DELLAs Control Plant Immune Responses by Modulating the Balance of Jasmonic Acid and Salicylic Acid Signaling

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DELLA proteins are plant growth repressors whose degradation is promoted by the phytohormone gibberellin [4]. Here, we show that DELLA stabilization contributes to flg22-induced growth inhibition. In addition, we show that DELLAs promote susceptibility to virulent biotrophs and resistance to necrotrophs, partly by altering the relative strength of salicylic acid and jasmonic acid (JA) signaling. A quadruple-DELLA mutant (which lacks four out of the five Arabidopsis DELLA proteins [5]) was partially insensitive to gene induction by Methyl-Jasmonate (MeJA), whereas the constitutively active dominant DELLA mutant gai [6] was sensitized for JA-responsive gene induction, implicating DELLAs in JA-signaling and/or perception. Accordingly, the elevated resistance of gai to the necrotrophic fungus Alternaria brassicicola and susceptibility to the hemibiotrophic Pseudomonas syringae pv. tomato strain DC3000 (Pto DC3000) was attenuated in the JA-insensitive cot1-16 mutant [7]. These findings suggest an explanation for why the necrotrophic fungus Gibberella fujikuroi, causal agent of the foolish-seeding disease of rice, makes gibberellin.

Results and Discussion

A series of Arabidopsis mutants impaired in hormone signaling or biosynthesis was used to test the involvement of known hormones in flg22-induced growth inhibition. Altered growth inhibition was detected only in mutants altered in gibberellin (GA) biosynthesis or signaling (Figures 1A and 1B; Figure S1 and Tables S1 and S2 available online). In Arabidopsis, GA is perceived by AtGID1a/b/c GA receptors [8]. Binding of GA to these receptors promotes AtGID1-DELLA protein-protein interactions, which in turn lead to polyubiquitination (through the E3 ubiquitin ligase SCFSLY1) and subsequent degradation of DELLAs by the 26S proteasome [8]. Mutants that stabilize one or more DELLAs, including sly1-10, the dominant gai, and the GA-deficient ga1-3, all showed a more pronounced flg22-induced growth inhibition (Figures 1A and 1B, Figure S1 and Tables S1 and S2). Conversely, growth of the loss-of-function mutant gai-t6 rga-t2 rgl1-1 rgl2-1 (quadruple-DELLA mutant, deficient in GAI, RGA, RGL1, and RGL2 protein [5]), was slightly, but statistically significantly (F = 9.18, 1 degree of freedom [df], p < 0.01, multivariate analysis of variance [MANOVA]; p < 0.01, Student’s t test), less inhibited in growth by flg22 than were La-er controls (Figure 1B, Table S2).

We next analyzed the effect of flg22 on the accumulation of the DELLA protein RGA. Flg22 treatment delayed GA-mediated degradation of a GFP-RGA fusion protein (in which the green fluorescent protein [GFP] is N-terminally fused with the DELLA protein RGA. Flg22 treatment delayed GA-mediated degradation of a GFP-RGA fusion protein (in which the green fluorescent protein [GFP] is N-terminally fused with the DELLA protein RGA). Flg22 treatment delayed GA-mediated degradation of a GFP-RGA fusion protein (in which the green fluorescent protein [GFP] is N-terminally fused with the DELLA protein RGA).

To test this hypothesis, we challenged GA-signaling and biosynthetic mutants with the hemibiotrophic Pseudomonas syringae Pto DC3000 and monitored bacterial growth in adult plants. Strikingly, gai, ga1-3, and sly1-10 mutant plants displayed an approximately 100-fold increase in bacterial titer compared to La-er-infected plants at both 2 and 4 days post-inoculation (dpi) (Figure 2A). In contrast, no significant difference in bacterial growth was observed with the avirulent Pto DC3000 (AvrRpt2) strain that elicits race-specific resistance (Figure 2A). In contrast, no significant difference in bacterial growth was observed with the avirulent Pto DC3000 (AvrRpt2) strain that elicits race-specific resistance (Figure 2A).

