A Phytochrome B-Independent Pathway Restricts Growth at High Levels of Jasmonate Defense

Ian T. Major,a Qiang Guo,a,b Jinling Zhai,a,2 George Kapali,a,d David M. Kramer,a,c and Gregg A. Howea,b,c,d,3,4

aDepartment of Energy-Plant Research Laboratory, Michigan State University, East Lansing, Michigan 48824
bDepartment of Plant Biology, Michigan State University, East Lansing, Michigan 48824
cDepartment of Biochemistry and Molecular Biology, Michigan State University, East Lansing, Michigan 48824
dPlant Resilience Institute, Michigan State University, East Lansing, Michigan 42284

ORCID IDs: 0000-0002-1727-2454 (I.T.M.); 0000-0002-6619-8211 (Q.G.); 0000-0003-0102-1359 (J.Z.); 0000-0003-2989-0302 (G.K.);
0000-0003-2181-6888 (D.M.K.); 0000-0002-9218-979X (G.A.H.).

The plant hormone jasmonate (JA) promotes resistance to biotic stress by stimulating the degradation of JASMONATE-ZIM-DOMAIN (JAZ) proteins, which relieves repression on MYC transcription factors that execute defense programs. JA-triggered depletion of JAZ proteins in Arabidopsis (Arabidopsis thaliana) is also associated with reduced growth and seed production, but the mechanisms underlying these pleiotropic growth effects remain unclear. Here, we investigated this question using an Arabidopsis JAZ-deficient mutant (jazD; jaz1–jaz7, jaz9, jaz10, and jaz13) that exhibits high levels of defense and strong growth inhibition. Genetic suppressor screens for mutations that uncouple growth-defense tradeoffs in the jazD mutant identified nine independent causal mutations in the red-light receptor phytochrome B (phyB). Unlike the ability of the phyB mutations to completely uncouple the mild growth-defense phenotypes in a jaz mutant (jazQ) defective in JAZ1, JAZ3, JAZ4, JAZ9, and JAZ10, phyB null alleles only weakly alleviated the growth and reproductive defects in the jazD mutant. phyB-independent growth restriction of the jazD mutant was tightly correlated with upregulation of the Trp biosynthetic pathway but not with changes in central carbon metabolism. Interestingly, jazD and jazD phyB plants were insensitive to a chemical inhibitor of Trp biosynthesis, which is a phenotype previously observed in plants expressing hyperactive MYC transcription factors that cannot bind JAZ repressors. These data provide evidence that the mechanisms underlying JA-mediated growth-defense balance depend on the level of defense, and they further establish an association between growth inhibition at high levels of defense and dysregulation of Trp biosynthesis.

Plants continuously integrate external and developmental cues to optimize their fitness in dynamic environments. Acclimation to stress is often associated with negative pleiotropic effects on plant growth and development, especially when resources are limited. Enhanced nutrient foraging and competitiveness, for example, can occur at the expense of resistance to biotic stress (Moreno et al., 2009; Ballardé, 2014). Conversely, plant resistance to herbivores and pathogens is frequently accompanied by reduced growth and reproductive output (Havko et al., 2016; Karasov et al., 2017; Züst and Agrawal, 2017). The antagonistic relationship between growth and defense has been interpreted as a symptom of metabolic competition for limited resources allocated to defense at the expense of growth, or vice versa (Hermes and Mattson, 1992; Heil and Baldwin, 2002; Stamp, 2003). Recent studies, however, have challenged this simple resource-based view of tradeoffs in favor of a more complex regulatory scenario in which interactions between hormone-based signaling networks evoke transcriptional changes that downwardly adjust growth rate upon activation of defense programs (Ullmann-Zeuner et al., 2013; Huot et al., 2014; Campos et al., 2016; Kliebenstein, 2016; Karasov et al., 2017; Züst and Agrawal, 2017; Machado et al., 2017; Guo et al., 2018a; Ballardé and Austin, 2019). A better understanding of mechanisms that constrain the upper limits of growth and defense traits has
potential implications for improving sustainable crop production (Ning et al., 2017; Guo et al., 2018a).

Induced resistance to biotic stress, like plant growth, is a highly complex process coordinated in large part by hormone-response pathways that integrate various developmental and environmental cues (Pieterse et al., 2009; Santner and Estelle, 2009; Bürger and Chory, 2019). Through their ability to both promote defense and inhibit growth, the lipid-derived jasmonates (JAs) exert strong control over the growth-defense balance (Baldwin, 1998; Wasternack and Hause, 2013; Guo et al., 2018a). The JA signaling pathway operates mainly in the nucleus and converges on a set of transcription factors that exert exquisite control over the amplitude of defense traits (Howe et al., 2018). In the unstressed state, JASMONATE ZIM-DOMAIN (JAZ) proteins bind to and repress the activity of cognate transcription factors such as MYC2 and its close relatives MYC3 and MYC4 (Chini et al., 2007; Dombrecht et al., 2007; Thines et al., 2007; Yan et al., 2007; Fernández-Calvo et al., 2011; Niu et al., 2011). In response to biotic challenge or developmental cues, the bioactive form of JAZ, jasmonoyl-L-Ile (JA-Ile), stimulates recognition of JAZ proteins by the F-box protein CORONATINE-SENSITIVE1 (COI1), which is the specificity determinant of the Skp/Cullin/F-box (SCF)-type E3 ubiquitin ligase complex, SCFCOI1. Ubiquitylation of JAZ substrates by SCFCOI1 marks JAZs for degradation via the 26S proteasome (Thines et al., 2007; Katsir et al., 2008; Fonseca et al., 2009; Yan et al., 2009; Howe et al., 2018). Rapid, stress-induced depletion of JAZ relieves repression on MYC and other client transcription factors to execute JA-mediated defense programs and concomitant growth restriction (Yan et al., 2007; Pauwels et al., 2008; Noir et al., 2013; Attaran et al., 2014). Consistent with this model, dominant mutations that impair the ability of Arabidopsis (Arabidopsis thaliana) MYC transcription factors to bind JAZ repressors lead to activation of a subset of JA responses. For example, the atr2D allele of MYC3 causes upregulation of genes encoding enzymes in the Trp biosynthetic pathway, which gives rise to the production of defensive compounds such as indole glucosinolates and camalexin (Smolen et al., 2002; Goossens et al., 2015).

