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BIG regulates stomatal immunity and jasmonate production in Arabidopsis

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Summary

- Plants have evolved an array of responses that provide them with protection from attack by microorganisms and other predators. Many of these mechanisms depend upon interactions between the plant hormones jasmonate (JA) and ethylene (ET). However, the molecular basis of these interactions is insufficiently understood.
- Gene expression and physiological assays with mutants were performed to investigate the role of Arabidopsis *BIG* gene in stress responses.
- *BIG* transcription is downregulated by methyl JA (MeJA), necrotrophic infection or mechanical injury. BIG deficiency promotes JA-dependent gene induction, increases JA production but restricts the accumulation of both ET and salicylic acid. JA-induced anthocyanin accumulation and chlorophyll degradation are enhanced and stomatal immunity is impaired by BIG disruption. Bacteria- and lipopolysaccaride (LPS)-induced stomatal closure is reduced in *BIG* gene mutants, which are hyper-susceptible to microbial pathogens with different lifestyles, but these mutants are less attractive to phytophagous insects.
- Our results indicate that BIG negatively and positively regulate the MYC2 and ERF1 arms of the JA signalling pathway. BIG warrants recognition as a new and distinct regulator that regulates JA responses, the synergistic interactions of JA and ET, and other hormonal interactions that reconcile the growth and defense dilemma in Arabidopsis.

Introduction

Jasmonates (JAs) act to amplify signals induced by wounding and negatively impact plant growth. This is exemplified in the so-called 'Bonsai effect', that is the over-activation of JA responses stunt plant growth by inhibiting cell production (Swiatek *et al.*, 2002; Pauwels *et al.*, 2008; Zhang & Turner, 2008; Wasternack & Hause, 2013). Under stress-free growth conditions, the inhibitory effects of JAs on plant growth are restrained by a group of nuclear proteins called JASMONATE-ZIM-DOMAIN (JAZ) repressors (Chini *et al.*, 2007), and by CYP94B3 that catalyzes the conversion of JA-Ile into the biologically inactive 12-hydroxy-JA-Ile (Koo *et al.*, 2011), as well as by the JAV1-JAZ8-WRKY51 (JJW) complex that binds and represses JA biosynthesis genes (Yan *et al.*, 2018). Although knowledge is still fragmentary, it seems likely that mechanical and biotic wounding causes rapid JA production through a positive feedback loop whereby JA signaling promotes its own synthesis (Staswick & Tiryaki, 2004; Chini *et al.*, 2007; Glauser *et al.*, 2009; Koo *et al.*, 2009; Wasternack & Hause, 2013; Yan *et al.*, 2018).

In plants, JA and ethylene (ET) interact to control plant immunity and development. Both *coi1-1* (JA insensitive) and *ein2-1* (ET insensitive) mutants are more susceptible to fungal infection than the wild-type Arabidopsis (WT) (Penninckx *et al.*, 1998; Thomma *et al.*, 1998, 1999; Wasternack, 2014) and the *ein3eil1* double mutant (encoding two transcription factors involved in ET signaling) is defective in JA-induced gene expression and root hair development (Zhu *et al.*, 2011). Apart from their synergistic action, JA and ET can interact negatively in regulating the expression of wound-responsive genes and the apical hook formation (Song *et al.*, 2014a).

Salicylic acid (SA) biosynthesis and SA signaling are required for local and systemic acquired resistance (SAR) against biotrophs. Plants unable to accumulate SA showed enhanced

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susceptibility to pathogen infection and resistance was restored when plants treated with SA or SA analogue (Verberne *et al.*, 2000; Spoel & Dong, 2008; Vlot *et al.*, 2009). Although some synergistic interactions are known, SA and JA usually have antagonistic effects as JA induction down-regulates the SA pathway, and vice versa (Kunkel & Brooks, 2002; Koornneef & Pieterse, 2008). The mutual antagonism of JA and SA is evolutionary conserved and has been reported in many plant species in various taxonomic groups (Doares *et al.*, 1995; Kloek *et al.*, 2001; Spoel *et al.*, 2003; Van der Does *et al.*, 2013).

Stomata are pores on the leaf surface that regulate the loss of water vapour and CO_2 uptake (Kim *et al.*, 2010; He & Liang, 2018). In addition to controlling gas exchange, stomata also contribute to plant defense. This is achieved by closing stomata upon pathogen perception. The net result is to restrict pathogen entry and thereby hamper colonization in plants (Melotto *et al.*, 2006; Faulkner & Robatzek, 2012). The available evidence indicates that defense through stomatal closure is an integral part of the SA-regulated innate immune system (Delaney *et al.*, 1994; Wildermuth *et al.*, 2001; Melotto *et al.*, 2006).

