

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

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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 repressors (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 JAZ 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.

disease resistance | plant growth | plant immunity | light response | plant defense

Sessile plants have evolved a dynamic regulatory network to adapt to the daily and seasonally fluctuating environment. Jasmonate (JA) is a lipid-derived plant hormone that regulates developmental processes, including pollen development, tendril coiling, fruit ripening and senescence, as well as response to biotic and abiotic stress (1–3). The F-box protein coronatine insensitive 1 (COI1), a component of the SCF E3 ubiquitin ligase, has been identified as a principal component of a receptor of JA in *Arabidopsis* and other plants (4–7), and the JA ZIM-domain (JAZ) family proteins are key regulators of JA signaling that repress transcription of JA-responsive genes through interaction with transcription factors, such as MYC2 (8–10). This transcriptional repression requires novel interactor of JAZ (NINJA) and TOPLESS corepressor proteins (11). Bioactive JA, the jasmonoyl-isoleucine conjugate, promotes physical interaction between COI1 and JAZ proteins that results in degradation of JAZs by the 26S proteasome, leading to initiation of JA responses (9, 10). Therefore, the SCF^{COI1}–JAZ protein complex acts as a core site of JA perception. As a regulatory loop, JA also activates *JAZ* gene transcription, leading to the

down-regulation of JA action and the dynamic nature of JA response (12).

Gibberellic acids (GAs) are plant growth-promoting hormones that play important roles in diverse aspects of plant growth and development, such as stem elongation, leaf expansion, flowering, seed development, and germination (13–17). The DELLA family proteins are key regulators of GA signaling that repress transcription of GA-responsive genes through interaction with growth-promoting transcription factors, such as phytochrome interacting factors (PIFs) (18, 19). Bioactive GAs bind to the GA insensitive dwarf1 (GID1) receptor, which, in turn, interacts with DELLA proteins (five members in *Arabidopsis* and a single SLR1 protein in rice) (16, 20). The GA-GID1-bound DELLA/SLR1 proteins are recognized by an F-box protein (SLY1 in *Arabidopsis* and GID2 in rice), resulting in proteasome-dependent degradation of DELLA/SLR1 repressors. Degradation of DELLA/SLR1 proteins derepresses the transcriptional activities of downstream transcription factors, including PIFs (16).

Activation of JA defense signaling is known to severely restrict plant growth, representing a prominent example of growth–defense tradeoff in plants. There have been several reports of crosstalk between JA and GA signaling pathways in *Arabidopsis*, mostly documenting the antagonistic effect of GA on JA signaling (1). A quadruple *della* mutant (which lacks four of the five *Arabidopsis* DELLA proteins) was shown to be partially insensitive to gene induction by JA, whereas the constitutively active dominant DELLA mutant *gai* was found to be sensitized for JA-responsive gene induction, implicating DELLAs in JA signaling and/or perception (21). Cheng et al. (22) found that GA promotes JA biosynthesis, thereby inducing the expression of *MYB21*, *MYB24*, and *MYB57* to promote stamen filament

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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. 1A and B), indicating that *OsCOI1* expression was effectively knocked down by RNAi.