The growth-defense balance in shoot tissues is controlled in part by interactions between the JAZ-MYC pathway and various regulators of cell expansion, including light and growth hormones (Ballaré, 2014; Huot et al., 2014; Havko et al., 2016). Increasing evidence implicates MYCs as conserved regulators of JA-induced shoot growth inhibition (Zhang and Turner, 2008; Major et al., 2017; Guo et al., 2018b; Penuelas et al., 2019). MYCs can influence leaf development by interfering with the activity of PHYTOCHROME-INTERACTING FACTORS (e.g. PIF4) and by promoting the activity of ELONGATED HYCOPOTYLS (HY5), a central regulator of photomorphogenesis (Zhang et al., 2018; Ortigosa et al., 2019). Antagonistic signal cross talk between JA and the red-light receptor phytochrome B (phyB) is thought to allow rapid growth for improved competitiveness with neighboring plants (Moreno et al., 2009; Cerrudo et al., 2012; de Wit et al., 2013; Chico et al., 2014). The transcription factors FAR-RED ELONGATED HYCOPOTYLS3 (FY3) and FAR-RED IMPAIRED RESPONSE1 (FAR1) were recently shown to connect phytochrome and JA signaling, providing a mechanism by which these pathways balance growth and defense (Liu et al., 2019). These phytochromes exert strong control over central metabolism (Yang et al., 2016; Krahmer et al., 2018) raising the additional possibility that JA and light signaling pathways interact to influence the partitioning of central metabolites during growth-defense transitions. Shoot growth is also modulated by antagonistic cross talk between the JA and gibberellin (GA) signaling pathways (Navarro et al., 2008; Huot et al., 2014; Machado et al., 2017). In Arabidopsis, multiple members of the JAZ family interact directly with DELLA repressors of GA signaling (Hou et al., 2010; Yang et al., 2012). JA-induced JAZ degradation can modulate the growth-defense balance by increasing the repressive activity of DELLA proteins on growth-promoting PIF transcription factors, thereby prioritizing defense over growth (Hou et al., 2010; Yang et al., 2012).

The function of JAZ proteins as negative regulators of JA responses has important implications for understanding the origins of induced resistance and its relationship to growth and reproductive success (Guo et al., 2018a; Monte et al., 2019). Consistent with the JAZ model of induced resistance (Howe and Jander, 2008; Erb and Reymond, 2019; Wang et al., 2019), the product of a single JAZ gene in the early land plant Marchantia polymorpha controls most, if not all, JA-mediated growth and defense responses (Monte et al., 2019). By comparison, the multimembered JAZ gene families of vascular plants have both overlapping and cell type-specific roles in growth- and defense-related processes (Thireault et al., 2015; Chini et al., 2016; Howe et al., 2018). In addition to hypermorphic MYC variants that are insensitive to JAZ repression (Smolen et al., 2002; Goossens et al., 2015), constitutive activation of JA responses in Arabidopsis has been achieved by combining loss-of-function mutations in multiple JAZ family members. Use of this approach to genetically “tune” JA responses in the absence of exogenous elicitors (e.g. JA treatment) provides a simple experimental system in which to understand how changes in the quantity and quality of defense impacts the growth-defense balance. For example, multimutants defective in five (jaz quin- tiple [jazQ]), 10 (jaz decuple [jazD]), or 11 (jaz unde- cuple; [jazU]) JAZ genes display increasing levels of defense concomitant with decreased growth and fecundity (Campos et al., 2016; Guo et al., 2018b). These phenotypes of JAZ deficiency provide mechanistic insight into a key prediction of the cost-benefit theory of plant defense, namely that resistance is costly in the absence of biotic stress (Simms and Rausher, 1987; Baldwin, 1998). A current gap in understanding the growth-defense balance is lack of knowledge of how JA
signaling restricts biomass accretion and reproductive output, which are generally regarded as costs of defense (Heil and Baldwin, 2002; Havko et al., 2016; Züst and Agrawal, 2017). jaz mutants in which JA responses are constitutively active provide new genetic tools to address this question and, more generally, to study how variable patterns of defense influence growth and reproductive success.

Central to understanding the underlying mechanisms of the growth-defense balance is the question of how plant growth and fitness are shaped by varying levels of defense (Bergelson and Purrington, 1996; Züst and Agrawal, 2017). Using Arabidopsis as a model system, we explored this question by assessing growth and fitness parameters of jaz mutants in which the level of defense is systematically altered, and by identifying suppressor mutations that mitigate the effects of elevated defense. A genetic screen for mutations that uncouple growth-defense antagonism in the jazQ mutant showed that phyB mutation completely rescued the mild growth defect of the jazQ mutant without affecting the level of defense (Campos et al., 2016). In demonstrating that enhanced defense in the jazQ mutant is not inextricably linked to growth restriction, we initiated this study to determine whether robust growth can also be achieved at even higher levels of defense, as might be expected if JA-mediated growth inhibition is attributed solely to the phyB pathway. A genetic screen for suppressor mutations that rescue the growth deficit of the jazD mutant without compromising defense identified multiple independent mutations in PHYB, thereby validating the importance of JA-phyB cross talk in managing growth-defense phenotypes. However, unlike the jazQ phyB mutant that grows and defends well at the same time, jazD phyB plants maintained the strong defense status of the jazD mutant but displayed only weak growth recovery. Thus, growth restriction associated with high levels of defense is attributed mainly to a phyB-independent pathway. In investigating this pathway, we found that the slow growth of jazD and jazD phyB plants is correlated with upregulation of the Trp biosynthetic pathway but not with changes in central carbon metabolism. Collectively, our results indicate that the mechanisms underlying JA-mediated growth-defense tradeoffs depend on the level of defense and suggest that dysregulation of Trp biosynthesis may contribute to growth restriction at high levels of defense.

RESULTS

jaz Mutations Likely Inhibit Growth Independent of GA Signaling

To understand the mechanism by which shoot growth is inhibited in the jazD mutant, we first tested a prevailing model (Fig. 1A) in which antagonistic cross talk between JAZ and DELLA proteins contributes to the growth-defense balance through reciprocal control of cognate MYC and PIF transcription factors (Hou et al., 2010; Yang et al., 2012). Based on this model, we hypothesized that genetic depletion of JAZs in jazQ and jazD mutants, similar to the effects of JA-induced JAZ degradation, may increase the repression of PIFs, thereby tempering the growth of jaz mutants (Fig. 1A). To test this, we measured the sensitivity of jazQ and jazD seedlings to exogenous GA3, a bioactive GA that promotes hypocotyl elongation. In the absence of GA3, jazQ and jazD hypocotyls were both shorter than wild-type hypocotyls (Fig. 1B). However, the extent to which exogenous GA3 promoted hypocotyl elongation in jazQ and jazD seedlings was similar to that observed for wild-type seedlings (Fig. 1B). This finding suggested that moderate (jazQ) or severe (jazD) JAZ depletion does not have a major effect on GA sensitivity under these conditions. Key evidence for the JAZ-DELLA model of growth-defense tradeoffs comes from studies showing that JA-induced JAZ degradation increases the accumulation of the DELLA proteins SLENDER RICE1 (SLR1) and REPRESSOR OF GA (RGA) in rice (Oryza sativa) and Arabidopsis, respectively (Yang et al., 2012). We therefore tested whether constitutive activation of JA responses in jazQ and jazD mutants, as a consequence of JAZ depletion, is associated with elevated levels of RGA. Contrary to this expectation, immunoblot analysis showed that RGA abundance was not increased in untreated jazQ or jazD seedlings relative to the wild type (Fig. 1C). These data suggest that changes in DELLA activity do not play a major role in restricting the shoot growth of jaz mutants.