Conclusion

Collectively, these results suggest that DELLA stabilization contributes to flg22-induced growth inhibition, although other growth-restraining processes are also likely to be involved. To identify putative downstream targets of DELLAs in the flg22 response, we compared microarray datasets generated from nonelicited ga1-3 mutant [9] and wild-type Arabidopsis seedlings treated with flg22 [1]. Surprisingly, approximately 30% of the transcripts that were more elevated in the ga1-3 mutant, as compared to La-er, were also upregulated by flg22 (Table S3). Among those, we identified defense-related genes including a subset of plant defense WRKY transcription factors [10]. Thus, DELLAs might contribute to a subset of plant defense responses in addition to their role in flg22-induced growth inhibition. To test this hypothesis, we challenged GA-signaling and biosynthetic mutants with the hemibiotrophic Pseudomonas syringae Pto DC3000 and monitored bacterial growth in adult plants. Strikingly, gai, ga1-3, and sly1-10 mutant plants displayed an approximately 100-fold increase in bacterial titer compared to La-er-infected plants at both 2 and 4 days post-inoculation (dpi) (Figure 2A). In contrast, no significant difference in bacterial growth was observed with the avirulent Pto DC3000 (AvrRpt2) strain that elicits race-specific resistance conferred by RPS2 [11] (Figure S2A). Moreover, we did not observe elevated bacterial growth when we used a Pto DC3000 hrcC mutant that is deficient in effector delivery to host cells via type III secretion [12] (Figure S2B). Thus, DELLAs promote susceptibility to virulent Pto DC3000 and have no discernible effect on either race-specific resistance or on resistance to Pto DC3000 mutants that cannot deliver effectors.
Role of DELLAs in Plant Immunity

We then analyzed whether DELLAs loss-of-function mutants showed altered resistance to virulent Pto DC3000. The quadruple-DELLA mutant displayed approximately 10-fold reduction in Pto DC3000 growth at 4 dpi compared to La-er plants (Figure 2B). Consistently, this mutant was also more resistant to the virulent oomycete Hyaloperonospora Arabidopsis race Cala2 (Figure S3). Trypan-blue staining of the quadruple-DELLA mutant displayed approximately 10-fold reduced in Pto DC3000 growth at 4 dpi compared to La-er plants (Figure 2B). Consistently, this mutant was also more resistant to the virulent oomycete Hyaloperonospora Arabidopsis race Cala2 (Figure S3). Trypan-blue staining of the quadruple-DELLA mutant showed altered resistance to virulent Pto DC3000. The quadruple-DELLA mutant displayed approximately 10-fold reduced SA content, data not shown) was significantly delayed in infected quadruple-DELLA plants versus La-er-infected plants at 24 hpi, whereas no significant difference between La-er and the quadruple-DELLA mutant was seen in uninfected plants (Figure 2E). Thus, DELLAs repress SA signaling and biosynthesis during Pto DC3000 infection.

Because the SA pathway is antagonistic to the JA/ET (ET: ethylene) pathway [13], we tested whether hyper-induction of SA-dependent genes, in the quadruple-DELLA infected mutant, could be correlated with a suppression of JA/ET-dependent gene expression. The induction of the JA/ET-dependent gene marker PDF1.2 by Pto DC3000 was significantly delayed in the quadruple-DELLA as compared to La-er-infected plants (Figure 2D). We further tested whether DELLAs could contribute to resistance toward Alternaria brassicicola, a necrotrophic fungus that is restricted by the JA/ET-defense pathway in Arabidopsis [14]. At 12 dpi, gai, sly1-10, and gai-1-3 mutants were significantly more resistant to A. brassicicola as represented by fewer and smaller lesions as compared to La-er-infected leaves (Figure 3A and data not shown). Conversely, the quadruple-DELLA-infected leaves, at 7 dpi, were necrotic and heavily colonized with fungal mycelium, whereas few lesions were observed in wild-type-infected leaves (Figure 3B, Figure S4A). The quadruple-DELLA mutant was also more susceptible to the necrotrophic fungus Botrytis cinerea (Figure S4B). Microscopy of trypan-blue-stained leaves infected with A. brassicicola revealed de novo spore formation in quadruple-DELLA leaves but not in La-er-infected leaves (Figure 3B). Furthermore, upregulation of PDF1.2 mRNA was delayed in infected quadruple-DELLA plants versus infected La-er controls (Figure 3C). Conversely, infected gai plants displayed earlier and stronger PDF1.2 mRNA induction.

