Plant hormone jasmonate prioritizes defense over growth by interfering with gibberellin signaling cascade

Dong-Lei Yang, Jian Yao, Chuan-Sheng Mei, Xiao-Hong Tong, Long-Jun Zeng, Qin Li, Lang-Tao Xiao, Tai-ping Sun, Jigang Li, Xing-Wang Deng, Chin Mei Lee, Michael F. Thomashow, Yinong Yang, Zuhua He, and Sheng Yang He

National Key Laboratory of Plant Molecular Genetics, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200032, China. Department of Energy Plant Research Laboratory, Michigan State University, East Lansing, MI 48824; Howard Hughes Medical Institute, Chevy Chase, MD 20815; Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701; Hunan Provincial Key Laboratory of Phytohormones, Hunan Agricultural University, Changsha 410128, China; Department of Biology, Duke University, Durham, NC 27705; Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT 06520; and Department of Plant Pathology and Huck Institutes of Life Sciences, Pennsylvania State University, University Park, PA 16802

Edited by Frederick M. Ausubel, Harvard Medical School, Massachusetts General Hospital, Boston, MA, and approved March 20, 2012 (received for review January 30, 2012)

Plants must effectively defend against biotic and abiotic stresses to survive in nature. However, this defense is costly and is often accompanied by significant growth inhibition. How plants coordinate the fluctuating growth-defense dynamics is not well understood and remains a fundamental question. Jasmonate (JA) and gibberellic acid (GA) are important plant hormones that mediate defense and growth, respectively. Binding of bioactive JA or GA ligands to cognate receptors leads to proteasome-dependent degradation of specific transcriptional regulators (the JAZ or DELLA family of proteins), which, at the resting state, represses cognate transcription factors involved in defense (e.g., MYCs) or growth (e.g., phytochrome interacting factors (PIFs)). In this study, we found that the coi1 JA receptor mutants of rice (a domesticated monocot crop) and Arabidopsis (a model dicot plant) both exhibit hallmark phenotypes of GA-hypersensitive mutants. JA delays GA-mediated DELLA protein degradation, and the DELLA mutant is less sensitive to JA for growth inhibition. Overexpression of a selected group of JA repressors in Arabidopsis plants partially phenocopies GA-associated phenotypes of the coi1 mutant, and JAZ9 inhibits RGA (a DELLA protein) interaction with transcription factor PIF3. Importantly, the pif quadruple (pifq) mutant no longer responds to JA-induced growth inhibition, and overexpression of PIF3 could partially overcome JA-induced growth inhibition. Thus, a molecular cascade involving the COI1–JAZ–DELLA–PIF signaling module, by which angiosperm plants prioritize JA-mediated defense over growth, has been elucidated.


The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Data deposition: The data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, www.ncbi.nlm.nih.gov/geo (accession nos. GSE28577 and GSM732294–GSM732299).

D.-L.Y., J.Y., and C.-S.M. contributed equally to this work.

Present address: Institute for Sustainable and Renewable Resources, Institute for Advanced Learning and Research, Danville, VA 24540.

*To whom correspondence may be addressed. E-mail: yuj@psu.edu, zhe@ibs.ac.kr, or hes@msu.edu.

This article contains supporting information online at www.pnas.org/cgi/doi/10.1073/pnas.1201616109/DCSupplemental.
el elongation. Most recently, it has been shown that DELLA repressors promote JA signaling through physically interacting with JAZ1 (23), suggesting a mechanism for GA-mediated down-regulation of JA defense responses. However, it remains unknown how JA could inhibit plant growth. In this study, through analysis of rice and Arabidopsis, we have elucidated a molecular cascade by which JA antagonizes GA signaling that explains how monocot and dicot plants prioritize JA defense over growth.

Results
Knockdown of Rice COI1 Genes Decreases JA Response. As a model monocot, rice (Oryza sativa L.) has been commonly used to study hormone signaling as well as defense responses in cereal crops. A number of studies have been conducted to dissect JA signaling and function in defense response and developmental process in rice (24–27). However, the role of COI1 in mediating JA signal perception is still unclear in rice. The rice genome contains two closely related COI1 genes, OsCOI1a (Os01g0853400) and OsCOI1b (Os05g0449500), which share 83% and 82% sequence identity at the DNA and protein levels, respectively. To determine the function of COI1 in rice, a double-strand RNAi construct containing the conserved sequence of OsCOI1a and OsCOI1b (Fig. S1A) was introduced into the model variety Nipponbare. More than 20 independent RNAi lines were produced (Fig. S1B). The transcript levels of both OsCOI1a and OsCOI1b were significantly reduced in these RNAi lines as detected by RNA blot and quantitative RT-PCR (qRT-PCR) analyses (Fig. 1 A and B), indicating that OsCOI1 expression was effectively knocked down by RNAi.

JA sensitivity was investigated in two stable RNAi lines, coi1-13 and coi1-18, that carry a single copy of the transgene. In this study, we used methyl JA (MeJA), which is converted to JA and then to bioactive jasmonyl-isoleucine in plants (3), to treat rice and Arabidopsis. As expected, the transgenic lines were much less sensitive to MeJA than the WT plants in the growth-inhibition assay (Fig. 1 C and D). Furthermore, JA-responsive genes such as OsVSP and OsMPK7 exhibited reduced expression in the OsCOI1-RNAi plants (Fig. 1E). These results demonstrate that OsCOI1 is required for JA signaling in rice.

OsCOI1-RNAi Plants Display Phenotypes Similar to Those of GA Overproduction. Intriguingly, when grown in the greenhouse or the paddy field, the coi1-13 and coi1-18 plants consistently showed increased plant height in comparison with the WT plants, a phenotype that mainly resulted from elongated internodes (Fig. 2 A–C). This elongated phenotype of the OsCOI1-RNAi plants is similar to that of the rice eui1 mutants, which contain a loss-of-function mutation in the P450 monooxygenase CYP714D protein that catalyzes the 16a,17-epoxidation reaction of GA deactivation (28, 29). Another similarity between the rice coi1 lines and the eui1 mutants was that they produce longer grains than the WT plants (Fig. S2). Plant growth is controlled by cell division and cell elongation. Cell length in the uppermost internode of the coi1-18 plants was found to be significantly increased in comparison with that of the WT plants (Fig. 2 D and E), indicating that increased plant height is mainly caused by cell elongation instead of cell division, a GA-related feature (30). Whole transcriptomic analysis of coi1-13 plants revealed that basal expression of several GA-related genes, including GA2ox, GA20ox, and OsWRKY71, was altered in the OsCOI1-RNAi rice (Fig. S1C).