Identification of jazD Suppressor Mutations

In the absence of evidence that the dwarf growth stature of the jazD mutant is caused by attenuation of GA-mediated growth responses, we conducted genetic suppressor screens to identify mutations that recover the growth of the jazD mutant without impeding the high level of defense. We visually screened a population of ~20,000 ethyl methanesulfonate (EMS)-mutagenized jazD plants (M2 generation) for individuals with increased rosette size and, as a proxy for defense, persistence of elevated leaf anthocyanin content (Supplemental Fig. S1). In anticipation of a potential contribution of light signaling to the reduced growth of jazD plants (Campos et al., 2016), we screened an additional 10,000 M2 seedlings for long hypocotyls, followed by rescreening of these long-hypocotyl plants at maturity for larger rosette size and increased anthocyanin levels. These combined screens identified 13 suppressor of jazD (sjd) mutants with partially improved rosette growth relative to the jazD parental line. The partial recovery of rosette diameter, leaf area, and biomass of each of these mutant lines was heritable in subsequent generations.

During the initial characterization of growth phenotypes, we observed that the increased leaf area and
biomass in a subgroup of nine *sjd* mutants (*sjd1*, *sjd4*, *sjd40*, *sjd83*, *sjd93*, *sjd109*, *sjd110*, *sjd111*, and *sjd113*) was associated with elongated hypocotyls and petioles, the latter of which contributed to increased rosette diameter (Supplemental Fig. S2). Given these distinct morphological features and their similarity to photomorphogenic mutants, subsequent experiments were focused on this subgroup of long-hypocotyl mutants. Tests of seedling responses to monochromatic light showed that all nine *sjd* lines had elongated hypocotyls under continuous red light (Fig. 2A), suggesting a potential defect in phyB signaling. In support of this hypothesis, the hypocotyl growth response of all nine mutants to far red or blue light was similar to that of the *jazD* mutant (Supplemental Fig. S3). Because phyB mutations impair photosynthesis in mature leaves (Boccalandro et al., 2009; Campos et al., 2016), we also assessed the chlorophyll fluorescence phenotypes of *sjd* mutants relative to *jazD* and an authentic *phyB*-9 mutant. In response to growth under dynamic light conditions, we found that the PSII quantum efficiency (ΦII) in all long-hypocotyl mutants was decreased in comparison to that in *jazD* and wild-type plants, and was in fact very similar to that of the *phyB*-9 mutant (Fig. 2B). Targeted DNA sequencing showed that all nine long-hypocotyl *sjd* mutants harbor point mutations in the *PHYB* gene, with most of these changes located in or near conserved domains of the protein (Fig. 2C). Allelic complementation tests further confirmed that the long-hypocotyl phenotype of *sjd* mutants under continuous white light was caused by the *phyB* mutations (Supplemental Fig. S4). To eliminate the possibility that additional EMS-induced mutations contribute to the growth phenotypes of this group of *sjd* mutants, we performed genetic crosses to reconstruct a *jazD phyB*-9 undecuple mutant carrying the *phyB*-9 null allele (Fig. 2A; Supplemental Fig. S5). All subsequent experiments were performed with this genetically reconstructed *jazD phyB*-9 line.

**The phyB Mutation Does Not Compromise Defense Phenotypes in the jazD Background**

We tested whether JA signaling is altered in *jazD phyB* plants by examining the sensitivity of roots and shoots to exogenous JA, which elicits strong hypersensitive reactions in the *jazD* mutant (Guo et al., 2018b). Root growth measurements showed that *phyB* and wild-type roots were of similar length on JA-free medium, whereas the loss of phyB in *jazD phyB* plants had no effect on the constitutive short-root phenotype of the *jazD* mutant. On media supplemented with JA, *jazD* and *jazD phyB* roots were similarly hypersensitive to the hormone compared to wild-type or *phyB* plants (Fig. 3A). We also assessed leaf sensitivity to JA by treatment with coronatine, which, as an agonist of the JA-Ile receptor, elicits strong JA responses when applied to Arabidopsis shoots (Feys et al., 1994; Attaran et al., 2014). *jazD* and *jazD phyB* leaves exhibited unrestrained responses to coronatine, as seen from
spreading necrosis and tissue death within 4 d of treatment. Wild-type and phyB leaves were much less sensitive to coronatine, showing only anthocyanin accumulation at the site of treatment (Fig. 3B). Together, these data indicate that the phyB mutation does not significantly alter the sensitivity of jazD roots or shoots to exogenous JA.

We next tested whether the loss of phyB affected the high constitutive expression of various JA-responsive markers in the jazD background. Consistent with our screen for sjd mutants that retain elevated anthocyanin content, the level of anthocyanin accumulation in rosette leaves of jazD phyB plants was about 3-fold higher than in the wild type, albeit not as high as in the jazD mutant (Fig. 3C). Transcript abundance measured by reverse transcription quantitative PCR (RT-qPCR) further showed that early JA-response genes, including ALLENE OXIDE SYNTHASE (AOS), OXOPHOTODIENOATE-REDUCTASE 3 (OPR3), and MYC2, were highly expressed to similar levels in jazD and jazD phyB plants (Fig. 3D). Likewise, defense genes associated with insect and pathogen attack, including VEGETATIVE STORAGE PROTEIN2 (VSP2), PLANT DEFENSIN1.2a (PDF1.2a), and THIONIN2.1 (Thi2.1), were also highly expressed in the jazD phyB mutant (Fig. 3E). These data indicate that defense-related transcriptional programs activated in the jazD mutant remain active in the jazD phyB mutant.

Transcriptional reprogramming in the jazD mutant involves the coordinated expression of primary and specialized metabolic genes involved in the biosynthesis of defensive compounds (Guo et al., 2018b). A prominent example is the upregulation of the Trp biosynthetic pathway, together with enhanced expression of genes encoding enzymes for the conversion of Trp to indole glucosinolates and related defense compounds (Fig. 4A). RT-qPCR analysis showed that among several Trp biosynthetic genes tested, all were upregulated in both jazD and jazD phyB plants relative to the wild-type and phyB backgrounds (Fig. 4B). Similarly, mRNAs encoding enzymes involved in the synthesis of the core glucosinolate structure (CYP79B3 and CYP83B1), as well as enzymes that modify the indole side chain (CYP81F2 and IGMT1; Fig. 4A), were generally more abundant in jazD and jazD phyB plants than in the wild type (Fig. 4C). To validate these findings, we used liquid chromatography-mass spectrometry (LC-MS) to determine the effect of the phyB mutation on the higher-intensity pulses (day 3, right). C, Sequencing of the PHYB gene in red-light-insensitive sjd lines identified mutations in all nine mutants. The diagram depicts locations of mutations relative to conserved domains of phyB (colored boxes). Asterisks denote nonsense mutations. One mutant (sjd83) harbors the same G-to-A transition mutation as the phyB-9 mutant allele. The N-terminal photosensory module includes a PAS-2 (Period/Arnt/Single-minded) domain, a GAF (cGMP phosphodiesterase/adenylyl cyclase/FhlA) domain for binding the bilin chromophore, and a PHY domain that stabilizes the photoactivated Pfr state. The C-terminal output module includes two PAS domains and a regulatory His kinase-related (HKR) domain.

