that receive a low R:FR signal (Izaguirre *et al.*, 2013). Because low R:FR is a signal of actual or potential shade, these parts are unlikely to be important contributors to the photosynthesis of the whole plant. Localized attenuation of defense responses under low R:FR could then be part of an evolved "self-pruning" strategy, where dispensable modules are left undefended to focus carbon and nutrients in more critical tissues. Another strategy might be based on the activation of volatile-mediated indirect defenses under conditions in which phyB is inactivated, as has been recently demonstrated in tomato (Cortés *et al.*, 2016).

Agricultural implications

Repression of defense responses under competition is likely to contribute to the increased susceptibility to pests and pathogens and dependence on pesticides in crops sown at high density (Ballaré *et al.*, 2012, Anten & Vermeulen, 2016). Suppression of SAS responses in crops has often been viewed as a promising strategy to increase yield, based on the idea that SAS represents a waste of resources in competition among crop plants and excessive stem elongation (Smith, 1992, Ballaré *et al.*, 1997, Ballaré & Casal, 2000, Carriedo *et al.*, 2016). The observation that low R:FR ratios

increase plant susceptibility to pathogens may represent an additional reason to suppress crop plant responses to phyB inactivation. However, a generalized repression of plant responses to low R:FR could come at a high cost, for example in terms of reduced light interception by the canopy or increased size inequality among crop plants (Ballaré *et al.*, 1997), which may have negative consequences for crop yield. Alternatives have been proposed, including targeting the manipulation of light responses to specific plant organs (Rousseaux *et al.*, 1997). Regarding the effects of shade-light cues on plant sensitivity to pathogens, the improved understanding of the mechanisms by which light quality regulates plant defense suggests new avenues to counter these effects –for example, via targeting genes that provide a critical link between phyB and JA signaling. The emerging evidence in Arabidopsis (present results and Campos *et al.*, 2016) suggests that inactivation of those *JAZ* genes that play an important role repressing JA signaling under light conditions that deplete the active form of phyB, such as *JAZ10*, could result in crop plants that express robust defenses against necrotrophic pathogens and insects even when grown at high densities.

CONFLICT OF INTERESTS

The authors have no conflicts of interest to declare.

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REFERENCES

Agrawal A., Kearney E., Hastings A. & Ramsey T. (2012) Attenuation of the jasmonate burst, plant defensive traits, and resistance to specialist monarch caterpillars on shaded common milkweed (*Asclepias syriaca*). *Journal of Chemical Ecology*, **38**, 893-901.

Anten N.P.R. & Vermeulen P.J. (2016) Tragedies and crops: Understanding natural selection to improve cropping systems. *Trends in Ecology & Evolution*, **31**, 429-439.

Augspurger C.K. & Kelly C.K. (1984) Pathogen mortality of tropical tree seedlings: experimental studies of the effects of dispersal distance, seedling density, and light conditions. *Oecologia*, **61**, 211-217.

Baldwin I.T. (1998) Jasmonate induced responses are costly but benefit plants under attack in native populations. *Proceedings of the National Academy of Sciences of the United States of America*, **95**, 8113-8118.

Ballaré C.L. (1999) Keeping up with the neighbours: phytochrome sensing and other signalling mechanisms. *Trends in Plant Science*, **4**, 97-102.