GA signaling regulates diverse biological processes, including α-amylase release, during seed germination in rice (31). We examined the effect of MeJA on the GA induction of α-amylase activity in embryoless seeds, and found that coinoculation with MeJA strongly suppressed the GA induction of α-amylase (Fig. S3A). Consequently, the seed germination rate was significantly decreased with MeJA treatment (Fig. S3B). In contrast, the seeds of coi1-13 and coi1-18 had significantly higher levels of α-amylase activity and germinated at a much quicker rate than the seeds of the WT (Fig. S3 C and D). These results indicated that the modulation of GA signaling by JA occurs not only in plant growth but also during seed germination. Taken together, these results suggest that interruption of JA signaling in the coi1 mutants augments the GA signal pathway in rice.

OsCOI1-RNAi Plants Are Hypersensitive to GA and Hyposensitive to GA Biosynthesis Inhibitor. To further confirm the alteration of GA signaling in the OsCOI1-RNAi rice plants, the growth of rice seedlings was examined in semisolid one-half Murashige–Skooog (MS) medium supplemented with different concentrations of GA3. The coi1-18 plants exhibited more sensitivity to exogenous application of GA3 in comparison with the WT plants (Fig. S4A). Consistent with their increased GA sensitivity, the coi1-18 plants exhibited reduced sensitivity to the GA biosynthesis inhibitor uniconazole compared with the WT plants (Fig. S4B). Therefore, the OsCOI1-RNAi plants were hypersensitive to exogenous GA and hyposensitive to GA biosynthesis inhibitor.

To examine the effect of the OsCOI1 silencing on the endogenous GA levels, the bioactive GAs, GA3 and GA4, were measured in both the RNAi and WT plants. In contrast to the eui1 mutants, which accumulate extremely high levels (30–100-fold) of GA3 and GA4 (28), the coi1-18 plants accumulated only slightly higher levels of GA4 (3.8-fold) than the WT plants in the elongating uppermost internode (Table S1). The levels of GA3 were similar in coi1-18 and WT plants. The modest change in the GA4 level may be correlated to the differential expression of
OsCOI1 plants show plant heights and internode lengths. (**) Mutant. We next wanted to determine the level of SLR1, coi1-18 grown in the isolated paddy or the WT plant because of the low basal morphology of and and is much longer than that of Nipponbare (**) in comparison with the RNAi plants. More than 30 plants of each line were analyzed. The difference between the control and transgenic plants is significant (**p < 0.001, Student’s t test). (C) Lengths of individual internodes in the WT and coi1-18 plants. Each internode of coi1-18 was longer than the counterpart of Nipponbare (**p < 0.01 and **p < 0.001, Student’s t test). (D) Microscopic sections of the elongating zone of the uppermost internodes from Nipponbare and coi1-18 grown in the isolated paddy field. (Scale bar, 40 μm.) (E) Cell lengths at the base of the elongating zone of the uppermost internodes in the WT and coi1-18 plants. The cell of coi1-18 is much longer than that of Nipponbare (**p < 0.001, Student’s t test).

Several GA metabolism genes in OsCOI1-RNAi plants (Fig. S1C). However, overall the defect in JA signaling does not appear to dramatically affect bioactive GA biosynthesis/accumulation in rice, even though OsCOI1-RNAi plants exhibited coi1-like growth phenotypes. Consistent with this observation, no significant difference in the transcript levels of EUI1 was found between coi1-18 and the WT (Fig. S5). Taken together, these results strongly suggest that the increased plant height and cellular elongation of the OsCOI1-RNAi plants is mainly a result of the hypersensitivity to GA.

Elongation of OsCOI1-RNAi Plants Is Inhibited by Attenuating GA Signaling. EUI1 overexpression resulted in a series of GA-deficient phenotypes, with drastic reduction of the bioactive GAs (28) and accumulation or stabilization of the DELLA protein SLR1 (32). We crossed the EUI1-overexpression plants (Eui1-OX) to the coi1-18 plant (Fig. 3D). The homozygous Eui1-OX/coi1-18 plants showed greatly reduced plant height (Fig. 3 A–C) and reduced cell size in the uppermost internode (Fig. 3 E and F), similar to Eui1-OX plants. In addition, the longer grain phenotype of the coi1-18 plants was reverted to that of the WT (Fig. 3 G and H). We also crossed coi1-18 with the GA receptor gid1-1 mutant. Again, the gid1-1/coi1-18 double mutant exhibited a dwarf phenotype like gid1-1 (Fig. S6). This result demonstrated that the GA receptor gene GID1 is required for the function of OsCOI1 in the GA pathway. These results suggest that the OsCOI1-RNAi morphology is dependent on the GA signaling pathway.

JA Antagonizes GA Signaling Pathway by Delaying GA-Induced SLR1 Degradation. We next wanted to determine the level of SLR1, a rice DELLA protein that functions as a key repressor of the GA signaling pathway (33). However, it was difficult to detect SLR1 in the coi1-18 or the WT plant because of the low basal level of SLR1. Instead, we found that SLR1 accumulated in the Eui1-OX/coi1-18 plant at a level comparable to the Eui1-OX plant (Fig. 4A). Therefore, the degradation dynamics of SLR1 protein was examined in Eui1-OX/coi1-18 in the presence of exogenous GA3. When transferred into the medium with 100 μM GA3 for 30 min, SLR1 was significantly degraded in the Eui1-OX/coi1-18 plants, whereas it was degraded only slightly even after 2 to 3 h with GA treatment in the Eui1-OX plants (Fig. 4Ab). This result suggested that JA signaling antagonizes GA-mediated reduction of the DELLA protein.