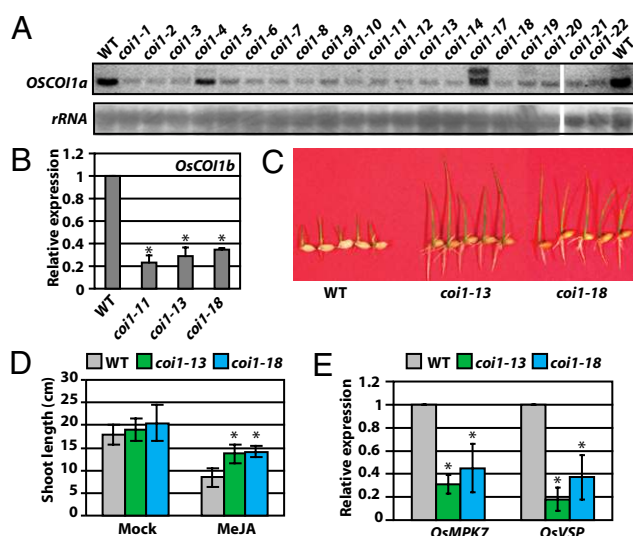


Fig. 1. Generation of *OsCOI1* RNAi transgenic rice with reduced JA sensitivity. (A) Suppression of *OsCOI1a* expression in transgenic RNAi lines as shown by RNA blot analysis. A fragment of 825 to 1,244 nt of *OsCOI1* was used as the probe, and 25S *rRNA* was used as the loading control. (B) Suppression of *OsCOI1b* expression in transgenic RNAi lines as shown by qRT-PCR. (C and D) The representative RNAi lines *coi1-13* and *coi1-18* show reduced sensitivity to MeJA treatment. Seedlings were grown in one-half MS medium with 0.6% agar supplemented with or without 20 μ M MeJA. Pictures were taken at day 7 and shoot length was measured at day 12. Data shown in D are the means from 12 plants. Error bars represent SD. Asterisks indicate significant difference between WT and *coi1* mutants based on Student's *t* test ($P < 0.01$). (E) Reduced expression of JA-response genes *OsMPK7* and *OsVSP* in *coi1-13* and *-18* plants revealed by qRT-PCR. Data shown in C and E are the means from two independent experiments. Error bars represent SD. Asterisks indicate the significant difference between WT and *coi1* mutants based on Student's *t* test ($P < 0.01$).

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 jasmonoyl-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. 1C 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. 2A–C). This elongated phenotype of the *OsCOI1*-RNAi plants is similar to that of the rice *eu1* mutants, which contain a loss-of-function mutation in the P450 monooxygenase CYP714D protein that catalyzes the 16 α ,17-epoxidation reaction of GA deactivation (28, 29). Another similarity between the rice *coi1* lines and the *eu1* 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. 2D 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 coinubation 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 those of the WT (Fig. S3C 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, GA₁ and GA₄, were measured in the RNAi and WT plants. In contrast to the *eu1* mutants, which accumulate extremely high levels (30- to 100-fold) of GA₁ 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 GA₁ were similar in *coi1-18* and WT plants. The modest change in the GA₄ level may be correlated to the differential expression of