Ballaré C.L. (2014) Light regulation of plant defense. *Annual Review of Plant Biology*, **65**, 335-363.
Ballaré C.L. & Casal J.J. (2000) Light signals perceived by crop and weed plants. *Field Crops Research*, **67**, 149-160.
Ballaré C.L., Mazza C.A., Austin A.T. & Pierik R. (2012) Canopy light and plant health. *Plant Physiology*, **160**, 145-155.
Ballaré C.L., Scopel A.L. & Sánchez R.A. (1997) Foraging for light: photosensory ecology and agricultural implications. *Plant, Cell and Environment*, **20**, 820-825.
Ballhorn D., Godschalx A., Smart S., Kautz S. & Schädler M. (2014) Chemical defense lowers plant competitiveness. *Oecologia*, **176**, 811-824.
Bednarek P., Pislewska-Bednarek M., Svatos A., Schneider B., Doubský J., Mansurova M., Humphry M., Consonni C., Panstruga R., Sanchez-Vallet A., Molina A. & Schulze-Lefert P. (2009) A glucosinolate metabolism pathway in living plant cells mediates broad-spectrum antifungal defense. *Science*, **323**, 101-106.
Brader G., Tas Ã. & Palva E.T. (2001) Jasmonate-dependent induction of indole glucosinolates in Arabidopsis by culture filtrates of the nonspecific pathogen *Erwinia carotovora*. *Plant Physiology*, **126**, 849-860.
Brown P.D., Tokuhisa J.G., Reichelt M. & Gershenzon J. (2003) Variation of glucosinolate accumulation among different organs and developmental stages of Arabidopsis thaliana. *Phytochemistry*, **62**, 471-481.
Browse J. (2009) Jasmonate passes muster: A receptor and targets for the defense hormone. *Annual Review of Plant Biology*, **60**, 183-205.
Burdon J.J. & Chilvers G.A. (1982) Host density as a factor in plant disease ecology. *Annual Review of Phytopathology*, **20**, 143-166.
Buxdorf K., Yaffe H., Barda O. & Levy M. (2013) The effects of glucosinolates and their breakdown products on necrotrophic fungi. *PLoS ONE*, **8**.
Campos M.L., Yoshida Y., Major I.T., de Oliveira Ferreira D., Weraduwage S.M., Froehlich J.E., Johnson B.F., Kramer D.M., Jander G., Sharkey T.D. & Howe G.A. (2016) Rewiring of jasmonate and phytochrome B signalling uncouples plant growth-defense tradeoffs. *Nat Commun*, **7**.
Carabelli M., Sessa G., Baima S., Morelli G. & Ruberti I. (1993) The Arabidopsis ATHB-2 and -4 genes are strongly induced by far-red-rich light. *The Plant Journal*, **4**, 469-479.
Cargnel M.D., Demkura P.V. & Ballaré C.L. (2014) Linking phytochrome to plant immunity: low red : far-red ratios increase Arabidopsis susceptibility to Botrytis cinerea by reducing the biosynthesis of indolic glucosinolates and camalexin. *New Phytologist*, **204**, 342-354.
Carriedo L.G., Maloof J.N. & Brady S.M. (2016) Molecular control of crop shade avoidance. *Current Opinion in Plant Biology*, **30**, 151-158.
Casal J.J. (2012) Shade Avoidance. *The Arabidopsis Book*, **10**, e0157.
Celenza J.L., Quiel J.A., Smolen G.A., Merrikh H., Silvestro A.R., Normanly J. & Bender J. (2005) The Arabidopsis ATR1 MYB transcription factor controls indolic glucosinolate homeostasis. *Plant Physiology*, **137**, 253-262.
Cerrudo I., Keller M.M., Cargnel M.D., Demkura P.V., de Wit M., Patitucci M.S., Pierik R., Pieterse C.M.J. & Ballaré C.L. (2012) Low red/far-red ratios reduce Arabidopsis resistance to *Botrytis cinerea* and jasmonate responses via a COI1-JAZ10-dependent, salicylic acid-independent mechanism. *Plant Physiology*, **158**, 2042-2052.
Cipollini D. (1997) Gibberellic acid treatment reduces the tolerance of filed-grown common bean to leaf removal. *Journal of Plant Growth Regulation*, **16**, 123-127.
Cipollini D. (2005) Interactive effects of lateral shading and jasmonic acid on morphology, phenology, seed production, and defense traits in *Arabidopsis thaliana*. *International Journal of Plant Sciences*, **166**, 955-959.

Cipollini D. (2007) Consequences of the overproduction of methyl jasmonate on seed production, tolerance to defoliation and competitive effect and response of *Arabidopsis thaliana*. *New Phytologist*, **173**, 146-153.

Cortés L.E., Weldegergis B.T., Boccalandro H.E., Dicke M. & Ballaré C.L. (2016) Trading direct for indirect defense? Phytochrome B inactivation in tomato attenuates direct anti-herbivore defenses whilst enhancing volatile-mediated attraction of predators. *New Phytologist*, **212**, 1057-1071.

Czechowski T., Stitt M., Altmann T., Udvardi M.K. & Scheible W.-R. (2005) Genome-Wide Identification and Testing of Superior Reference Genes for Transcript Normalization in Arabidopsis. *Plant Physiology*, **139**, 5-17.

Chico J.-M., Fernández-Barbero G., Chini A., Fernández-Calvo P., Díez-Díaz M. & Solano R. (2014) Repression of jasmonate-dependent defenses by shade involves differential regulation of protein stability of MYC transcription factors and their JAZ repressors in Arabidopsis. *The Plant Cell*, **26**, 1967-1980.