Having shown that turning down the JA pathway could increase the GA signaling output, we next examined the possibility that turning on JA signaling might antagonize the GA signaling pathway. Indeed, whereas growing WT rice seedlings in the presence of 10 μM GA3 leads to elongation of the second leaf sheath by approximately 120%, addition of MeJA greatly reduced the GA-triggered elongation in a dose-dependent manner (Fig. 4B and C). Furthermore, in the presence of MeJA, GA-induced SLR1 degradation was significantly inhibited as long as 6 h after treatment (Fig. 4D). Consistent with this observation, seedling growth was inhibited by MeJA in a dose-dependent manner, with decreased shoot length (Fig. 4E and F). In addition, the SLR1 protein accumulated in the plants grown in the medium supplemented with MeJA (Fig. 4G), whereas no change was observed in the SLR1 transcript level (Fig. S7). Finally, the growth inhibition effect of MeJA was significantly suppressed in the SLR1 loss-of-function mutant slr1 in comparison with the WT (Fig. 5), further supporting that JA-mediated growth inhibition is in part dependent on the DELLA repressor. These results collectively demonstrated that JA represses rice growth through antagonizing GA signaling at least partly via affecting the level of the DELLA protein SLR1.

Arabidopsis coi1 Mutant Also Exhibits GA-Related Phenotypes. The significant GA hypersensitivity phenotypes of the OsCOI1 RNAi lines was somewhat unexpected because such phenotypes were not previously reported for the Arabidopsis coi1 mutants (6, 34). We therefore looked for GA-related phenotypes in the Arabidopsis coi1 mutant plants. We found that Arabidopsis coi1 plants have several robust phenotypes that resemble GA hypersensitivity, including longer hypocotyls and petioles under low-in...
Fig. 3. The GA-deficiency mutation reverses the phenotype of col1-18 plants. (A) Morphological phenotype of Eui1-OX/coi1-18. (B) The average plant height of WT, col1-18, Eui1-OX, and Eui1-OX/coi1-18 plants. (C) The length of each internode of col1-18 decreased in the Eui1-OX/coi1-18 plants. (D) Expression of OsCOI1 in Eui1-OX/coi1-18. (E and F) Cell lengths at the bases of the uppermost internodes in col1-18, Eui1-OX/coi1-18, and Eui1-OX plants. (Scale bar, 40 μm.) (G) and (H) Grain lengths of col1-18, Eui1-OX/coi1-18, and Eui1-OX plants. Letters on the columns in B, C, F, and H indicate significant differences determined by Tukey-Kramer multiple comparison test (P < 0.05).

tensity light condition and early flowering (Fig. 6). Moreover, transgenic overexpression of JAZ repressors, which mimics the effect of col1 mutations, was found to phenocopy the col1 mutant. Among eight AtJAZ genes (AtJAZ1, 3, 4, 5, 6, 9, 10, and 11) we were able to overexpress, AtJAZ1, 3, 4, 9, 10, and 11 produced the early flowering phenotype, but, interestingly, AtJAZ5 and 6 overexpression plants did not (Fig. S8). We also checked AtJAZ9 overexpression plants for GA-mediated germination response and found that they were more resistant to the GA biosynthesis inhibitor paclobutrazol (Fig. S9), which is a GA-hypersensitivity phenotype.

Next, we investigated whether, like in rice, JA could antagonize GA signaling by affecting the level of DELLA proteins in Arabidopsis. A well characterized DELLA protein, RGA, was monitored in these experiments. Consistent with what was observed in rice, when Arabidopsis seedlings were continuously treated with JA, the RGA protein level increased, whereas the RGA transcript level did not change (Fig. 7). As internal controls, JA induced degradation of JAZ9 and expression of a known JA-responsive gene, AOS (Fig. 7). Taken together, these results collectively show that disruption of JA perception and signaling affects GA phenotypes in Arabidopsis and that JA negatively regulates GA responses through modulating the level of DELLA repressors in rice and Arabidopsis.

Fig. 4. Levels of the rice DELLA protein SLR1 and the antagonistic effect of MeJA on GA-mediated plant growth. (A) The GA-mediated degradation of the DELLA protein SLR1 was promoted in the col1-18 plants. The 10-d-old seedlings grown on one-half MS plates with 0.6% agar were transferred to liquid one-half MS medium with 100 μM GA₃, and the SLR1 protein was detected with an SLR1 antibody at the indicated time points. (B and C) MeJA inhibited GA-induced shoot elongation in WT plants: 1, control plant; and 2 to 5, representative plants treated with GA₃ and MeJA in the same order as in Fig. 4C. The relative growth is indicated by the length of second leaf sheath after being treated with 10 μM GA₃ and various concentrations of MeJA. (D) MeJA delayed SLR1 degradation induced by GA₃. The concentration of GA₃ and MeJA used were 10 μM and 100 μM, respectively. (E and F) MeJA inhibited WT rice seedling growth and second sheath elongation in a dose-dependent manner. Asterisks indicate significant difference between mock and treatments (P < 0.01, Student’s t test). (G) MeJA treatment promoted the accumulation of SLR1 in a dose-dependent manner in WT plants. The rice plants were grown on one-half MS plates with 0.6% agar supplemented with MeJA (final concentration indicated on Top).

Fig. 5. The rice slr1 mutant is insensitive to MeJA. (A) WT and slr1 seedling were grown on one-half MS plates with 0.6% agar supplemented with or without 25 μM MeJA. (B) Relative growth of slr1 and WT plants is indicated by percentages of the second leaf sheath lengths with MeJA treatment compared with those without MeJA treatment (**P < 0.001, Student’s t test).
continuous white light at 22 °C. Della proteins were recently shown to interact with the JAZ family of proteins, which are transcriptional repressors that regulate gene expression in response to various environmental signals. The interaction of Della proteins with the JAZ family is thought to involve the GRAS domain of Della proteins and the JAZ N terminus. This interaction is critical for modulating growth and development in Arabidopsis and other plants. The study by Yang et al. further supports this interaction and demonstrates its role in modulating growth in Arabidopsis under various conditions.