Figure 2. Long-hypocotyl sjd mutants are impaired in phytochrome B signaling. A, Hypocotyl lengths of long-hypocotyl sjd mutants under monochromatic red light. Seedlings of the indicated genotype were grown for 7 d on LS medium in continuous red light at a fluence rate of 25 μE m⁻² s⁻¹. Data points are means ± so (n = 8–10 plants per genotype). The dashed line indicates the length of jazD hypocotyls. Asterisks denote significant differences at P < 0.05 relative to jazD by Dunnett’s Test. B, Heat map of $\Phi_i$, in which chlorophyll fluorescence values for the indicated mutants were normalized to Col-0. Photosynthetic performance was monitored over 3 d of 16 h/d light intensity regimes: constant light (day 1, left); a sinusoidal increase and decrease in light intensity (day 2, middle); and a sinusoidal light regime with
constitutive accumulation of indole glucosinolates in jazD leaves. As shown in Figure 4D (and Supplemental Fig. S6A), virtually all identifiable glucosinolates that were more abundant in the jazD mutant (relative to the wild type) were also elevated in the jazD phyB mutant, with a trend that levels in the jazD phyB mutant were slightly lower than in the jazD mutant. Principal component analysis of the complete glucosinolate profile in each genotype explained 88% of the total variance among the genotypes (Supplemental Fig. S6B). This analysis also showed that the overall glucosinolate profile of jazD phyB leaves was similar to that of jazD but distinct from that of wild-type and phyB leaves. We conclude that the constitutive production of indole glucosinolates in the jazD mutant remains largely intact in the jazD phyB mutant.

Finally, we tested whether the enhanced resistance of the jazD mutant to insect herbivory by Trichoplusia ni or infection by the necrotrophic fungal pathogen Botrytis cinerea depends on phyB signaling. jazD leaves displayed strong resistance to both T. ni and B. cinerea challenge (Fig. 5), as previously reported (Guo et al., 2018b). Whereas T. ni larvae reared on wild-type and phyB plants achieved similar weights after 10 d of feeding under these experimental conditions, insect performance on jazD phyB leaves was dramatically reduced to levels observed on jazD plants (Fig. 5, B and C). Likewise, B. cinerea infection assays showed that the size of spreading lesions on jazD and jazD phyB leaves was comparable, and much smaller than that on wild-type and phyB leaves (Fig. 5, D and E). These data indicate that the loss of phyB signaling does not significantly compromise the high level of JA-mediated resistance conferred by the jazD mutations.

The phyB Mutation Weakly Recovers the Growth and Reproductive Phenotypes of the jazD Mutant

Our initial characterization of long-hypocotyl sjd mutants suggested that the phyB mutation only weakly
recovered the rosette growth of the \textit{jazD} mutant (Supplemental Fig. S2), in contrast to the full growth recovery reported for the \textit{jazQ phyB} mutant (Campos et al., 2016). To validate this observation, we compared the growth of \textit{jazD phyB} and \textit{jazQ phyB} plants, together with appropriate control lines, in a set of plants grown side by side under long-day conditions. The moderate reduction in rosette biomass of \textit{jazQ} plants was fully recovered by the \textit{phyB} mutation, with the leaf area of the \textit{jazQ phyB} mutant being even greater than that of the wild type (Fig. 6). By comparison, the rosette biomass and leaf area of the \textit{jazD phyB} mutant were similar to those of the \textit{jazD} mutant. Under these conditions, however, the petiole length and rosette diameter of the \textit{jazD phyB} plants were greater than those of \textit{jazD} plants (Supplemental Fig. S7A), consistent with our visual identification of these \textit{sjd} mutants in the suppressor screen.

We noticed that \textit{jazD phyB} plants grown under the short-day (8-h days) conditions used for our insect and pathogen bioassays appeared to have greater growth recovery than those grown under long-day (16-h days) conditions (Fig. 5A). Comparison of \textit{jazD} and \textit{jazD phyB} plants grown under long- and short-day conditions indeed showed that the growth recovery of \textit{jazD phyB} plants (relative to \textit{jazD}) was stronger under short days (Supplemental Fig. S7). For example, in short-day-grown \textit{jazD phyB} plants, the rosette diameter and petiole length returned to wild-type levels, while the rosette fresh weight and leaf area were improved by \textasciitilde 50\% of that of the wild type (Supplemental Fig. S7). These data indicate that genetic interactions between \textit{jazD} and \textit{phyB} affect shoot growth in a photoperiod-dependent manner.

The inability of the \textit{phyB} mutation to fully recover the slow shoot growth in the \textit{jazD} background extended to reproductive phenotypes of the \textit{jazD} mutant (Guo et al., 2018b). Specifically, we found that traits indicative of the poor reproductive performance of \textit{jazD} plants, including shorter siliques and fewer seeds per siliqua, were only partially recovered in \textit{jazD phyB} plants (Supplemental Fig. S7A). We also observed that the strong delay in time to flowering in the \textit{jazD} mutant was not affected by the loss of \textit{phyB} in \textit{jazD phyB} plants (Supplemental Fig. S7C). These results show that, similar to shoot growth phenotypes, the \textit{phyB} mutation does not fully recover the reproductive phenotypes of the \textit{jazD} mutant.

### Figure 4. Glucosinolate accumulation remains elevated in the \textit{jazD phyB} mutant.

#### A. Simplified pathway for the biosynthesis of indole glucosinolates from central carbon metabolites erythrose 4-phosphate (E4P) and phosphoenolpyruvate (PEP). Trp is both an essential amino acid for growth and a precursor for defensive glucosinolates.

#### B. RT-qPCR measurements of relative transcript levels of Trp biosynthesis genes (ASA1, PAT1, IGPS, TSA1, and TSB2; B) and indole glucosinolate biosynthetic genes (CYP79B3, CYP83B1, CYP81F2, and IGMT1; C) in rosette leaves of the indicated genotypes. Expression levels were normalized to the reference gene PP2a. Data points show the means \( \pm SD \) \( (n = 3 \text{ plants per genotype}) \).

#### C. Accumulation of indole glucosinolates in rosette leaves of wild-type (WT), \textit{jazD}, \textit{phyB}, and \textit{jazD phyB} plants. Data points are means \( \pm SD \) \( (n = 4 \text{ plants per genotype}) \). Lowercase letters represent a significant difference at \( P < 0.05 \), determined by Tukey's HSD mean-separation test. ASA1, Anthranilate synthase alpha subunit 1; PAT1, anthranilate phosphoribosyltransferase1; IGPS, indole-3-glycerol-phosphate synthase; TSA1, Trp synthase \textalpha{} chain1; TSB2, Trp synthase \textbeta{} chain2; IGMT1, indole glucosinolate O-methyltransferase1; I3M, indol-3-ylmethyl (glucobrassicin); 4OH-I3M, 4-hydroxyindol-3-ylmethyl (hydroxyglucobrassicin); 4MOI3M, 4-methoxyindol-3-ylmethyl (methoxyglucobrassicin); 1OH-I3M, 1-hydroxyindol-3-ylmethyl; 1MOI3M, 1-methoxyindol-3-ylmethyl (neoglucobrassicin).
Reduced Growth of the jazD phyB Mutant Is Not Strongly Correlated with Changes in Central Metabolism