Chini A., Fonseca S., Fernandez G., Adie B., Chico J.M., Lorenzo O., Garcia-Casado G., Lopez-Vidriero I., Lozano F.M., Ponce M.R., Micol J.L. & Solano R. (2007) The JAZ family of repressors is the missing link in jasmonate signalling. *Nature*, **448**, 666-671.

Dávila-Flores A.M., DeWitt T.J. & Bernal J.S. (2013) Facilitated by nature and agriculture: Performance of a specialist herbivore improves with host-plant life history evolution, domestication, and breeding. *Oecologia*, **173**, 1425-1437.

de Wit M., Spoel S.H., Sanchez Perez G.F., Gommers C.M.M., Pieterse C.M.J., Voesenek L.A.C.J. & Pierik R. (2013) Perception of low Red:Far-red ratio compromises both salicylic acid- and jasmonic acid- dependent pathogen defences in Arabidopsis. *The Plant Journal*, **75**, 90-103.

Demianski A.J., Chung K.M. & Kunkel B.N. (2012) Analysis of Arabidopsis *JAZ* gene expression during *Pseudomonas syringae* pathogenesis. *Molecular Plant Pathology*, **13**, 46-57.

Di Rienzo J., Casanoves F., Balzarini M., Gonzalez L., Tablada M. & Robledo C. (2011) InfoStat versión 2011. *Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. URL http://www.infostat.com.ar*.

Djakovic-Petrovic T., de Wit M., Voesenek L.A.C.J. & Pierik R. (2007) DELLA protein function in growth responses to canopy signals. *The Plant Journal*, **51**, 117-126.

Donaldson J.R., Kruger E.L. & Lindroth R.L. (2006) Competition- and resource-mediated tradeoffs between growth and defensive chemistry in trembling aspen (*Populus tremuloides*). *New Phytologist*, **169**, 561–570.

Elad Y. (1991) An inhibitor of polyamine biosynthesis "Difluoromethylornithine" and the polyamine spermidine for the control of gray mold (*Botrytis cinerea*). *Phytoparasitica*, **19**, 201-209.

Fraser D.P., Hayes S. & Franklin K.A. (2016) Photoreceptor crosstalk in shade avoidance. *Current Opinion in Plant Biology*, **33**, 1-7.

Frerigmann H. (2016) Glucosinolate Regulation in a Complex Relationship – MYC and MYB – No One Can Act Without Each Other. In: *Advances in Botanical Research*, pp. doi:10.1016/bs.abr.2016.1006.1005. Academic Press.

Goossens J., Fernández-Calvo P., Schweizer F. & Goossens A. (2016) Jasmonates: signal transduction components and their roles in environmental stress responses. *Plant Molecular Biology*, 1-17.

Guo R., Shen W., Qian H., Zhang M., Liu L. & Wang Q. (2013) Jasmonic acid and glucose synergistically modulate the accumulation of glucosinolates in *Arabidopsis thaliana*. *Journal of Experimental Botany*, **64**, 5707-5719.

Havko N., Major I., Jewell J., Attaran E., Browse J. & Howe G.A. (2016) Control of carbon assimilation and partitioning by jasmonate: an accounting of growth–defense tradeoffs. *Plants*, **5**, 7.

Hou X., Lee L.Y.C., Xia K., Yan Y. & Yu H. (2010) DELLAs modulate jasmonate signaling via competitive binding to JAZs. *Developmental Cell*, **19**, 884-894.

Huot B., Yao J., Montgomery B.L. & He S.Y. (2014) Growth-defense tradeoffs in plants: a balancing act to optimize fitness. *Molecular Plant*, **7**, 1267-1287.

Izaguirre M.M., Mazza C.A., Astigueta M.S., Ciarla A.M. & Ballaré C.L. (2013) No time for candy: passionfruit (*Passiflora edulis*) plants down-regulate damage-induced extra floral nectar production in response to light signals of competition. *Oecologia*, **173**, 213–221.

Izaguirre M.M., Mazza C.A., Biondini M., Baldwin I.T. & Ballaré C.L. (2006) Remote sensing of future competitors: impacts on plant defenses. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 7170-7174.