**JAZ Repressors Directly Interfere with DELLA–PIF Interaction.**

DELLA proteins have been shown to interact and repress growth-promoting transcription factors, such as PIFs in Arabidopsis (18, 19). Interestingly, the Della proteins were recently found to also interact with AtJAZ1 in Arabidopsis (23). By using multiple methods, we independently observed multiple JAZ–DELLA interactions in plant or yeast, and found that, in the case of the JAZ9–GAI interaction, the N terminus of JAZ9 and the GRAS domain of GAI are important for interaction in yeast (Fig. 8 A and B and Fig. S10). Although Hou et al. focused their study on how GA antagonizes JA signaling through the AtJAZ1–GAI interaction, we noticed a striking correlation between the ability of AtJAZ overexpression to confer early flowering and the ability of JAZ9 overexpression to confer early flowering (Fig. 8 S8) and physical interaction with DELLA proteins: AtJAZ1, 3, 4, 9, 10, and 11, but not AtJAZ5 and 6, interacted with Della proteins and produced the early flowering phenotype (Fig. S10). We therefore investigated the intriguing possibility that AtJAZ repressors may impede the DELLA–PIF interaction. We first confirmed the interaction between the GRAS domain of RGA and PIF3 in a yeast two-hybrid assay (Fig. 8C). We found that expression of AtJAZ9 inhibited the RGA–PIF3 interaction in yeast three-hybrid assays (Fig. 8C), without affecting the protein levels of RGA and PIF3 (Fig. 8D). The effect of JAZ9 on the RGA–PIF3 interaction could also be observed in plant cells by using Nicotiana tabacum–based transient expression assays. Again, RGA interacted with PIF3 in this system; however, coexpression with AtJAZ9 could effectively prevent RGA–PIF3 interaction (Fig. 8E).

Our results suggest that JAZ-mediated interference with the DELLA–PIF interaction is a key mechanism that modulates plant growth. To obtain genetic evidence for or against this possibility, we analyzed the responses of pif mutants and PIF3 overexpression plants to JA treatment. We found that the pif quadruple mutant (pifq) grew more slowly compared with WT plants, and were no longer able to respond to JA-mediated inhibition of hypocotyl growth (Fig. 9 A and B). This result suggests that PIFs are likely the main, if not the only, growth-promoting transcription factors that are targeted by JA-induced growth inhibition. More interestingly, overexpression of PIF3 alone was sufficient to partially overcome JA-induced inhibition of hypocotyl growth (Fig. 9 A and B). Our results contrast with those from a recent report that showed that PIF4 transgenic overexpression plants exhibited enhanced JA-induced growth inhibition (23). Also, although the delta quadruple mutant (delaq) showed only a slightly lower sensitivity to JA-mediated inhibition of hypocotyl growth in the study by Hou et al. (23), under our experimental conditions, the delta quintuple mutant (gai-6/rga-12/gl1-1/gl2-1/gl3-1) was almost completely insensitive to JA-induced hypocotyl inhibition (Fig. S11). Finally, we found that expression of two examined DELLA/PIF-regulated genes—expansin 10 (EXP10, AT1G26770) and xyloglucan:xyloglucan transferase 33 (XTH33, AT1G0530)—was altered in a predicted manner upon JA treatment: JA up-regulates the expression of EXP10, which is down-regulated by PIFs, whereas JA down-regulates XTH33, which is up-regulated by PIFs (Fig. 9 C and D). Taken together, our results strongly suggest that JAZ-mediated interference with the DELLA–PIF interaction is a critical part of a mechanism by which JA antagonizes GA signaling in modulating growth.

**Discussion**

The growth–defense conflict is a widely known phenomenon in plants, although the underlying molecular mechanism is not well understood. Our study provides insights into the mechanisms underlying the JAZ-mediated inhibition of hypocotyl growth, which is a key component of the growth-defense conflict in Arabidopsis. The interaction between Della proteins and JAZ family members, particularly the JAZ9–GAI interaction, is a critical part of a mechanism by which JA antagonizes GA signaling in modulating growth. This finding not only contributes to our understanding of the molecular basis of growth-defense conflicts but also provides insights into the regulation of growth and development in plants.
characterized. JA is an important plant hormone that plays a prominent role in plant defense against diverse pathogens and herbivores (3, 38). Despite rapid progress on dissecting the JA signaling pathway in recent years, a mechanistic explanation for how plants effectively balance growth and defense in response to the activation of JA signaling has remained elusive. In this study, we show that modulation of the level of DELLA repressors and interference with DELLA interaction with growth-promoting PIF transcription factors are two key mechanisms underlying JA-mediated growth inhibition in monocot rice and dicot Arabidopsis, illustrating a potentially widely conserved strategy by which angiosperm plants coordinate a major form of growth/defense tradeoff. Future research shall address whether the two mechanisms function independently of each other and how JA signaling modulates the level of DELLA proteins.

The DELLA proteins were first identified as key repressors of the GA pathway (39), and were subsequently shown to impact other hormone pathways such as auxin, abscisic acid, and ethylene (36, 40–42); plant photomorphogenesis (18, 19, 43); and plant survival under abiotic stress (44–46). Our results further support the notion that DELLA proteins act as key regulators/switches in integrating hormone and environmental signals and in fine-tuning plant growth and stress responses (47), which are critical for plant survival under harsh conditions (44, 46). We have provided clear evidence that JA treatment increases SLR1 levels in rice and RGA in Arabidopsis, which are predicted to result in the repression of plant growth. Conversely, we found that JAZ9 could effectively interrupt RGA–PIF3 interaction, suggesting that, in the absence of JA signaling, some DELLA repressors could be titrated out by JA proteins, which would allow more PIF transcription factors to activate growth programs. This mechanism could explain the GA-hypersensitivity phenotypes observed in the coi1 mutants of rice and Arabidopsis, and in transgenic Arabidopsis plants overexpressing those JAZ proteins (e.g., AJJAZ1, 3, 4, 9, 10, and 11) that interact with DELLA proteins. It can also explain why not only the della quintuple mutant was largely insensitive to JA-induced growth inhibition, as expected, but also why overexpression of PIF3 could partially counter JA inhibition of growth (Fig. 9A). In short, we have provided experimental evidence for a signaling cascade, involving the COI1–JAZ–DELLA–PIF signaling module, that underlies the growth inhibition during JA defense activation.