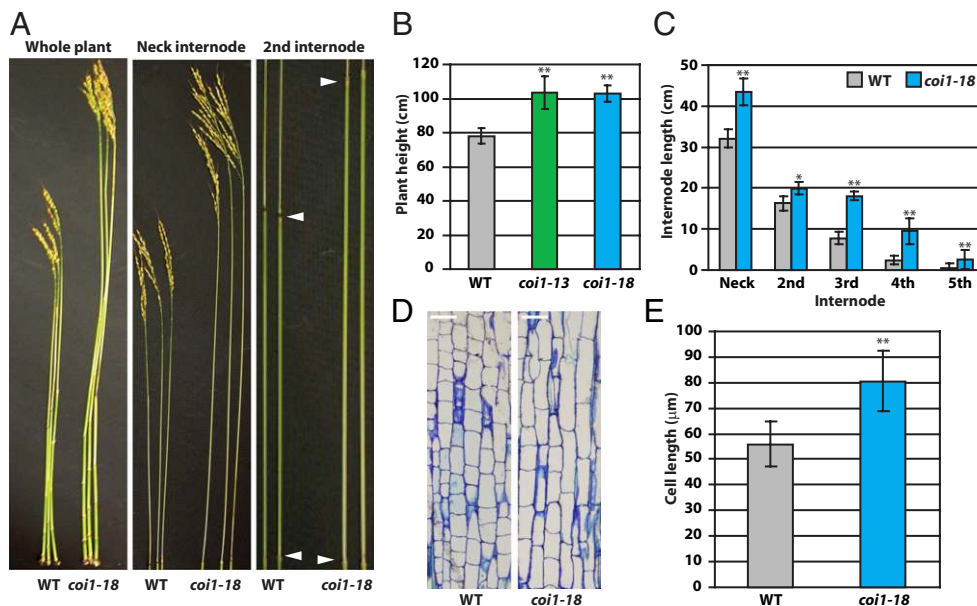


Fig. 2. Morphological phenotypes of *coi1-18* plants. (A) Images of WT (Nipponbare) and *coi1-18* plants show plant heights and internode lengths. (B) Quantification of heights of the WT, *coi1-13*, and *coi1-18* plants. More than 30 plants of each line were analyzed. The difference between the control 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 *OsCOII*-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 *OsCOII*-RNAi plants exhibited *eui1*-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 *OsCOII*-RNAi plants is mainly a result of the hypersensitivity to GA.

Elongation of *OsCOII*-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. 3A–C) and reduced cell size in the uppermost internode (Fig. 3E and F), similar to *Eui1*-OX plants. In addition, the longer grain phenotype of the *coi1-18* plants was also reverted to that of the WT (Fig. 3G 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 *OsCOII* in the GA pathway. These results suggest that the *OsCOII*-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 GA_3 . When transferred into the medium with 100 μM GA_3 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. 4A). 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 GA_3 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 *OsCOII* 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-

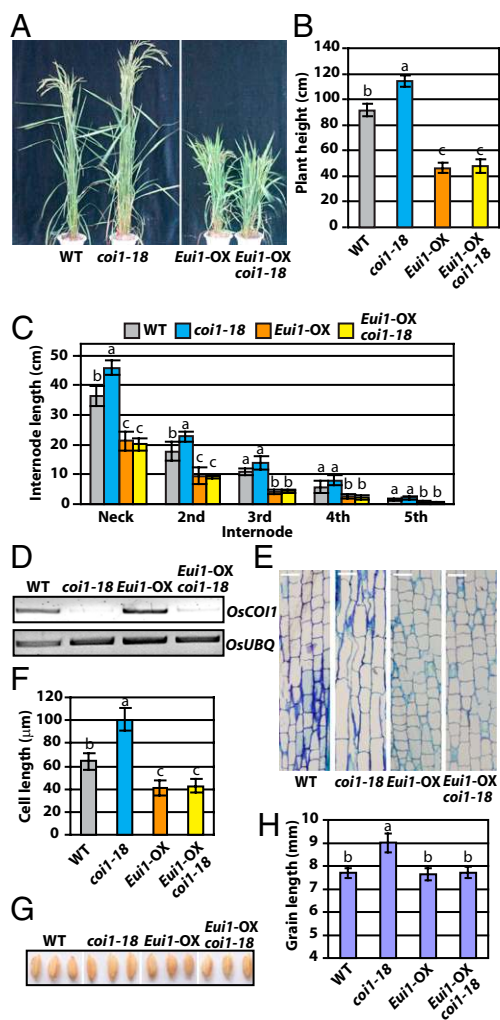


Fig. 3. The GA-deficiency mutation reverses the phenotype of *coi1-18* plants. (A) Morphological phenotype of *Eui1-OX/coi1-18*. (B) The average plant height of WT, *coi1-18*, *Eui1-OX*, and *Eui1-OX/coi1-18* plants. (C) The length of each internode of *coi1-18* decreased in the *Eui1-OX/coi1-18* plants. (D) Expression of *OsCOI1* in *Eui1-OX/coi1-18*. (E and F) Cell lengths at the bases of the uppermost internodes in *coi1-18*, *Eui1-OX/coi1-18*, and *Eui1-OX* plants. (Scale bar, 40 μm .) (G and H) Grain lengths of *coi1-18*, *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 *coi1* mutations, was found to phenocopy the *coi1* 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-