Izaguirre M.M., Scopel A.L., Baldwin I.T. & Ballaré C.L. (2003) Convergent responses to stress. Solar ultraviolet-B radiation and *Manduca sexta* herbivory elicit overlapping transcriptional responses in field-grown plants of *Nicotiana longiflora*. *Plant Physiology*, **132**, 1755-1767.

Kazan K. & Manners J.M. (2012) JAZ repressors and the orchestration of phytohormone crosstalk. *Trends in Plant Science*, **17**, 22-31.

Keller M.M., Jaillais Y., Pedmale U.V., Moreno J.E., Chory J. & Ballaré C.L. (2011) Cryptochrome 1 and phytochrome B control shade-avoidance responses in *Arabidopsis* via partially-independent hormonal cascades. *The Plant Journal*, **67**, 195-207.

Kurashige N.S. & Agrawal A.A. (2005) Phenotypic plasticity to light competition and herbivory in *Chenopodium album* (Chenopodiaceae). *American Journal of Botany*, **92**, 21-26.

Leone M., Keller M.M., Cerrudo I. & Ballaré C.L. (2014) To grow or defend? Low red : far-red ratios reduce jasmonate sensitivity in Arabidopsis seedlings by promoting DELLA degradation and increasing JAZ10 stability. *New Phytologist*, **204**, 355-367.

Mazza C.A. & Ballaré C.L. (2015) Photoreceptors UVR8 and phytochrome B cooperate to optimize plant growth and defense in patchy canopies. *New Phytologist*, **207**, 4-9.

Mazza C.A., Boccalandro H.E., Giordano C.V., Battista D., Scopel A.L. & Ballaré C.L. (2000) Functional significance and induction by solar radiation of ultraviolet-absorbing sunscreens in field-grown soybean crops. *Plant Physiology*, **122**, 117-125.

McGuire R. & Agrawal A.A. (2005) Trade-offs between the shade-avoidance response and plant resistance to herbivores? Tests with mutant *Cucumis sativus*. *Functional Ecology*, **19**, 1025-1031.

Mewis I., Appel H.M., Hom A., Raina R. & Schultz J.C. (2005) Major signaling pathways modulate Arabidopsis glucosinolate accumulation and response to both phloem-feeding and chewing insects. *Plant Physiology*, **138**, 1149-1162.

Moreno J.E., Tao Y., Chory J. & Ballaré C.L. (2009) Ecological modulation of plant defense via phytochrome control of jasmonate sensitivity. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 4935-4940.

Oerke E.C. (2006) Crop losses to pests. *Journal of Agricultural Science*, **144**, 31-43.

Pauwels L. & Goossens A. (2011) The JAZ proteins: A crucial interface in the jasmonate signaling cascade. *The Plant Cell*, **23**, 3089-3100.

Pierik R. & de Wit M. (2014) Shade avoidance: phytochrome signalling and other aboveground neighbour detection cues. *Journal of Experimental Botany*, **65**, 2815-2824.

Rasmann S., Kollner T.G., Degenhardt J., Hiltpold I., Toepfer S., Kuhlmann U., Gershenzon J. & Turlings T.C.J. (2005) Recruitment of entomopathogenic nematodes by insect-damaged maize roots. *Nature*, **434**, 732-737.

Redman A.M., Cipollini D.F. & Schultz J.C. (2001) Fitness costs of jasmonic acid-induced defense in tomato, *Lycopersicon esculentum*. *Oecologia*, **126**, 380-385.

Reed J.W., Nagpal P., Poole D.S., Furuya M. & 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. *The Plant Cell*, **5**, 147-157.

Roberts M.R. & Paul N.D. (2006) Seduced by the dark side: integrating molecular and ecological perspectives on the influence of light on plant defence against pests and pathogens. *New Phytologist*, **170**, 677-699.

Rodriguez-Saona C., Vorsa N., Singh A.P., Johnson-Cicalese J., Szendrei Z., Mescher M.C. & Frost C.J. (2011) Tracing the history of plant traits under domestication in cranberries: potential consequences on anti-herbivore defences. *Journal of Experimental Botany*, **62**, 2633-2644.

Rosenthal J.P. & Dirzo R. (1997) Effects of life history, domestication and agronomic selection on plant defence against insects: Evidence from maizes and wild relatives. *Evolutionary Ecology*, **11**, 337-355.

Rousseaux M.C., Ballaré C.L., Jordan E.T. & Vierstra R.D. (1997) Directed overepression of PHYA locally suppresses stem elongation and leaf senescence responses to far-red radiation. *Plant, Cell and Environment*, **20**, 1551-1558.