In this study, we noticed interesting differences between rice, a domesticated monocot crop, and Arabidopsis, a wild dicot, in that prominent GA phenotypes of coi1 mutants are displayed under different conditions for rice and Arabidopsis. Whereas OsCOI1-RNAi plants exhibit exaggerated stem elongation and other GA-related phenotypes under strong light conditions in the greenhouse and in the field, Atcoi1 plants show most obvious GA phenotypes under dim light conditions (10 μmol m⁻² s⁻¹ continuous white light; Materials and Methods), but not under other growth conditions previously reported (6, 34). Therefore, although the core JA and GA pathways are likely conserved in monocot and dicot plants, divergence in JA and GA signaling and/or the crosstalk between JA and GA signaling in dicot and monocot species might exist. Recently, Robson et al. (48) found that the coi1 mutant flowered earlier and developed longer

Fig. 8. JAZ9 interferes with the interaction between RGA and PIF3. (A) 3xHA-JAZ9 interacts with 9xMyc-GAI or 9xMyc-RGA protein when expressed transiently in N. tabacum leaves. Protein extracts were immunoprecipitated with an anti-HA antibody and analyzed by Western blot with an anti-Myc antibody. (B) JAZ9 interacts with RGA protein in 3xHA-JAZ9 transgenic Arabidopsis plants. Protein extracts from 12-d-old seedlings were immunoprecipitated with an anti-HA antibody and analyzed by Western blot with an anti-Myc antibody. (C) JAZ9 inhibits the interaction between RGA and PIF3 in yeast. The activity of the reporter gene HIS3, which indicates the interaction between RGA and PIF3, is greatly reduced [indicated by reduced growth on medium lacking histidine (-His)] in the presence of JAZ9 [induced in medium without methionine (-Met)]. (D) Western blot shows that all proteins analyzed in the Y3H assay (C) were expressed as expected. (E) 3xHA-JAZ9 interferes with the interaction between 9xMyc-RGA and 3xFLAG-PIF3 when transiently expressed in N. tabacum leaves. Protein extracts were immunoprecipitated with an anti-Myc antibody and analyzed by Western blot with anti-FLAG, anti-HA, or anti-Myc antibody.
hypocotyls under low red/far-red light conditions than the WT. It is possible that these phenotypes could also be related to GA phenotypes studied here because light and GA signals integrate to regulate Arabidopsis growth (18, 19, 44), with DELLA proteins functioning in plant photomorphogenesis (43). We therefore propose that DELLA-mediated integration of JA, GA, and light signaling may give rise to a fundamental framework and needed flexibility in JA-induced growth–defense tradeoff in adaptation to and/or reflecting extraordinarily diverse growth habitats and domestication histories of angiosperm plants.

Materials and Methods

Plant Materials and Growth Condition. Rice plants (cv. Nipponbare) were grown in a greenhouse or in the isolated paddy field. All Arabidopsis plants were described here were derived from Col-0 except for the delta mutant, which is in Landsberg erecta (Ler) background. The jaz9-1, del1aqs (gai-t6 erga-t2/lrg1-t1rg2-t1rg3-1), and pifq (pif1-t1pif3-3pif4-2pif5-3) mutants, as well as the Pnaj1::GFP-RGA and PIF3OE transgenic lines, have been previously described (9, 18, 35, 49, 50). The jaz9-3 (SM_3-34031; Fig. S12) and coil-30 (SALK_039548; Fig. S13) mutants were characterized in the present study. All Arabidopsis seeds were ordered from the Biological Resource Center.

Arabidopsis seeds were stratified for 3 d at 4 °C before planting. Surface-sterilized seeds were sown on MS medium containing 0.8% agar and 5 mM Mes (pH 5.8), and placed in a growth chamber with 10 μmol m⁻²s⁻¹ continuous cool-white fluorescent light at 22 °C or in a long-day growth chamber with a 16-h day (120 μmol m⁻²s⁻¹ cool-white fluorescent light, 22 °C) and 8-h night (18 °C) cycle. The soil-grown plants were placed in the long-day growth chamber.

Transgenic Expression. For generation of OsC011::RNAi transgenic rice, two fragments of the OsC011 genes were amplified by using the primer pairs OsC011-F1/R1 and OsC011-F2/R2 (Dataset S1), respectively. The fragments were inversely inserted into the pCAMBIA1300S that contained a double 3SS promoter and a terminator. The resulting OsC011::RNAi construct was introduced into the model variety rice Nipponbare (Oryza sativa L. sp. japonica) by using Agrobacterium-mediated transformation. Independent RNAi rice lines were analyzed and confirmed by Southern and Northern blot analyses, as well as qRT-PCR assays. All transgenic plants were grown in a greenhouse or in the isolated paddy field for measurement of plant height and other morphological traits.

For production of transgenic Arabidopsis lines expressing P35S::3xHA::AtJAZ9::Bx::His, the coding sequence of AtJAZ9, excluding the stop codon, was amplified by PCR by using PfuUltra II DNA polymerase (Agilent Technologies) and Arabidopsis cDNA, which was obtained from a 28-d-old Col-0 plant by using the AtJAZ9-F and -R primer set (Dataset S1). The PCR product was first cloned into vector pGEM-T easy (Promega), and then moved into a binary vector pYOP003 (SI Materials and Methods) to create a 3xHA-AtJAZ9::Bx::His fusion construct. Col-0 plants were transformed by Agrobacterium-mediated transformation. Primary transfectants were selected based on BASTA resistance, and the JAZ9 expression level was determined by Western blotting. Transgenic lines with a 3:1 (resistant:sensitive) segregation ratio for BASTA resistance were selected, and several homozygous lines were identified in the T3 generation.

The Gateway entry clones containing GAI, RGA, or PIF3 were identified from the REGIA Arabidopsis transcription factor library (S1). Inserts were transferred into pYOP006 or pYOP012 by LR recombination (Life Technologies) to create P35S::9xGAI, P35S::9xRGA, and P35S::3xFLAG-PIF3, which were used for transient expression in tobacco leaves as previously described (S2).