Free-SA content (but not conjugated-SAs, data not shown) was approximately 2-fold higher in the quadruple-DELLA-infected plants versus La-er-infected plants at 24 hpi, whereas no significant difference between La-er and the quadruple-DELLA mutant was observed (Figure 3D). Thus, DELLAs repress SA signaling and biosynthesis during Pto DC3000 infection.

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Similar gene-expression profiles were obtained for the JA-dependent markers lipoxygenase 2 (LOX2) [15] and tyrosine aminotransferase-1 (AtTAT1) [16] over a 6 hr time-course (Figure 3C), suggesting a role for DELLAs in JA perception and/or signaling.

To test this hypothesis, we treated the quadruple-DELLA mutant with MeJA and analyzed the kinetics of PDF1.2, LOX2, and TAT1 mRNA induction. Upregulation of these transcripts was delayed in treated quadruple-DELLA mutant versus control La-er plants (Figure 4A). PDF1.2 and LOX2 mRNAs were delayed in treated quadruple-DELLA mutant versus control La-er plants (Figure 4A).

Figure 2. DELLAs Promote Disease Susceptibility to Pto DC3000 and Repress the SA-Defense Pathway

(A) DELLA gain-of-function mutants are more susceptible to Pto DC3000. Growth of Pto DC3000 on La-er, gai, ga1-3, and sly1-10 plants was assessed 2 and 4 days postinoculation (dpi) of 10⁵ colony-forming units (cfu/ml) bacterial concentration. Inoculation was performed by syringe infiltration on 6- to 7-week-old plants. Error bars represent the standard error of log-transformed data from five independent samples, and similar results were obtained in two independent experiments.

(B) The quadruple-DELLA loss-of-function mutant is more resistant to Pto DC3000. Bacterial growth was performed on 4- to 5-week-old plants as described in (A). Results are presented as in (A), and similar results were obtained in two independent experiments.

(C) DELLAs repress cell death during Pto DC3000 infection. Five-week-old La-er and quadruple-DELLA plants were syringe infiltrated with either MgCl₂ (Mock) or Pto DC3000 at a concentration of 10⁶ cfu/ml for 18 hr, and trypan-blue-stained leaves were further analyzed by microscopy. La-er plants were similarly treated with the avirulent Pto DC3000 (AvrRpt2) bacterium (at 10⁵ cfu/ml) as a positive control for cell-death development. Similar results were obtained in three independent experiments.

(D) The quadruple-DELLA mutant promotes SA-dependent-, and suppresses JA/ET-dependent-, gene induction in response to Pto DC3000. Four-week-old quadruple-DELLA mutant plants were syringe infiltrated with Pto DC3000 at a concentration of 2 × 10⁶ cfu/ml. PR1 (At2g14610), PR2 (At3g57260), and PDF1.2 (At5g44420) mRNA levels were monitored by semiquantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis over a 24 hr time course. Actin2 (At5g18780) was used to control equal cDNA amount in each reaction.

(E) The quadruple-DELLA mutant displays higher free-SA content in response to Pto DC3000. Five-week-old La-er (control: C.) and quadruple-DELLA (mutant: m.) plants were treated with either MgCl₂ or Pto DC3000 (at 10⁶ cfu/ml) for 24 hr, and free-SA content was measured. Error bars represent the standard error of three independent samples, and similar results were obtained in three out of four independent experiments.
were also rapidly upregulated in treated gai plants (as early as 1 hr after Methyl-Jasmonate [MeJA] treatment), whereas no significant induction was observed in treated La-er plants at this time point (Figure 4B). Additionally, TAT1 transcripts displayed an approximately 1000-fold induction in MeJA-treated gai plants versus an approximately 5-fold induction in La-er controls (Figure 4B), indicating that JA-responsive gene induction is sensitized in gai. Collectively, these results implicate DELLAs in JA perception and/or signaling.