We next explored the hypothesis that the slow growth of jazD phyB plants is associated with changes in central metabolism resulting from strong (jazD), but not from moderate (jazQ), defense levels. We reasoned that any metabolic changes interfering with phyB-mediated growth recovery of the jazD mutant would persist in the jazD phyB plants but would be absent in jazQ and jazQ phyB mutants. Gas exchange measurements showed that the photosynthetic rate per unit leaf area relative to the wild type was not affected in the jazD mutant but was reduced in both phyB and jazD phyB plants (Supplemental Fig. S9, A and B). Analysis of the CO2 response curves further indicated that the reduced net assimilation of CO2 in jazD phyB leaves reflects limitations in the activity of Rubisco and electron transport (Supplemental Fig. S9A), which is remarkably similar to the photosynthetic phenotype of jazQ phyB plants in which growth is fully restored (Campos et al., 2016). We also employed gas exchange experiments to measure the day- and night-time respiration rates in the various genotypes. Respiration rates in the jazD mutant were slightly higher than in wild-type and phyB plants (Supplemental Fig. S9, C and D), as previously reported (Guo et al., 2018b). Respiration rates in the jazD phyB mutant were intermediate between those of the jazD and phyB mutants, but the differences were not significant. These data suggest that changes in photosynthesis and respiration do not account for the reduced growth of jazD phyB plants.

We previously observed that the heightened defense status and slow growth of the jazD mutant is associated with symptoms of carbon limitation, including increased expression of sugar starvation marker genes and modest reduction in the levels of Suc and starch (Guo et al., 2018b). In comparisons across genotypes, end-of-day Suc levels were lower in jazQ and jazD but higher in phyB plants relative to the wild type (Fig. 7A), consistent with previous studies (Guo et al., 2018b; Yang et al., 2016). Suc content was recovered in both jazQ phyB and jazD phyB plants (Fig. 7A). Thus, variations in Suc levels do not strictly correlate with growth. A similar trend was observed for starch, where the phyB mutation tended to increase starch levels in both the jazQ and jazD genetic backgrounds (Fig. 7B). We next queried the transcript abundance of four sugar starvation marker genes (BRANCHED-CHAIN AMINO ACID TRANSFERASE2 [BCAT2], DARK INDUCIBLE1 [DIN1], DARK INDUCIBLE6 [DIN6], and BETA-GALACTOSIDASE4 [BGAL4]) that are induced in response to extended darkness (Baena-González et al., 2007). In soil-grown plants maintained under our standard long-day conditions, transcripts of all four marker genes were more abundant in jazQ phyB leaves than in wild-type, phyB, and jazQ leaves (Fig. 7C). BCAT2 and BGAL4 expression remained elevated in the jazD phyB mutant, whereas that of DIN1 and DIN6 returned to wild-type levels (Fig. 7C). These findings indicate that although the expression of

Figure 5. The enhanced resistance of the jazD mutant to biotic stress is not compromised by the loss of phyB. Plants of the indicated genotype were grown under short-day conditions and challenged with neonate T. ni larvae or B. cinerea infection. A, Photograph of control (Con) and insect-challenged (T. ni) plants at the end of the feeding trial. Scale bars = 2 cm. WT, Wild type. B and C, Photograph of representative T. ni larvae (B) and larval weights (C) measured after 10 d of feeding on the indicated genotype. Scale bar = 0.5 cm. Data points show the means ± se (n = 14, where each sample is the mean of three larvae per plant). Lowercase letters in C and E represent a significant difference at P < 0.05, determined by Tukey’s HSD mean-separation test. D and E, Photograph of representative B. cinerea lesions (D) and areas of spreading necrotic lesions (E) after 5 d of infection on detached leaves of the indicated genotype. Scale bars = 0.5 cm. Data points show the means ± se (n = 15–18 infected leaves per genotype).
which elevated expression of Trp biosynthetic genes correlates with the growth phenotype of the six relevant genotypes (wild type, jazQ, jazD, phyB, jazQ phyB, and jazD phyB). This analysis showed that the expression of genes encoding enzymes for the conversion of chorismate to Trp was elevated to similar levels in the two growth-inhibited mutants (jazD and jazD phyB) but not in the genotypes having modest (jazQ) or no (wild type, phyB, and jazQ phyB) growth reduction (Supplemental Fig. S10, A and B). Very similar results were obtained for genes in the phospho-Ser pathway, which supplies Ser for Trp biosynthesis (Supplemental Fig. S10, A and B). Consistent with these results, we also found that the levels of Trp and Ser in jazD and jazD phyB leaves were elevated relative to the other four genotypes (Fig. 8A).

The initial step in Trp biosynthesis is catalyzed by anthranilate synthase (AS), which is subject to feedback inhibition by Trp (Fig. 8B). As an independent approach to correlate changes in Trp metabolism with the strength of growth-defense tradeoffs, we tested the sensitivity of jazQ and jazD seedlings to a toxic analog of Trp, 5-methyl-Trp (5-MT). 5-MT also exerts feedback inhibition on AS, but unlike Trp, it cannot be used to support protein synthesis (Fig. 8B; Li and Last, 1996). Interestingly, we found that jazD roots were more resistant than wild-type roots to a broad range of 5-MT concentrations, whereas jazQ roots were as sensitive as wild-type roots (Fig. 8C). This finding is consistent with the fact that AS and other Trp biosynthetic enzymes are strongly upregulated in the jazD mutant, but not in the jazQ mutant (Supplemental Fig. S10; Guo et al., 2018b). Using a concentration of 5-MT (15 μM) that strongly inhibits the growth of jazQ and wild-type roots, but not jazD roots, we next compared the 5-MT sensitivity of all six genotypes. Similar to the results obtained for other Trp biosynthetic markers, jazD and jazD phyB roots were completely insensitive to 5-MT, whereas wild-type, jazQ, phyB, and jazQ phyB roots were fully sensitive to the inhibitor (Fig. 8D). These collective data show that increased Trp metabolism is associated not only with elevated production of defense compounds and biotic resistance, but also with reduced growth.

**DISCUSSION**

JAZ transcriptional repressors promote growth and reproductive success in plants by preventing over-activation of defense responses, a function that is conserved in ancient land plants (Guo et al., 2018a, 2018b; Monte et al., 2019). Here, we used a jaz decuple mutant (jazD) of Arabidopsis that is mostly devoid of the JAZ repressors as a model to explore the nature of growth and reproductive constraints that accompany high levels of defense. To further address how growth-defense tradeoffs are alleviated and, specifically, whether the mitigation of tradeoffs by rewiring of JA-linked transcriptional circuits is dependent on the level of defense, we employed a genetic suppressor screen of the jazD mutant. We reasoned that if genetic uncoupling of
growth-defense antagonism, as observed previously in the jazQ phyB mutant (Campos et al., 2016), is independent of the level of defense, it should be possible to identify sjd mutants in which high levels of defense (i.e. jazD-like) are accompanied by robust growth (i.e. wild type-like) and reproductive performance. Although this expectation was not borne out, our identification of phyB as the causal mutation in 9 of the 13 sjd suppressors validates the role of phyB activity in JA-induced growth inhibition (Campos et al., 2016) and, given the weak growth recovery of jazD phyB mutants, also reveals a phyB-independent pathway for growth restriction. Our selection of a subset of sjd mutants for long-hypocotyl phenotypes likely biased the screen in favor of light signaling defects. Nevertheless, we note that of the four remaining (non-phyB) sjd suppressors identified, none exhibited obvious light-related phenotypes (e.g. elongated hypocotyls) or complete uncoupling of growth and defense phenotypes. Further characterization of these non-phyB suppressor mutants may provide additional insight into the underlying mechanisms of the JA-mediated growth-defense balance. Because our sjd suppressor screen was not saturated for genes other than PHYB, additional suppressor screens with the jazD mutant or other higher-order jaz mutants may be informative.