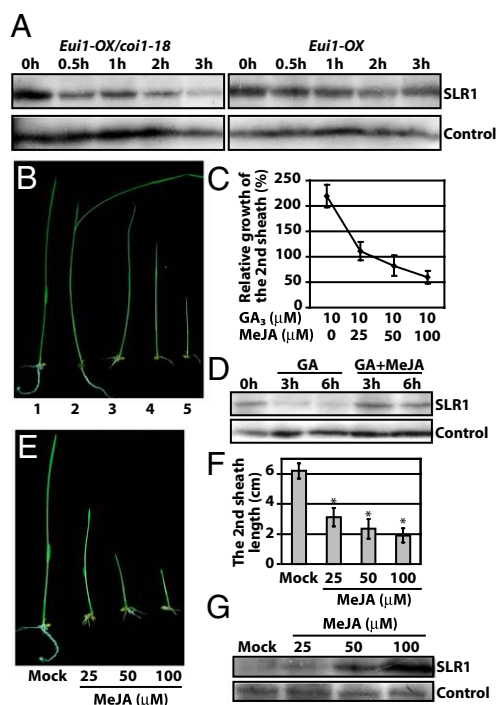


Fig. 4. Levels of the rice DELLA protein SLR1 and the antagonistic effect of MeJA on GA-mediated plant growth. (A) The GA-mediated degradation of the DELLA protein SLR1 was promoted in the *coi1-18* plants. The 10-d-old seedlings grown on one-half MS plates with 0.6% agar were transferred to liquid one-half MS medium with 100 μM GA_3 , 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 GA_3 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 GA_3 and various concentrations of MeJA. (D) MeJA delayed SLR1 degradation induced by GA_3 . The concentration of GA_3 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 pattern. 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).

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*.

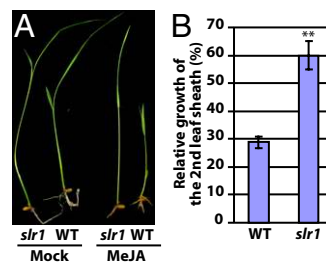


Fig. 5. The rice *slr1* mutant is insensitive to MeJA. (A) WT and *slr1* seedling were grown on one-half MS plates with 0.6% agar supplemented with or without 25 μM MeJA. (B) Relative growth of *slr1* and WT plants is indicated by percentages of the second leaf sheath lengths with MeJA treatment compared with those without MeJA treatment (** $P < 0.001$, Student's *t* test).

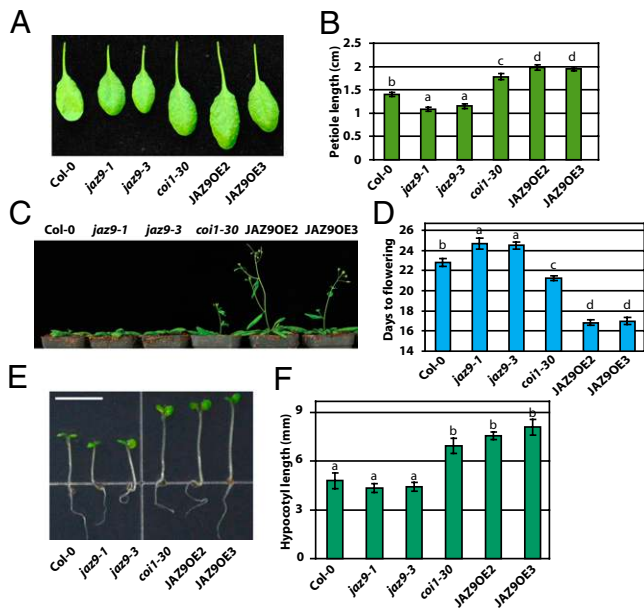


Fig. 6. GA-associated phenotypes of the *Arabidopsis coi1* mutant and JAZ9 overexpression plants. Image (A) and quantification (B) of petioles of *coi1-30* and JAZ9 overexpression plants. The plants were grown in a long-day growth chamber (16 h 120 $\mu\text{mol m}^{-2}\text{s}^{-1}$ light/8 h dark, 22 °C/18 °C). The third true leaves of 21-d-old plants were imaged, and their petiole lengths were measured. Image of 28-d-old plants (C) and quantification (D) of early flowering phenotypes of JAZ9 overexpression lines and *coi1-30* plants. The plants were grown under the same conditions as in A. Image (E) and quantification (F) of hypocotyls of *coi1-30* and JAZ9 overexpression plants when grown under 10 $\mu\text{mol m}^{-2}\text{s}^{-1}$ continuous white light at 22 °C for 6 d. (Scale bar, 5 mm.) Data shown in B, D, and F are the means from 12 plants. Error bars represent SD. Letters on columns indicate significant differences ($P < 0.05$, Tukey–Kramer multiple comparison test).