Hormone Treatment and Growth Assay. For rice, the seeds were sterilized and incubated on one-half MS medium with 0.6% agar and supplemented with different concentrations of GAs and MeJA. Seedling (i.e., shoot) growth and the lengths of the second sheath were measured 12 d after treatment. For Western blotting, 10-d-old seedlings grown in one-half MS medium with 0.6% agar were transferred to liquid one-half MS medium supplemented with 100 μM GA₃ with or without 100 μM MeJA. Samples were harvested at different time points and frozen at −80 °C for RNA and protein extraction. Arabidopsis seeds used for growth assays were harvested on the same day from plants grown side by side. Seedlings were grown on MS plates for 4 d before being transferred onto soil. Seedlings of homozygous coil-30 plants were selected on MS plates containing 10 μM MeJA (Sigma-Aldrich). Plants were kept in a long-day growth chamber unless indicated otherwise. Flowing time was determined when floral buds became visible at the center of rosette. Pediole lengths of the third true leaves were measured on day 21. At least 16 plants of each line were assessed.

Arabidopsis seedlings were also grown on plates with or without 10 μM MeJA under continuous light for 6 d. Seedlings were then placed on a new plate and scanned at a resolution of 600 dpi. The hypocotyl length was measured by using ImageJ software (National Institutes of Health).

Endogenous GA Assay in Rice. The elongating uppermost internodes of the transgenic and WT plants were harvested and lyophilized at −20 °C. GAs were extracted, and GA₄ and GA₃ were assayed by LC-MS with internal standards as described previously (S3).
RNA blot and Transcript Analysis. Total RNA was isolated from rice leaf tissues by using TRIzol reagent according to the manufacturer's protocol (Invitrogen). RNA and blotting was performed by using standard protocol with PerfectHyb buffer (Sigma), and relative gene expression was quantified by using a Phosphorimagr (Amerham Biosciences). For qRT-PCR, total RNA was first treated with DNase I and the first-strand cDNA was then synthesized by using the oligo dT primer and SuperScript II reverse transcriptase (Invitrogen). Rice ubiquitin 1 gene (UBQ1; Os06g0681400) was used as an internal control to normalize samples. Quantitative PCR was performed on the MX3000P real-time PCR system (Agilent Technologies) with a Quantitect SYBR Green PCR kit (Qiagen). Semiquantitative RT-PCR was conducted by using the SuperScript III First-Strand Synthesis System (Invitrogen). The primers used to detect the transcripts of the target genes are listed in Dataset S1.

For analysis of Arabidopsis transcripts, total RNA was extracted by using an Ambion TOYALL RNA Total RNA Isolation Kit (Life Technologies) according to the manufacturer's manual. After DNase I (Roche) treatment, RNA was further purified by using an RNasey Mini Kit (Qiagen). First-strand cDNA was synthesized by using M-MLV reverse transcriptase (Invitrogen) and oligo(dT) as primers. act2 (AT3G18780), cap-binding protein 20 (CBP20; AT5G44200), protein phosphatase 2A subunit A3 (PP2A3; AT1G3320), and ubiquitin-conjugating enzyme 21 (UBC21; AT5G25760) (54) were used as internal controls to normalize target gene expression by geological methods (55). Quantitative PCR was performed on an ABI7500 Fast Real-time PCR System with Fast SYBR Master Mix (Applied Biosystems) according to the manufacturer's protocol. The primers used to detect specific transcripts are listed on Dataset S1.

Protein Extraction, Quantification, and Immunoblots. Total proteins were extracted using plant protein extraction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 0.1% SDS, 1 mM EDTA, and 1 mM DTT) with 1% protease inhibitor for plant cell and tissue extract (Sigma-Aldrich) and 100 μM MG132 (Cayman Chemicals). Protein content was quantified by using the Protein DC assay kit (Bio-Rad). Equal amounts of protein were subjected to SDS/PAGE followed by Western blotting analysis. Immunodetection of GFP-RGA, HA-JAZ9, RGA, Myc-RGA, Myc-GAI, FLAG-PIF3, and SLR1 were performed by using anti-GFP antisera (Abcam), anti-HA antibody (Roche Applied Science), anti-RGA antisera (49), anti-Myc antisera (Abcam), anti-FLAG antibody (Sigma-Aldrich), and anti-SLR1 (33), respectively. Corresponding HRP conjugated secondary antibodies and SuperSignal West Pico Chemiluminescent Substrate (Thermo Fisher Scientific) were used for detection.

Coinmunoprecipitation Assay. Total proteins were extracted from Arabidopsis seedlings or tobacco leaves by using lysis buffer (50 mM Tris-HCl, pH 7.5, 75 mM NaCl, 0.2% Triton X-100, 5 mM EDTA, 5 mM EGTA, 1 mM DTT, and 1% protease inhibitor for plant cell and tissue extract (Sigma-Aldrich) and 100 μM MG132 (Cayman Chemicals). Protein content was quantified by using the Protein DC assay kit (Bio-Rad). Equal amounts of protein were subjected to SDS/PAGE followed by Western blotting analysis. Immunodetection of GFP-RGA, HA-JAZ9, RGA, Myc-RGA, Myc-GAI, FLAG-PIF3, and SLR1 were performed by using anti-GFP antisera (Abcam), anti-HA antibody (Roche Applied Science), anti-RGA antisera (49), anti-Myc antisera (Abcam), anti-FLAG antibody (Sigma-Aldrich), and anti-SLR1 (33), respectively. Corresponding HRP conjugated secondary antibodies and SuperSignal West Pico Chemiluminescent Substrate (Thermo Fisher Scientific) were used for detection.

Rice Microarray Assay. Whole transcriptomic analysis was performed with the Affymetrix GeneChip Rice Genome Array, representing 51,279 transcripts and 3143 probe sets. Total RNA was extracted using plant protein extraction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Triton X-100, 0.1% SDS, 1 mM EDTA, and 1 mM DTT) with 1% protease inhibitor for plant cell and tissue extract (Sigma-Aldrich) and 100 μM MG132 (Cayman Chemicals). Protein content was quantified by using the Protein DC assay kit (Bio-Rad). Equal amounts of protein were subjected to SDS/PAGE followed by Western blotting analysis. Immunodetection of GFP-RGA, HA-JAZ9, RGA, Myc-RGA, Myc-GAI, FLAG-PIF3, and SLR1 were performed by using anti-GFP antisera (Abcam), anti-HA antibody (Roche Applied Science), anti-RGA antisera (49), anti-Myc antisera (Abcam), anti-FLAG antibody (Sigma-Aldrich), and anti-SLR1 (33), respectively. Corresponding HRP conjugated secondary antibodies and SuperSignal West Pico Chemiluminescent Substrate (Thermo Fisher Scientific) were used for detection.