The opposing effects of the jaz and phyB mutations on leaf architecture may provide, at least in part, a physiological explanation for how loss of phyB mitigates the slow growth of constitutive JA-response mutants. The progressive negative effect of the jazQ and jazD mutations on leaf area and petiole length leads to increased leaf overlap and, as a consequence, a reduction in whole-plant leaf area available to intercept light (Guo et al., 2018b). We previously showed that phyB increases the leaf area of the jazQ mutant while also reducing leaf thickness, which reduces leaf construction costs, elevates the whole-plant photosynthetic rate, and likely contributes to growth recovery (Campos et al., 2016; Weraduwage et al., 2018). These architectural features of jazQ phyB leaves are similar to those observed here for the jazD phyB mutant, particularly the accentuated growth of jazD phyB plants relative to jazD under short-day conditions in which a longer total growth period compounds the effect of leaf area on light capture and photosynthetic performance (Weraduwage et al., 2015). Future studies are needed to better understand the mechanisms by which JAZ and phyB regulate leaf architecture, including potential impacts on cell division and cell wall remodeling (Bömer et al., 2018; Mielke and Gasperini, 2019).

The JA pathway is part of a larger, integrated signaling network that controls growth and development in response to changing environmental factors (Kazan and Manners, 2011; Ballaré, 2014; Huot et al., 2014). A key node of hormone cross talk involves the mutual antagonism between JA and GA responses, which is controlled in part by JAZ-DELLA interactions (Hou et al., 2010; Yang et al., 2012). Our results do not...
play a role in jaz-mediated growth inhibition, which is supported by recent studies in Arabidopsis and the liverwort Marchantia polymorpha (Major et al., 2017; Penuelas et al., 2019). Emerging evidence further indicates that MYC transcription factors contribute to JA-phyB cross talk by influencing other photomorphogenic regulators, including PIF4, HY5, and FHY3 (Zhang et al., 2018; Chakraborty et al., 2019; Liu et al., 2019; Ortigosa et al., 2019). A better understanding of how JA and phyB signaling pathways intersect to modulate the growth-defense balance is likely to emerge from systems-level analysis of the action of cognate transcription factors, including G-box-binding MYCs and PIFs that occupy the promoter regions of many JA- and light-responsive genes (Franklin and Quail, 2010; Zander et al., 2020).

The modest recovery of rosette biomass and leaf area of the jazD phyB mutant contrasts with the restoration of wild-type-sized rosettes in jazQ phyB plants (Campos et al., 2016). The inability of the phyB mutation to fully restore growth in the jazD background indicates the existence of one or more phyB-independent pathways for growth inhibition that operate at high defense levels. A clue to the incomplete growth recovery of the jazD phyB mutant comes from the observation that this mutant maintains high levels of defense associated with major reprogramming of primary and specialized metabolism, including symptoms of carbon limitation and dysregulation of amino acid metabolism (Guo et al., 2018; Chakraborty et al., 2019; Liu et al., 2019; Ortigosa et al., 2019). A better understanding of how JA and phyB signaling pathways intersect to modulate the growth-defense balance is likely to emerge from systems-level analysis of the action of cognate transcription factors, including G-box-binding MYCs and PIFs that occupy the promoter regions of many JA- and light-responsive genes (Franklin and Quail, 2010; Zander et al., 2020).

Our finding that Trp metabolism remains elevated in jazD phyB plants but not in jazQ or jazQ phyB plants establishes a correlation between high defense levels, increased Trp metabolism, and growth constraint. Interestingly, some Trp-related phenotypes of jazD and jazD phyB plants are reminiscent of the effects of dominant mutations (e.g. atr2D) that impede the binding of MYC transcription factors to JAZ repressors, leading to hyperactive MYC activity (Smolen et al., 2002; Goossens et al., 2015). The severe growth restriction of MYC3atr2D-overexpressing plants (Smolen et al., 2002) resembles that of the jazD mutant and supports the notion of MYCs as growth inhibitory factors (Major et al., 2017). The insensitivity of jazD and MYC3atr2D mutants to 5-MT further suggests that hyperactivation of MYC3 contributes to the upregulation of Trp metabolism in jazD plants (Smolen et al., 2002; this study). It remains to be determined whether other gain-of-function MYC mutants, such as MYC2D105N (Goossens et al., 2015), are affected in 5-MT sensitivity or the production of Trp-derived defense compounds. We

support the hypothesis that the growth restriction of jaz mutants results from inhibition of GA responses, consistent with other work showing that the effects of JA on growth are largely independent of GA (Zhang and Turner, 2008; Ortigosa et al., 2019). We favor an alternative hypothesis in which MYC transcription factors
also note that the MYC3atr2D mutant does not recapitulate all phenotypes of the jazD mutant. For example, whereas the MYC3atr2D mutant is associated with lower levels of free Trp in leaves (Smolen et al., 2002), we found that Trp levels in jazD leaves are slightly higher than in the wild type. This observation suggests a broader regulatory function for JAZs in coordinating primary metabolism (e.g. amino acid biosynthesis) with the production of specialized defense compounds (Bolton, 2009).

Stringent control of amino acid metabolism during the growth-to-defense transition may reflect a mechanism to avoid pleiotropic negative effects on fitness while providing sufficient primary building blocks for defense. Although it is clear that Trp metabolism is required to support the production of indole glucosinolates and other defensive compounds in Arabidopsis, a direct link between altered Trp metabolism and JA-induced growth restriction remains to be established. It seems unlikely that increased levels of free Trp are responsible for the slow growth of the jazD and jazD phyB mutants, because elevated Trp levels in various Trp biosynthetic mutants (e.g. trp5) are not associated with reduced growth (Li and Last, 1996). An alternative interpretation is that the growth constraint in the jazD mutant reflects a compensatory strategy to avoid metabolic perturbations resulting from changes in primary metabolism required to supply defense pathways, analogous to amino acid biosynthetic mutants that exhibit severe growth restriction (Dong et al., 2017; de Oliveira et al., 2019). The relationship between altered Trp metabolism and growth is further complicated by potential perturbations in levels of auxin, whose predominant biosynthesis from Trp could be affected by the allocation of Trp to defense (Mashiguchi et al., 2011; Malka and Cheng, 2017) or through the formation of Trp conjugates that interfere with auxin transport (Staswick, 2009; Staswick et al., 2017). It is also possible that a metabolite derived from the Trp pathway acts via a conserved energy sensing pathway to modulate growth (Malinovsky et al., 2017). At present, we cannot formally exclude the possibility that genetic polymorphisms introduced from non-Columbia-0 (Col-0) accessions during the construction of the jazD mutant contribute to the slow growth of this line; whereas the five jaz insertion mutations used to construct the jazQ mutant were all derived from Col-0 strains, two of the additional five jaz mutations used for construction of jazD were introgressed from other accessions (Guo et al., 2018b). Such genetic linkage effects have been implicated as a cost of resistance in breeding crop plants for R gene-mediated resistance (Karasov et al., 2017). In future studies it will be informative to determine whether the reduced growth of the jazD mutant can be recovered by loss of the COI1 receptor or the MYC transcriptional regulators, as was shown to be the case for the jazQ mutant (Major et al., 2017).