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–DELLA interaction, we noticed a striking correlation between the ability of AtJAZ overexpression to confer early flowering (Fig. 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

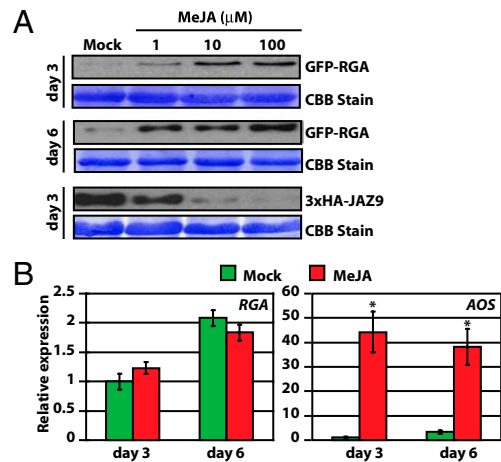


Fig. 7. MeJA increases the level of the *Arabidopsis* DELLA protein RGA. Seedlings were grown under 10 $\mu\text{mol m}^{-2}\text{s}^{-1}$ continuous white light at 22 °C. (A) Increased accumulation of the RGA protein in *Arabidopsis* seedlings treated with different concentrations of MeJA for 6 d. RGA and JAZ9 (positive control) were detected by anti-GFP and anti-HA antibody, respectively. (B) MeJA treatment does not affect the RGA transcript level in *Arabidopsis*. *Arabidopsis* seedlings were treated with 0.1% ethanol (mock) or 100 μM MeJA for 6 d. Total RNA was purified and used for qRT-PCR analysis. Data shown are the means of three biological replicates. Error bars represent SD. JA-inducible expression of AOS was used as a positive control for JA treatment. Asterisks indicate significant difference between mock and MeJA treatment ($P < 0.01$, Student's *t* test).

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 *della* quadruple mutant (*della*) 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 *della* quintuple mutant (*gai-t6/rga-t2/rgl1-1/rgl2-1/rgl3-1*) 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:xyloglucosyl transferase 33* (*XTH33*, *At1G10550*) (35–37)—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-regulated *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

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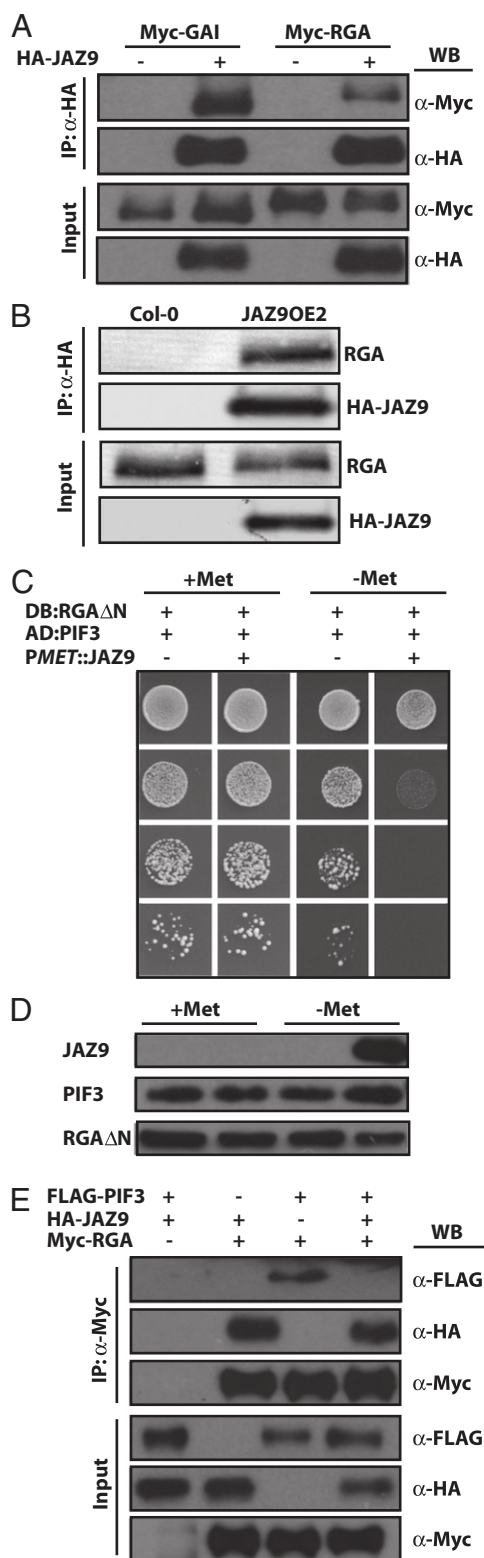