Salter M.G., Franklin K.A. & Whitelam G.C. (2003) Gating of the rapid shade-avoidance response by the circadian clock in plants. *Nature*, **426**, 680-683.

Smith H. (1992) The ecological functions of the phytochrome family. Clues to a transgenic programme of crop improvement. *Photochemistry and Photobiology*, **56**, 815-822.

Smith H. (1995) Physiological and ecological function within the phytochrome family. *Annual Review of Plant Physiology and Plant Molecular Biology*, **46**, 289-315.

Thatcher L.F., Cevik V., Grant M., Zhai B., Jones J.D.G., Manners J.M. & Kazan K. (2016) Characterization of a JAZ7 activation-tagged Arabidopsis mutant with increased susceptibility to the fungal pathogen Fusarium oxysporum. *Journal of Experimental Botany*, **67**, 2367-2386.

Thines B., Katsir L., Melotto M., Niu Y., Mandaokar A., Liu G., Nomura K., He S.Y., Howe G.A. & Browse J. (2007) JAZ repressor proteins are targets of the SCF^{COI1} complex during jasmonate signalling. *Nature*, **448**, 661-665.

Thireault C., Shyu C., Yoshida Y., St. Aubin B., Campos M.L. & Howe G.A. (2015) Repression of jasmonate signaling by a non-TIFY JAZ protein in Arabidopsis. *The Plant Journal*, **82**, 669-679.

Yan Y., Stolz S., Chetelat A., Reymond P., Pagni M., Dubugnon L. & Farmer E.E. (2007) A downstream mediator in the growth repression limb of the jasmonate pathway. *The Plant Cell*, **19**, 2470-2483.

Yang D.-L., Yao J., Mei C.-S., Tong X.-H., Zeng L.-J., Li Q., Xiao L.-T., Sun T.-p., Li J., Deng X.-W., Lee C.M., Thomashow M.F., Yang Y., He Z. & He S.Y. (2012) Plant hormone jasmonate prioritizes defense over growth by interfering with gibberellin signaling cascade. *Proceedings of the National Academy of Sciences of the United States of America*, **109**, E1192-E1200.

Yu J., Zhang Y., Di C., Zhang Q., Zhang K., Wang C., You Q., Yan H., Dai S.Y., Yuan J.S., Xu W. & Su Z. (2016) JAZ7 negatively regulates dark-induced leaf senescence in Arabidopsis. *Journal of Experimental Botany*, **67**, 751-762.

Zavala J.A. & Baldwin I.T. (2006) Jasmonic acid signalling and herbivore resistance traits constrain regrowth after herbivore attack in *Nicotiana attenuata*. *Plant, Cell and Environment*, **29**, 1751-1760.

Zavala J.A., Patankar A.G., Gase K. & Baldwin I.T. (2004) Constitutive and inducible trypsin proteinase inhibitor production incurs large fitness costs in *Nicotiana attenuata*. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 1607-1612.

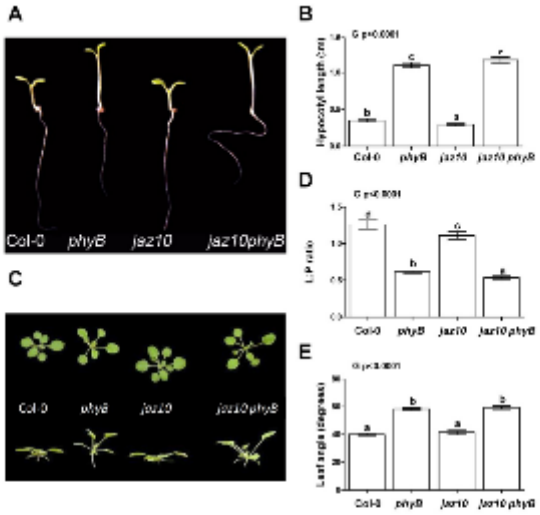

Figure 1

Figure 1. Mutation of the *JAZ10* gene does not compromise SAS morphological responses elicited by phyB inactivation. **A**, Representative seedlings of each genotype after 7 d of growth in 0.7 % agar, 1 % sucrose and Murashigue and Skoog medium. **B,** Hypocotyl length of 7-day-old seedlings. **C**, Representative photographs of 17-day-old plants grown in soil. **D**, Lamina/petiole ratio and **E**, Leaf angle of 17-d old rosettes. In all panels, error bars indicate ±1 SE (n = 20 plant replicates). The p-values for the effect of genotype (G) in the ANOVA are indicated in each panel; different letters indicate significant differences between genotype means ($p < 0.05$). Plants were grown in a growth chamber under short-days (8 h) and 150 µmol m^{-2} s^{-1} of PAR.