In summary, our results indicate that the mechanisms underlying JA-mediated growth-defense trade-offs depend on the level of defense activation. As indicated by studies of the jazQ mutant (Campos et al., 2016; Major et al., 2017; Guo et al., 2018b), growth restriction at low to moderate levels of defense are not associated with major defects in reproductive output under laboratory conditions and can be suppressed by loss of phyB signaling. Moderate growth reduction at this intermediate level of defense may be an integral feature of induced resistance, which downwardly adjusts growth without necessarily penalizing fitness (Smith and Stitt, 2007; Guo et al., 2018a; Ballaré and Austin, 2019). At high levels of investment in defense, our data suggest that massive metabolic reprogramming geared toward the production of chemical defense compounds generates both growth and reproductive constraints that are independent of phyB. This form of growth-defense tradeoff may be manifested only under extreme conditions in which the high levels of defense necessitate major adjustments to primary metabolism. It is possible that strong allocation of primary metabolites to pathways for chemical defense, which in Arabidopsis are largely derived from amino acids, results in metabolic imbalances that cannot support normal growth. This hypothesis is consistent with the idea that plants have the capacity to sense changes in the availability of amino acids and other primary metabolites, and to respond by adjusting the growth rate to a level that matches the availability of resources (Smith and Stitt, 2007; Guo et al., 2018a; de Oliveira et al., 2019). A challenge for future studies will be to determine how specific changes in metabolism during the growth-to-defense transition are mechanistically linked to growth.

**MATERIALS AND METHODS**

**Plant Material and Growth Conditions**

Arabidopsis (Arabidopsis thaliana) ecotype Columbia-0 (Col-0), was the wild-type genetic background for all experiments. The jazD phyB mutant was constructed by combining the jazD (Guo et al., 2018b) and jazQ phyB mutants (Campos et al., 2016) as described in Supplemental Figure S5. PCR-based genotyping of the jazD phyB mutant used primer sets flanking T-DNA insertion sites, with a third primer specific for the T-DNA border (Supplemental Table S1; Campos et al., 2016; Guo et al., 2018b). The phyB-9 allele used in this study was recently shown to carry a second site phyB-nine-enhancer (bnen) mutation in the PHENOSA4 gene that alters photosynthetic traits and leaf growth (Yoshida et al., 2018). We confirmed that the bnen mutation was lost during the construction of the jazQ phyB and jazD phyB mutants (Supplemental Fig. S5D). PCR reactions for genotyping were performed with GoTaq Green Master Mix (Promega) and the following programmed conditions: 5 min at 95°C, followed by 35 cycles of denaturation (90 s at 95°C), annealing (90 s at 56°C), and elongation (90 s at 72°C), with a final 10-min elongation step at 72°C. The bnen mutation was detected using a dCAPS marker. The PCR-amplified products were digested with DmeI restriction enzyme (New England Biolabs) as per the manufacturer’s instructions.

Seeds were stratified for 3 to 4 d at 4°C in the dark to improve the rate and synchrony of germination. After sowing seeds on soil, pots were covered with a transparent plastic dome for 10 d to increase humidity. Unless stated otherwise, soil-grown plants were maintained with 21°C days at a light intensity of 90–110 μmol m⁻² s⁻¹ from cool-white fluorescent lights and 20°C nights. For experiments with plate-grown seedlings, seeds were surface sterilized in a 60% (v/v) bleach solution for 10 min and washed at least six times before stratification. Seeds were sown on square culture plates (Thermo Fisher Scientific) containing one-half strength Linsmaier and Skoog (LS; Caisson Labs) salts with 0.2% (w/v) 

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phytoblend (Caisson Labs) agar and 0.8% (w/v) Suc, unless stated otherwise. Plates were maintained at 21°C with 16 h at a light intensity of 80 \mu M\text{m}^{-2}\text{s}^{-1} and 8 h dark.

**jazD Suppressor Screen**

Approximately 30,000 jazD seeds were mutagenized by immersion in a solution of 0.1% or 0.2% (v/v) EMS (Sigma-Aldrich) for 16 h at room temperature, with constant agitation (Campos et al., 2016). Seeds (M1 generation) were thoroughly washed with water, stratified in the dark at 4°C for 2 d and then immediately sown on soil for growth at 21°C under long-day conditions. M2 seed was collected from 24 pools of self-pollinated M1 plants (≈1,000 M1 plants/pool). Soil-grown M2 plants (≈30,000 total) were visually screened for individuals with either larger rosette size or longer hypocotyl length compared to the jazD mutant, together with persistence of anthocyanin accumulation (see Supplemental Fig. S1). Putative jsl (suppressor of jazD) mutants were rescreened in the M3 generation to confirm heritability of phenotypes.

**Measurements of Shoot and Root Growth**

Root growth inhibition assays were performed with seedlings grown on media oriented vertically and supplemented with the indicated concentrations of methyl-JA (Sigma-Aldrich; Shyu et al., 2012) or 5-MT dissolved in 0.5M HCl media oriented vertically and supplemented with the indicated concentrations of 0.1% or 0.2% (v/v) EMS (Sigma-Aldrich) for 16 h at room temperature (8-h day, 16-h night) or for 7 weeks under short-day conditions (9- to 11-d-old seedlings using an extraction buffer containing 50 mM NaCl, 1% (v/v) Triton X-100, 50 \mu M MC-132, 1 \mu M phenylmethylsulfonyl fluoride, and 1X protease inhibitor cocktail. The resulting protein was quantified by BCA assay (ThermoFisher). Proteins were separated on a 10% SDS-polyacrylamide gel and electro-transferred to a polyvinylidene difluoride membrane. Western blot was performed with rabbit anti-RGA primary antibody (Agriera) and donkey antirabbit secondary antibody (goat HR, ThermoFisher). The blot was incubated for 5 min with SuperSignal West Pico Chemiluminescent Substrate (ThermoFisher) and proteins were detected with a ChemiDoc MP Imaging System. Protein extracted from the transfer DNA (T-DNA) insertion mutant rge-28 (SALK_089146) was used as a control for specificity of the anti-RGA antibody.