Fig. 8. JAZ9 interferes with the interaction between RGA and PIF3. (A) 3xHA-JAZ9 interacts with 9xMyc-GAI or 9xMyc-RGA protein when expressed transiently in *N. tabacum* leaves. Protein extracts were immunoprecipitated with an anti-HA antibody and analyzed by Western blot with an anti-Myc antibody. (B) 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-RGA antibody. (C) JAZ9 inhibits the interaction between RGA and PIF3 in yeast. The activity of the reporter gene *HIS3*, which indicates the

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 JAZ proteins, which would allow more PIF transcription factors to activate growth programs. This mechanism could explain the GA-hypersensitivity phenotypes observed in the *coil* mutants of rice and *Arabidopsis* and in transgenic *Arabidopsis* plants overexpressing those JAZ proteins (e.g., AtJAZ1, 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 *coil* mutants are displayed under different conditions for rice and *Arabidopsis*. Whereas *OscOII*-RNAi plants exhibit exaggerated stem elongation and other GA-related phenotypes under strong light conditions in the greenhouse and in the field, *Atcoil* plants show most obvious GA phenotypes under dim light conditions (10 $\mu\text{mol m}^{-2}\text{s}^{-1}$ 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 *coil* mutant flowered earlier and developed longer

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.

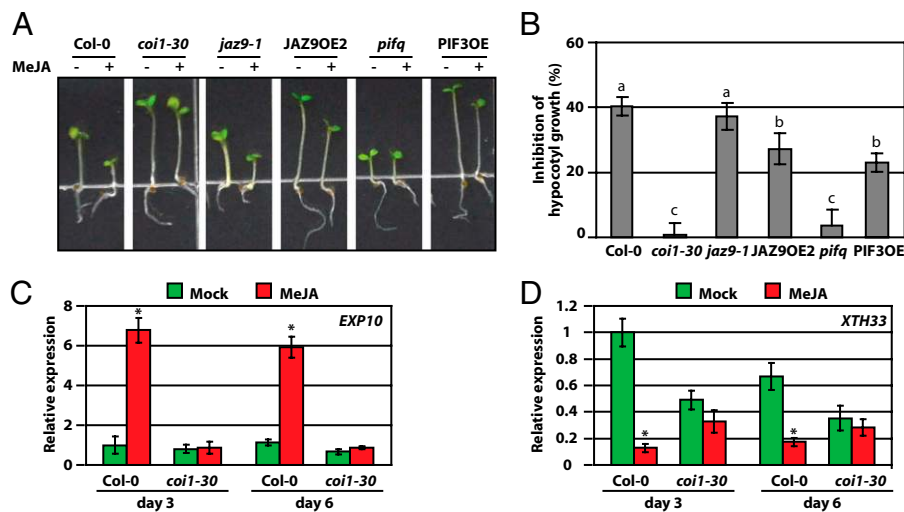


Fig. 9. JA sensitivity of PIF-overexpressing plants and *pif* mutants. Seedlings were grown on MS medium with or without 10 μM of MeJA under 10 $\mu\text{mol m}^{-2}\text{s}^{-1}$ continuous white light at 22 $^{\circ}\text{C}$ for 6 d. Image (A) and quantification (B) of the effect of MeJA on *Arabidopsis* hypocotyl elongation. The hypocotyl lengths were measured and the inhibition of hypocotyl growth was calculated as $(1 - \text{treated} / \text{untreated}) \times 100\%$. Data shown are the means from 16 seedlings. Error bars represent SD. Letters on columns indicate significant differences ($P < 0.05$, Tukey–Kramer multiple comparison test). (C and D) MeJA has antagonizing effects on the expression of *XTH33* (PIF-up-regulated) and *EXP10* (down-regulated) genes in *Arabidopsis*. Total RNAs were purified and used for qRT-PCR analysis. Data shown are the means of three biological replicates. Error bars represent SD. Asterisks indicate significant difference between mock and MeJA treatment ($P < 0.05$, Student's *t* test).