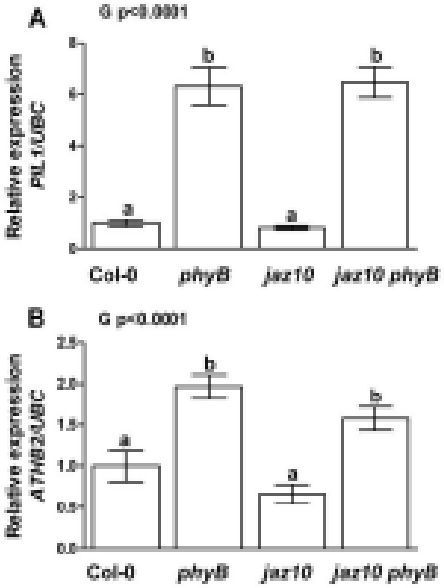

Figure 2

Figure 2. The up-regulation of shade markers in *phyB* is not affected by the *jaz10* mutation. mRNA levels were measured by qPCR in 18-day-old, soil-grown Arabidopsis rosettes and are expressed relative to Col-0. **A**, Relative expression of *PIL1*. **B**, Relative expression of *ATHB2*. Error bars indicate ±1 SE (n = 6 biological replicates; each biological replicate is a pool of three individual plants). The p-values for the effect of genotype (G) in the ANOVA are indicated in each panel; different letters indicate significant differences between genotype means ($p < 0.05$).

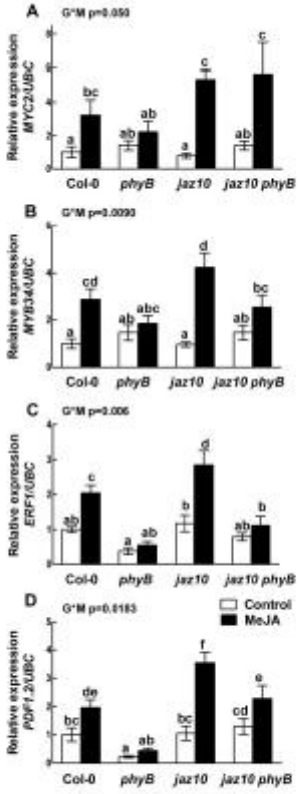

Figure 3

Figure 3. The *jaz10* mutation enhances the induction of JA response marker genes in the *phyB* background. mRNA levels were measured by qPCR 3 h after treatment of 18-day-old, soil-grown Arabidopsis rosettes with MeJA (200 µM), and are expressed relative to the Col-0 control. **A**, Relative expression of *MYC2*. **B**, Relative expression of *MYB34*. **C**, Relative expression of *ERF1*. **D**, Relative expression of *PDF1.2*. Error bars indicate ±1 SE (n = 6 biological replicates; each biological replicate is a pool of three individual plants). The p-values for the relevant terms in the factorial ANOVA are indicated in each panel. When the genotype x MeJA (G*M) interaction term was significant ($p<0.05$), differences between means are indicated by different letters; when the interaction term was not significant, different letters indicate significant differences between genotype means. MeJA, methyl jasmonate treatment.

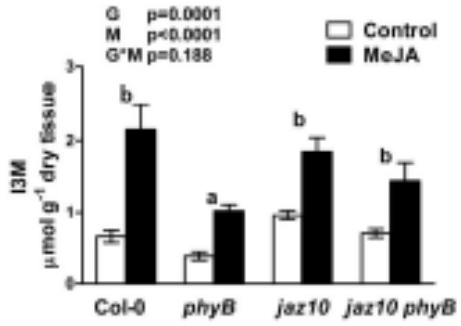

Figure 4

Figure 4. The *jaz10phyB* double mutant has wild-type concentrations of indol-3-ylmethyl glucosinolate (I3M). I3M was quantified from leaf tissue by HPLC two days after treatment of 28-day-old, soil-grown Arabidopsis rosettes with MeJA (200 µM). Error bar represent ±1 SE (n = 4 biological replicates; each biological replicate is a pool of three individual plants). The p-values for the various terms of the factorial ANOVA are shown (G = effect of genotype, M = effect of MeJA treatment). Different letters indicate significant differences between genotype means. MeJA, methyl jasmonate tratment.