**Western Blot Analysis**

Total protein was extracted from shoot tissue of 16-d-old plate-grown seedlings using an extraction buffer containing 50 mM Tris (pH 8.0), 150 mM NaCl, 1% (v/v) Triton X-100, 50 \mu M MC-132, 1 \mu M phenylmethylsulfonyl fluoride, and 1X protease inhibitor cocktail. The resulting protein was quantified by BCA assay (ThermoFisher). Proteins were separated on a 10% SDS-polyacrylamide gel and electro-transferred to a polyvinylidene difluoride membrane. Western blot was performed with rabbit anti-RGA primary antibody (Agriera) and donkey antirabbit secondary antibody (goat HR, ThermoFisher). The blot was incubated for 5 min with SuperSignal West Pico Chemiluminescent Substrate (ThermoFisher) and proteins were detected with a ChemiDoc MP Imaging System. Protein extracted from the transfer DNA (T-DNA) insertion mutant rge-28 (SALK_089146) was used as a control for specificity of the anti-RGA antibody.

**Metabolite Measurements**

Metabolites were measured from plants grown for 4 weeks on soil under long-day conditions. Rosettes were harvested, weighed, and homogenized with a Tissuelyser II (Qiagen) after freezing in liquid nitrogen and were stored at –80°C until analysis. For anthocyanin measurements, tissue was extracted overnight at 4°C in MeOH containing 1% (v/v) HCl, and then clarified by centrifugation. Bulk anthocyanin content was determined spectrophotometrically by A320 – 0.25 (A657) and normalized to extracted tissue weight (Campos et al., 2016). For glucosinolate measurements, tissue was extracted in 80% (v/v) MeOH as described previously (Glauser et al., 2012). Samples were diluted 1:10 in water and analyzed in the Michigan State University Mass Spectrometry and Metabolomics Facility with a Xevo G2-XS UPLC QTOF (Waters), as described previously (Koo et al., 2009; Glauser et al., 2012). Sinigrin was used as an internal standard and glucosinolate levels were reported relative to the wild type after normalization to extracted tissue weight.

To determine levels of Trp and Ser, leaf tissue (~10 mg) was incubated at 90°C in 70\% (v/v) water containing labeled standards (13C,15N-labeled amino acids; Sigma Aldrich) for 5 min. After cooling on ice, the extract was clarified by centrifugation and filtered through 0.2-\mu m, low-binding hydrophilic polytetrafluoroethylene centrifugal filters (Millipore). Filtrates were diluted 2-fold in 20 \mu M perfuroheptanoic acid (Sigma Aldrich) and analyzed in the Michigan State University Mass Spectrometry and Metabolomics Facility with a Quattro Micro API LC-MS/MS (Waters) equipped with an Acquity UPLC BEH T3 1.8 \mu m column (2.1 × 100 mm, 1.8-\mu m particle size). Waters) with multiple reaction monitoring, as described previously with modifications (Gu et al., 2007). The LC method was modified to a 13-min run with 10 \mu M perfuroheptanoic acid solvent to better retain and separate amino acids. The MS/MS method was modified to include transitions for stable-labeled amino acid internal standards and divided into three resolved functions (0–4.5 min, 4.5–6.3 min, and 6.3–13 min) for data acquisition to allow sufficient dwell time for each analyte (Supplemental Table S2). Endogenous amino acid concentrations were determined according to external standard curves and normalized to extracted tissue weight.

For starch and Suc measurements, leaf tissue was frozen in liquid nitrogen, lyophilized, weighed, and homogenized with a Tissuelyser II. Tissue was extracted in 3.5% (v/v) perchloric acid on ice for 5 min and clarified by centrifugation at 4°C. The resulting supernatant was neutralized to pH 7–8 with neutralizing buffer (2 \mu M KOH, 150 \mu M HEPES, and 10 \mu M KCl), frozen to precipitate salts, and clarified by centrifugation. The resulting supernatant was used for Suc determination. The perchloric acid pellet was washed twice with water, twice with 80% (v/v) ethanol, resuspended in 0.2 \mu M KOH and incubated

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at 95°C for 30 min. After cooling, 1 M acetic acid was added to adjust the pH to ~5 and starch was digested to Glc by incubating with 50 U α-amylase (Megazyme) and 1 U amyloglucosidase (Megazyme) at room temperature for 2 d. Samples were clarified by centrifugation and the supernatant was used for determination of starch content. Glc content was determined spectrophotometrically with a NADP(H)-linked assay (Lowry and Passonneau, 1972). Samples were incubated until reaction completion in assay buffer (150 mM HEPES [pH 7.2], 15 mM MgCl₂, and 3 mM EDTA) containing 500 nmol NADP, 500 nmol ATP, and 1 U Glc-6-phosphate dehydrogenase (Sigma). A baseline was determined at A₅₄₀. Samples were then incubated until reaction completion with 1 U hexokinase (Sigma), and the absolute Glc content was determined from the increase in A₅₄₀ from an extinction coefficient of 6,220 L mol⁻¹ cm⁻¹ for NADPH at 340 nm. To measure Suc content, samples were further incubated until reaction completion with 50 U invertase (Sigma) and the Glc content was determined from the increase in A₅₄₀.

Insect and Pathogen Assays

Plants for insect feeding and pathogen infection assays were grown for 6 weeks on soil under short-day (8-h day, 16-h night) conditions. For insect feeding assays, three neonate Trichoplusia ni larvae (Bemzony Research) were reared on each of at least nine plants per genotype for 10 d, after which photographs were taken of plants and larvae, and larval weights were measured (Herde et al., 2013). For pathogen infection assays, detached leaves were placed on filter paper moistened with sterile water in culture plates. Each leaf was inoculated with a 4-µL drop of Botrytis cinerea spore suspension (5,000 spores/ml in 50% [v/v] grape juice) and 5 and starch was digested to Glc by incubating with 50 U -amylase (Megazyme) and 1 U amyloglucosidase (Megazyme) at room temperature for 2 d.

Supplemental Data

The following supplemental materials are available.
Supplemental Table S1. Primers used for genotyping and RT-qPCR.
Supplemental Table S2. Details of LC-MS/MS gradient and functions.
Supplemental Figure S1. Genetic screen for suppressor of jazD (sjd) mutants.
Supplemental Figure S2. Suppressor of jazD (sjd) mutants with long hypocotyls have improved growth.
Supplemental Figure S3. Hypocotyl lengths of long-hypocotyl (sjd) mutants under monochromatic far red and blue light.
Supplemental Figure S4. Allelic complementation of long-hypocotyl (sjd) mutants with jazD phyB.
Supplemental Figure S5. Genetic reconstitution of jazD phyB.
Supplemental Figure S6. jazD and jazD phyB plants have similar glucosinolate profiles.
Supplemental Figure S7. phyB partially recovers growth of jazD in a photoperiod-dependent manner.
Supplemental Figure S8. phyB mutation only weakly recovers reproductive phenotypes of jazD.
Supplemental Figure S9. jazD and phyB interact to modulate photosynthesis and respiration.
Supplemental Figure S10. Elevated Trp metabolism persists in jazD phyB.

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