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 described here were derived from Col-0 except for the *della* mutant, which is in Landsberg *erecta* (Ler) background. The *jaz9-1*, *dellaq* (*gai-t6/rga-t2/rgl1-1/rgl2-1/rgl3-1*), and *pifq* (*pif1-1/pif3-3/pif4-2/pif5-3*) mutants, as well as the $P_{RGA}::GFP-RGA$ and PIF3OE transgenic lines, have been previously described (9, 18, 35, 49, 50). The *jaz9-3* (SM_3_34031; Fig. S12) and *coi1-30* (SALK_035548; Fig. S13) mutants were characterized in the present study. All *Arabidopsis* seeds were ordered from the *Arabidopsis* Biological Resource Center.

Arabidopsis seeds were stratified for 3 d at 4 $^{\circ}\text{C}$ 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 $\mu\text{mol m}^{-2}\text{s}^{-1}$ continuous cool-white fluorescent light at 22 $^{\circ}\text{C}$ or in a long-day growth chamber with a 16-h day (120 $\mu\text{mol m}^{-2}\text{s}^{-1}$ cool-white fluorescent light, 22 $^{\circ}\text{C}$) and 8-h night (18 $^{\circ}\text{C}$) cycle. The soil-grown plants were placed in the long-day growth chamber.

Transgenic Expression. For generation of *OscO1*-RNAi transgenic rice, two fragments of the *OscO1* genes were amplified by using the primer pairs *OscO1*-F1/R1 and *OscO1*-F2/R2 (Dataset S1), respectively. The fragments were inversely inserted into the pCambia1300S that contained a double 35S promoter and a terminator. The resulting *OSCO1* RNAi construct was introduced into the model variety rice Nipponbare (*Oryza sativa* L. ssp. *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 $P_{35S}::3\times\text{HA-AtJAZ9-8}\times\text{His}$, 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 pJYP003 (SI Materials and Methods) to create a $3\times\text{HA-AtJAZ9-8}\times\text{His}$ fusion construct. Col-0 plants were transformed by *Agrobacterium*-mediated transformation. Primary transformants 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 (51). Inserts were transferred into pJYP006 or pJYP012 by LR recombination (Life Technologies) to create $P_{35S}::9\times\text{Myc-GAI}$, $P_{35S}::9\times\text{Myc-RGA}$, and $P_{35S}::3\times\text{FLAG-PIF3}$, which were used for transient expression in tobacco leaves as previously described (52).

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 GA_3 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 GA_3 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 *coi1-30* plants were selected on MS plates containing 10 μM MeJA (Sigma-Aldrich). Plants were kept in a long-day growth chamber unless indicated otherwise. Flowering time was determined when floral buds became visible at the center of rosette. Petiole 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 GA_1 and GA_4 were assayed by LC-MS with internal standards as described previously (53).