ACKNOWLEDGMENTS. We thank Dr. M. Matsuoka (Nagoya University) for the gid1-1 mutant and the antibody against SLR1, Dr. Q. Qian (China National Rice Research Institute) for the slender rice 1 mutant, Dr. J.-M. Li for use on the research, Dr. P. Quail (University of California) for pifq mutant and PIF3 antibody, H. Zhang, R. K. Kari, and J. Withers for help on the growth of the mutants and J.-O. Li for microscopy assistance. This work was supported by Natural Science Foundation of China Grants 90817102 and 30730064 (to Z.H.), National Key Basic Research and Development Program 2013CB110070 (to Z.H.), US Department of Energy (DOE) Biological and Environmental Research (BER), National Institute of Biotechnology Initiative Grant 2003-33117-17873 (to Y.Y.), National Science Foundation Plant Genome Research Program Grant DBI-0922747 (to Y.Y.), National Institutes of Health Grant R01AI068718 (to S.Y.H. for experiments with jasmonate signaling), and US Department of Energy (Chemical Sciences, Geosciences, and Biosciences Division, Office of Basic Energy Sciences, Office of Science) Grant DE-FG02-91ER20021 (to S.Y.H. for experiments with growth and GA signaling). S.Y.H. is a Howard Hughes Medical Institute and Gordon and Betty Moore Foundation Investigator.


SI Materials and Methods

Binary Vector Constructions. Oligonucleotides that encode the 3xHA, 9xMYC, and 3xFLAG tags were synthesized (Integrated DNA Technologies) and inserted into the NcoI and Nhel sites of pET42a (Novagen) to create pET42a-3xHA, -9xMYC, and -3xFLAG, respectively. The resulting 3xHA-8xHis, 9xMYC-8xHis, and 3xFLAG-8xHis inserts were released by NcoI and AvrII and inserted into the same sites of pFGC5941 (Chromatin Database, www.chromdb.org), in which the EcoRI and XhoI sites were destroyed to create pJYP003, -004, and -011, respectively. An attR Gateway cassette was cloned into the EcoRI and XhoI sites of pJYP003, -004, and -011 to create Gateway cloning-compatible pJYP005, -006, and -012, respectively.

Molecular Cloning. Coding sequences of AtJAZ1-12, AtJAZ9.2, various deletions of AtJAZ9 (1), and AtMYC2, excluding the stop codon, were amplified by PCR using PfuUltra II DNA polymerase (Agilent Technologies) and Arabidopsis cDNA, which was obtained from a 28-d-old Col-0 plant by using primers listed in Dataset S1. PCR products were first cloned into vector pGEM-T easy (Promega), and then moved into the binary vector pJYP003 to create 3xHA-AtJAZ-8xHis fusion constructs or into a gateway entry vector to create gateway entry clones. The Gateway entry clones containing RGL1, RGL2, or RGL3 were identified from REGIA collections. All AtJAZ genes were cloned into both pDEST32 ((Life Technologies) and pDEST-GBKT7 (2) to create GAL4DB bait vectors. AtMYC2 and all DELLA genes were cloned into both pDEST22 (Life Technologies) and pDEST-GADT7 (2) to create GAL4 prey vectors.

Yeast Two-Hybrid Assay. The GAL4 bait and prey vectors were transformed into yeast strain AH109 (Clontech) or MaV203 (Life Technologies) and assayed following the manufacturer’s manual.

Production of Transgenic Plants in Arabidopsis. The p35S:3xHA-AtJAZ-8xHis constructs were transformed into WT Col-0 plants by Agrobacterium-mediated transformation. Primary transformants were selected for BASTA resistance, and jasmonate ZIM-domain (JAZ) expression levels were determined by Western blotting. Transgenic lines with a 3:1 (resistant:sensitive to BASTA) segregation ratio were selected, and several homozygous lines were identified in the T3 generation.

Seed Germination Assay. Approximately 120 surface-sterilized Arabidopsis seeds were sown on plates with or without 5 μM of paclobutrazol (PAC; Sigma-Aldrich). After stratification, plates were placed in a long-day growth chamber. The percentages of seeds that had germinated with fully expanded green cotyledons were scored at day 5.


**Fig. S2.** OsCOI1-RNAi lines have longer grains, which resemble the eui1 mutant. (A) Grain sizes of WT, coi1-13, and coi1-18. (B) Average grain lengths of WT, coi1-13, and coi1-18. There is significant difference between the WT and transgenic OsCOI1-RNAi lines (***P < 0.001, Student’s t test). (C) Grain sizes of TP309 (WT) and Eui1-RNAi line S73. (D) Average grain lengths of TP309 and Eui1-RNAi lines S73. There is a significant difference between the WT and transgenic lines (**P < 0.001, Student’s t test).

**Fig. S3.** Effect of methyl jasmonate (MeJA) on seed germination. (A) MeJA repressed α-amylase induction by GA. The embryoless seeds are used to test α-amylase induction by 1 μM GA₃ alone or in combination with 50 μM MeJA. (B) MeJA repressed seed germination. Seed soaking times were 24 h (Left) and 36 h (Right). (C) OsCOI1-RNAi seeds show higher levels of α-amylase activity than WT Nipponbare seeds. The deembryonated half seeds were imbibed in 1.0 μM of GA₃ solution in the dark at 28 °C for 2 d. The α-amylase activity is presented as A540/mg protein/min, with SEs displayed. In B and C, asterisks indicate significant difference between WT and coi1 mutants (P < 0.01, Student’s t test). (D) Seed germination is promoted in the OsCOI1-RNAi seeds. Soaking times were 24 h (Left) and 36 h (Right). Letters on columns indicate significant differences (P < 0.05, Tukey-Kramer multiple comparison test).