We crossed the gai mutant to the JA-insensitive coi1-16 mutant [7] and analyzed the resistance of the double coi1-16 gai mutant toward Pto DC3000 and A. brassicicola infections. A partial restoration of both disease resistance to Pto DC3000 and disease susceptibility to A. brassicicola was observed in the coi1-16 gai (versus gai controls; Figures 4C and 4D).

Consistent with this observation, PDF1.2 was not induced in infected gai coi1-16 plants (compare with infected coi1-16 plants; Figure S5). Thus, the enhanced resistance to A. brassicicola and susceptibility to Pto DC3000, observed in the gai mutant, is partly dependent on the JA-signaling pathway.

We show here that DELLAs contribute to flg22-induced growth restraint (Figures 1A and 1B; Figure S1 and Tables S1 and S2) and that flg22 also delays the GA-mediated degradation of GFP-RGA in an FLS2-dependent manner (Figures 1C and 1D). A significant proportion of the flg22-induced genes were more elevated in the GA-deficient gai-3 (Table S3). Among those, some WRKY transcription factors might represent downstream targets of DELLAs. This is consistent with other findings reporting a role for WRKYS in GA signaling [17, 18]. For example, the rice OsWRKY71, which regulates plant defense [19], also represses GA-induced Amy32b α-amylase promoter in aleurome cells through interaction with W-box elements present in the Amy32b promoter [18]. Like DELLAs, the OsWRKY71 protein is nuclear localized and rapidly destabilized upon GA treatment [18]. Importantly, the likely Arabidopsis orthologs AtWRKY18 and AtWRKY40 are upregulated in the flg22 response [1, 20], and overexpression of AtWRKY18 in Arabidopsis also results in dwarfism [21]. Investigation of whether some WRKYS interact with, and/or act downstream of, DELLAs might provide a possible molecular mechanism by which flg22 induces both defense and growth inhibition.

The enhanced disease susceptibility toward Pto DC3000 infection in the DELLA-stabilized mutants (Figure 2A) is intriguing given that flg22 (1) retards GA-directed degradation of GFP-RGA (Figure 1C) and (2) promotes resistance to Pto DC3000 [1]. It is possible that flg22 induces similar events to those activated in plants in response to necrotrophs. Consistent with this idea, flg22 rapidly induces JA- as well as ET-responsive genes [22] that are also strongly activated during necrotroph infections [14]. Moreover, flg22 was shown to promote resistance against B. cinerea [23]. This result indicates that a branch of the flg22-induced signaling pathway, which may involve DELLA stabilization, contributes to resistance against B. cinerea and perhaps other necrotrophs.

The role for DELLAs in resistance to necrotrophs and susceptibility to biotrophs suggests that GA might promote resistance to biotrophs and susceptibility to necrotrophs (by degrading DELLAs and thus changing the balance of SA/JA signaling). Consistent with this idea, pretreatment with exogenous GA elevates resistance to Pto DC3000 and susceptibility to A. brassicicola (Figure S6), thus suggesting that GA should act as a virulence factor for necrotrophs.

GA was originally identified from the necrotrophic fungus Gibberella fujikuroi (Fusarium moniliforme) [24], the causal agent of the bakanae or foolish-seedling disease of rice. Disease symptoms include extensive seedling elongation with slender leaves and cause a drastic reduction in rice crop yields. However, the mechanism by which GA might promote pathogen virulence was unknown. We propose that Gibberella might secrete GA as a virulence factor to degrade DELLAs and disable JA-mediated necrotroph resistance in plants, resulting concomitantly in loss of DELLA-mediated growth restraint.

Experimental Procedures

Plant Materials

All the Arabidopsis thaliana mutants used in this work were in La-er (gai, ga1-3, sly1-10, quadruple-DELLA, fls2-17) [3, 5, 6, 25–27] or Col-0 (coi1-16, sid2-1, ein3-1, tir1-1) [7, 28–30] accessions.
Seeds were grown for 7–8 days on plates containing 1x Murashige-Skoog (MS) medium (Duchefa), 1% sucrose, and 1% agar under a 16 hr photoperiod at 22°C. Seedings were then transferred into MS liquid medium (one seedling per 500 µl of medium in wells of 24-well plates) and fig22 (QRLSTGSRINSKDDAQLQIA) or fig22A. brasiccicola-GFP (ARVSGLRIGADSDNAAYWSIA) peptides supplied at a final concentration of 10 nM. The fresh weight of fig22A. brasiccicola-GFP versus fig22GFP-treated seedlings from La-er and quadruple-DELLA genotypes was measured 7–8 days after. Fig22-triggered growth inhibition in genotypes La-er and quadruple-DELLA was compared with MANOVA and the Student’s t test.