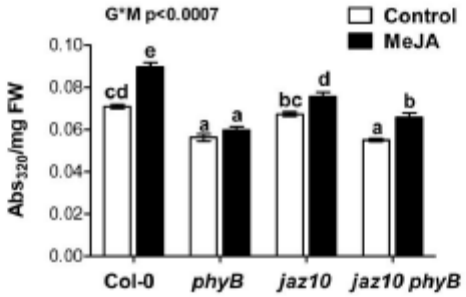

Figure 5

Figure 5. The *jaz10* mutation restores the ability of the *phyB* mutant to respond to JA with increased accumulation of phenolic compounds. Soluble phenolic compounds were measured 72 h after treatment of 21-day-old, soil-grown Arabidopsis rosettes with MeJA (200 µM). Error bars indicate ±1 SE (n = 6 plants per genotype). G*M = genotype x MeJA interaction; differences between means are indicated by different letters. MeJA, methyl jasmonate treatment.

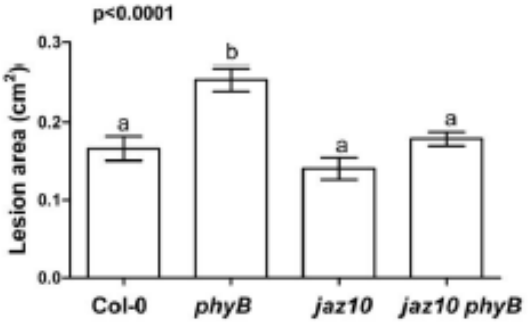

Figure 6

Figure 6. The effect of *phyB* mutation increasing Arabidopsis sensitivity to *B. cinerea* is lost in the *jaz10phyB* double mutant. Lesion areas were measured 48 h after inoculation in plants grown under white light in a growth chamber. Each bar represents the mean ±1 SE (n = 10 plants per genotype). Three leaves per plants were infected with a drop containing *B. cinerea* spores (for details, see the Materials and Methods). The p-value from the ANOVA is shown. Different letters indicate significant differences between means ($p<0.05$, Duncan test).

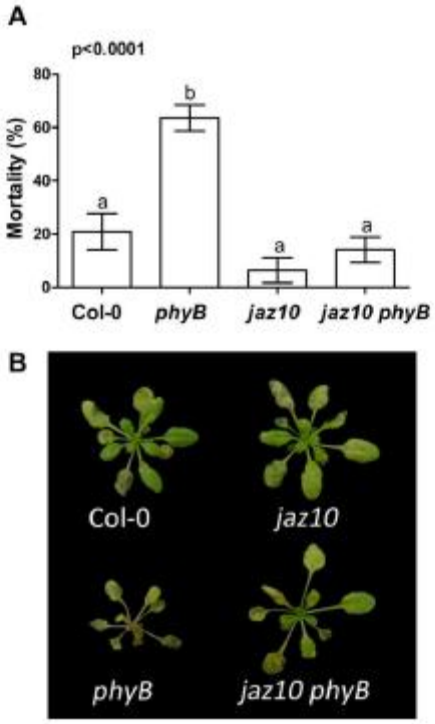

Figure 7

Figure 7. The *phyB* mutant is highly susceptible to *B. cinerea* even under natural high-light conditions, and introduction of the *jaz10* mutation restores plant resistance to the fungus. **A**, Mortality rates 8 d after spraying the canopies with *B. cinerea* spore suspensions. Each bar represents the mean ±1 SE (n = 4 replicates). The p-value from the ANOVA is shown. Different letters indicate significant differences between means (p<0.05, Duncan test). **B**, Representative plants of each genotype at the end of the experiment. Note that the chlorotic areas in *Col-0*, *jaz10*, and *jaz10phyB* plants are concentrated in the older leaves of the rosette, whereas the young, expanding leaves and the apex are green and do not display symptoms of tissue damage. In contrast, in *phyB*, even the young tissues show extensive necrosis, which eventually leads to the death of the plant. The plants were grown in a greenhouse under natural light conditions and kept in the same greenhouse during the infection period (for details, see the Materials and Methods).