**Fig. S4.** coi1-18 plants are hypersensitive to gibberellin. (A) Second sheath lengths of coi1-18 and WT plants grown in one-half Murashige–Skoog (MS) medium with 0.6% agar supplemented with various concentrations of GA₃. Note that coi1-18 is more sensitive to exogenous GA₃ than the WT. (B) The coi1-18 plants are more resistant to uniconazole, a GA biosynthesis inhibitor, than WT plants, as indicated by increased elongation of the second leaf sheath (**P < 0.01, Student’s t test).

**Fig. S5.** Transcript levels of Eui1 in OsCOI1-RNAi plants. RNA was isolated from the uppermost internode of Nipponbare, coi1-13, and coi1-18 plants grown in the isolated paddy field. Ubiquitin transcript was used as the internal control. PCR cycles: Eui1, 36; Ubiquitin, 25.
**Fig. S6.** The gid1 mutation reduced the shoot length of coi1-18. The plant height of coi1-18/gid1-1 is comparable to that of the gid1-1 mutant.

**Fig. S7.** MeJA treatment does not alter the transcript level of SLR1. Total RNAs were extracted from 7-d-old seedlings grown in one-half MS medium with 0.6% agar with or without 50 μM and 100 μM MeJA. Number of PCR cycles: SLR1, 31; Ubiquitin, 25.

**Fig. S8.** Overexpression of several JAZs in Arabidopsis promotes early flowering. (A) Images of 28-d-old plants taken on the same day. Plants were grown in a long-day growth chamber (16 h 120 μmol m⁻² s⁻¹ light/8 h dark, 22 °C/18 °C). (B) Flowering time of plants indicated. Data shown are the means from 12 plants. Error bars represent SD. (C) Expression of JAZ proteins in transgenic Arabidopsis; 50 μg of total proteins from rosette leaves of 28-d-old Arabidopsis plants were loaded into each lane for immunoblot analysis with an anti-HA antibody. Coomassie brilliant blue (CBB) was used to stain the blotted membrane to show equal loading of proteins.

**Fig. S9.** Overexpression of AtJAZ9 reduces inhibition of seeds germination by PAC. (A) Arabidopsis seeds were germinated on MS plates with or without 5 μM PAC. (B) Data shown are the mean of three independent experiments. Error bars represent SD. Letters on columns indicate significant differences (P < 0.05, Tukey–Kramer multiple comparison test).
Fig. S10. Characterization of interaction between Arabidopsis JAZ and DELLa proteins in yeast. (A) DELLa proteins interact with multiple AtJAZ proteins in yeast. Growth assay was performed on selective medium (-His, -Ade). Yeast growth indicates positive interaction between two proteins. AtJAZs were cloned into pGBK7 and DELLa were cloned into pDEST-GADT7. Yeast strain AH109 was used for the assay. (B) AtJAZ9 interacts with GAI and RGA through its N terminus. Growth assay was performed on selective medium (-His). The medium also contains X-α-gal to monitor MEL1 activity. Yeast growth with blue color indicates positive interaction between two proteins. AtJAZ9 and its derivatives were cloned into pDEST32. MYC2, GAI, and RGA were cloned into pDEST22. Yeast strain MaV203 was used for the assay. (C) AtJAZ9 interacts with the GRAS domain of GAI. Growth assay on selective medium (-His, -Ade). The growth of yeast indicates positive interaction between two proteins. AtJAZ9 was cloned into pGBK7. GAI and its derivatives were cloned in pACT2 (1). Yeast strain AH109 was used for the assay.


Fig. S11. MeJA does not inhibit hypocotyl elongation of a quintuple della mutant. (A) Arabidopsis seedlings were grown on MS medium with or without 10 μM of MeJA under 10 μmol m⁻² s⁻¹ continuous white light for 6 d. (B) The hypocotyl lengths were measured and inhibition of hypocotyl growth was calculated as (1 – treated / untreated) × 100%. Data shown are the means from 16 seedlings. Error bars represent SD. Asterisks indicate significant difference between Landsberg erecta (Ler) and the quintuple della mutant (P < 0.01, Student’s t test).
Fig. S12. Characterization of Arabidopsis jaz9 KO mutants and JAZ9 overexpression lines. (A) Line (SM_3.34031; atjaz9) was isolated from the John Innes Center SM line collection (1). The red arrows indicate the transferred DNA (T-DNA)/transposon insertion sites in jaz9-1 (2) and jaz9-3. (B) RT-PCR indicates no JAZ9 transcripts in jaz9-1 or jaz9-3 plants. (C) Screening of JAZ9 overexpression lines by Western blot. Twenty micrograms of total proteins from 10-d-old seedling were loaded in each lane for immunoblot analysis with an anti-HA antibody. Coomassie brilliant blue (CBB) was used to stain the blotted membrane to show equal loading of proteins.


Fig. S13. Characterization of the coi1-30 line. (A) A new coi1 mutant (SALK_035548) was isolated from SALK T-DNA mutagenesis lines (1). The red arrow points to the T-DNA insertion site in COI1. RT-PCR indicates no detectable COI1 transcript in coi1-30 plants. (B) coi1-30 is resistant to Pseudomonas syringae pv. tomato DC3000 infection. The plants were vacuum-infiltrated with 1 \times 10^6 cfu/mL of bacteria. Images were taken after 3 d. (C and D) coi1-30 is resistant to MeJA treatments, and complemented coi1-30/p35S:YFP:COI1 is sensitive to MeJA. Seedlings were grown on MS medium with or without 10 μM of MeJA for 10 d. The root lengths were measured. Data shown are the means from 12 seedlings. Error bars represent SD. (E) coi1-30 is male-sterile.


### Table S1. Levels of bioactive GAs in internodes of WT and coi1-18 plants

<table>
<thead>
<tr>
<th>Genotype</th>
<th>GA\textsubscript{4}</th>
<th>GA\textsubscript{1}</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>0.98 ± 0.8</td>
<td>12.54 ± 2.07</td>
</tr>
<tr>
<td>coi1-18</td>
<td>3.75 ± 0.8</td>
<td>12.33 ± 1.65</td>
</tr>
</tbody>
</table>

*Significant difference detected between WT and coi1-18 (P < 0.05 Student’s t test). GA amounts are in ng per gram of dry weight.

Other Supporting Information Files

Dataset S1 (XLS)

Yang et al. www.pnas.org/cgi/content/short/1201616109