**Pathogen and MeJA Treatments**

The bacterial strains used in this study were *Pseudomonas syringae* pv. *tomato* DC3000 (Pto DC3000), Pto DC3000 carrying the avirulence gene *avrRpt2* and Pto DC3000 deficient in effector delivery to host cells (Pto DC3000 *hrcC* mutant). Bacterial inoculations were performed by syringe infiltration on 5-week-old quadruple-DELLA mutant and on 6- to 7-week-old *gai*, *gai-1*, and *sly-1* mutant plants. For measurement of bacterial titers, a concentration of 10^6 colony-forming units (cfu/ml) of Pto DC3000 and Pto DC3000 (AvrRpt2) or a concentration of 10^7 cfu/ml of Pto DC3000 *hrcC* mutant were used. So that defense-marker genes could be monitored, a concentration of 2 × 10^5 cfu/ml of Pto DC3000 was used. The necrotrophic fungi used in this study are *Alternaria brassicicola* and *Botrytis cinerea*. *A. brassicicola* spores were sprayed with a concentration of 5 × 10^4 spores/ml on 4- to 5-week-old quadruple-DELLA plants and on 6- to 7-week-old *gai*, *gai-1*, and *sly-1* mutant plants. So that the size of *A. brassicicola*-triggered lesions and profile defense marker genes could be measured, 5-week-old quadruple-DELLA plants were inoculated with two drops of 5 µl leaf with an inoculum of 5 × 10^9 spores/ml. *B. cinerea* spores were sprayed with a concentration of 5 × 10^5 spores/ml on 5-week-old quadruple-DELLA mutant plants. Spores of the oomycete *Hyaloperonospora Arabidopsis* race Cala2 were sprayed at a concentration of 4 × 10^6 spores/ml on 3-week-old quadruple-DELLA mutant plants. MeJA was sprayed on 4- to 5-week-old La-er, quadruple-DELLA, or *gai* mutant plants at a concentration of 10 µM.

**Histochemical Staining and Microscopy**

GFP fluorescence from RGAp::GFP-RGA root tips was observed by confocal microscopy as previously described [31]. Fungal hyphal structures as well as dead cells were observed by staining of infected leaves with trypan blue. Infected leaves were transferred in a trypan blue solution (10 ml lactic acid [DL Sigma L-1258], 10 g glycerol, 10 ml H2O, and 10 mg trypan blue) diluted in ethanol 1:1 and boiled for 1–2 min. The leaves were then destained overnight in chloral hydrate, washed several times with water, and observed under a Leica (DMR) microscope.

**SA Measurements**

Five-week-old *Arabidopsis* La-er and quadruple-DELLA leaves were collected 24 hpi of Pto DC3000 at a concentration of 10^6 cfu/ml. Samples were prepared as described in the Supplemental Data, and SA contents were measured with an Agilent 1100 High Performance Liquid Chromatography coupled to a Mass Spectrometry (HPLC-MS) as described in Supplemental Data.

**RT-PCR Analyses**

Total RNA was extracted with Rneasy Plant Mini kit (QiAGEN). RNA samples were treated with DNase Turbo DNA-free (Ambion) and reverse transcribed into complementary DNA (cDNA) with SuperScript III reverse transcriptase (Invitrogen). The cDNA was further quantified with a SYBR Green qPCR kit (DyNAmo HS SYBR GREEN qPCR kit from FINNZYMES) and gene-specific primers as described in the Supplemental Data. Semiquantitative RT-PCR was performed as previously described [22].

**Supplemental Data**

Additional Experimental Procedures, six figures, and three tables are available at http://www.current-biology.com/cgi/content/full/18/9/650/DC1/.
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References