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 gel blotting was performed by using standard protocol with PerfectHyb buffer (Sigma), and relative gene expression was quantified by using a PhosphorImager (Amersham 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 ToTALLY 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 RNeasy Mini Kit (Qiagen). First-strand cDNA was synthesized by using M-MLV reverse transcriptase (Invitrogen) and oligo(dT) as primers. *actin2* (*AT3G18780*), *cap-binding protein 20* (*CBP20*; *AT5G44200*), *protein phosphatase 2A subunit A3* (*PP2AA3*; *AT1G13320*), and *ubiquitin-conjugating enzyme 21* (*UBC21*; *AT5G25760*) (54) were used as internal controls to normalize target gene expression by GeNORM method (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 μ M 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.

Coimmunoprecipitation 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, 1% Sigma Protease inhibitor mixture, 100 μ M MG132, 10 mM NaF, and 2 mM Na_3VO_4). The immunocomplexes were captured by anti-HA or anti-Myc agarose beads (Sigma-Aldrich), washed, and released by 2 \times SDS sampling buffer, and were then subjected to SDS/PAGE followed by further Western blotting analysis.

Rice Microarray Assay. Whole transcriptomic analysis was performed with the Affymetrix GeneChip Rice Genome Array, representing 51,279 transcripts with three biological replicates. Raw data were analyzed with Affymetrix GeneChip Operating Software (GeneSpring, version 11.0) using Affymetrix default analysis settings and global scaling as normalization method. The GA, JA, and defense-related gene was analyzed by MAPMAN. The microarray

data has been deposited into the National Center for Biotechnology Information Gene Expression Omnibus database (accession nos. GSE29577 and GSM732294–GSM732299).

Assays for α -Amylase Activity. The release of α -amylase was assayed as described by Zhang et al. (29). The sterilized embryoless half seeds were incubated on agar plates containing 0.2% starch with 1 μ M GA_3 or 1 μ M GA_3 and 50 μ M MeJA for 3 d at 28 °C in darkness. These plates were exposed to iodine vapor for a few minutes. The reaction between starch and iodine turned the agar plates a blue-purple color. The agar around half seeds with α -amylase activity remained colorless because of hydrolysis of starch by α -amylase. The α -amylase protein was extracted from deembryonated half seeds imbibed in 1.0 μ M of GA_3 solution in the dark at 28 °C for 2 d (56). The α -amylase activity was assayed by quantifying reducing sugar released from substrate starch (57).

Microscopic Observation. Rice internodes and sheaths were sampled for resin sectioning. The samples were first fixed in FAA [3.7% (vol/vol) formaldehyde, 5% (vol/vol) acetic acid, 50% (vol/vol) ethanol], followed by dehydration through a graded ethanol series. The samples were embedded in resin and polymerized at 58 °C. Sections (8–10 μ m) were examined under a microscope (DMLB; Leica) and documented by photography.

Yeast Three-Hybrid Assay. The coding sequence corresponding to the GRAS domain of RGA was amplified by PCR by using the RGAdN-F and -R primer set ([Dataset S1](#)) and cloned into pGBridge (*SI Materials and Methods*) to create a Gal4DB-Myc-RGA Δ N construct. The *AtJAZ9* was cloned into pET42a-3 \times FLAG (*SI Materials and Methods*) to create a 3 \times FLAG-JAZ9 cassette, which was further transferred into pBridge (Clontech) to create $P_{MET}::3\times\text{FLAG-JAZ9}$. The $P_{MET}::3\times\text{FLAG-JAZ9}$ insert was transferred into the Gal4DB-Myc-RGA Δ N fusion plasmid to create the yeast three-hybrid (Y3H) bait vector. The *AtPIF3* was cloned into pDEST-GADT7 (58) by LR recombination to create the prey vector. The bait and prey vectors were transformed into the yeast strain AH109 (Clontech). Y3H assays were performed following the manufacturer's protocol (Clontech).

Statistic Analysis. Unless indicated otherwise, one-way ANOVA was performed for all data sets. Tukey–Kramer multiple comparison test was used to compare the means of the treatments at an α level of 0.05.

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