Originally isolated as a mutant with defects in the resistance to the growth-inhibiting effects of 1-*N*-naphthylphthalamic acid, a widely used potent inhibitor of auxin transport, *BIG* encodes a putative large calossin-like protein with a MW of 560 kDa, participating in different processes including auxin distribution, hormone and light responses, phosphate stress, vesicle trafficking and endocytosis (Li *et al.*, 1994; Ruegger *et al.*, 1997; Gil *et al.*, 2001; Kanyuka *et al.*, 2003; López-Bucio *et al.*, 2005; Paciorek *et al.*, 2005; Yamaguchi *et al.*, 2007). *BIG* was re-annotated based on the sequences of an overlapping set of RT-PCR products. The gene encodes a predicted 5077 amino acid protein which is required for stomatal response to elevated concentration of CO_2 (He *et al.*, 2018). Mutations in *BIG* restrict overall plant growth and development, leading to plant architecture reshaping and delayed flowering (Ruegger *et al.*, 1997; Gil *et al.*, 2001; Yamaguchi *et al.*, 2007; Guo *et al.*, 2013). Intriguingly, both the enhanced and reduced resistance against fungal pathogens *F. oxysporum* and *A. brassicicola* have been ascribed to the perturbed auxin pathway in *big* mutants (Kazan & Manners, 2009; Qi *et al.*, 2012).

Here we show that defects in development and defence observed in *big* mutants can be largely ascribed to the activation of the JA pathway. MeJA downregulates the expression of *BIG* at the transcription level. *BIG* also regulates an array of JAs-mediated plant development and defense responses in a COI1-dependent manner. Loss of BIG function potentiated the buildup of JAs that represses the SA pathway in Arabidopsis. ET synthesis was impaired by BIG deficiency and BIG respectively, negatively and positively regulates the MYC2 and ERF1 arms of the JA signalling pathway. Moreover, BIG is required in hook formation of seed germination for seedling soil emergence and also likely in the survival of etiolated seedling to light exposure, two key adaptive traits of agronomic impact. Taken together, BIG represents a new regulator of JA pathway and a point of convergence for the interactions of JA and ET and other hormones, which is important to reconcile the growth and defense dilemma in plant.

Materials and Methods

Plant material, growth conditions

All Arabidopsis (*Arabidopsis thaliana* L.) lines used were in the Columbia background (Col-0). Two well characterized alleles of *big* mutant used in this study, *doc1-1* and *big-1* were obtained from NASC (the European Arabidopsis Stock Centre, http://Arabidopsis.org.uk). Mutant lines *coi1-1* (Xie *et al.*, 1998) and *coi1-2* (Xu *et al.*, 2002) were previously described. Arabidopsis plants were grown in ½MS medium or in soil in a glasshouse at 22°C : 18°C, day : night cycles with a 10 h : 14 h, light : dark photoperiod (light intensity 120 μmol photons $m^{-2}\,s^{-1}$).

Botrytis cinerea growth and plant inoculation

Fungi cultivation and plant inoculation were performed essentially as described (Yuan *et al.*, 2017). In brief, *B. cinerea* strain (B05.10) was cultured on potato dextrose plates at 22°C for 10–14 d. Spores were collected and suspended in liquid PDA sodium. Leaves of 5-wk-old plants were inoculated with spores at a final concentration of 1.5×10^6 spores ml^{-1}. Infected plants were transferred to an artificial environmental plant growth chamber with a setup as 10 h : 14 h, light : dark photoperiod at 22°C : 18°C, day : night cycles with a light intensity 100 μmol photons $m^{-2}\,s^{-1}$ and a constant 90% humidity.

ET induction was performed according to Zander *et al.* (2012) with slight modifications. Briefly, 4-wk-old plants were sprayed with 0.5 mM of the ethylene precursor 1-aminocyclopropane-carboxylic acid (ACC) (Sigma). Water (the solvent) spraying served as the mock control. Forty-eight hours later, the ACC treated and control plants were inoculated with spores at a final concentration of 1.5×10^6 spores ml^{-1} before transferred to environmental controlled plant growth chamber as described above. Symptoms on rosette leaves were analyzed 4 d after inoculation.

Bacteria growth assay

P. syringae strains *Pst* DC3000 and *Pst* DC3000 (avrB) used in this study were obtained and prepared as previously described (Yuan *et al.*, 2017). Bacteria were grown on a plate of a low salt Luria Bertani (LB) medium with appropriate antibiotics at 28°C for 1–2 d. Then the bacteria were transferred to liquid sodium with appropriate antibiotics grown shaking for 12 h. The culture was centrifuged at 2500 ***g*** for 10 min and the bacteria are resuspended in 10 mM $MgCl_2$. Five-week-old plants were sprayed with a bacterial suspension at a concentration of 5×10^8 CFU ml^{-1} containing 0.02% Silwet L-77. Following inoculation, the plants were immediately transferred to a growth room that had a setup of 10 h : 14 h light : dark photoperiod at 22°C : 18°C, day : night cycles with a light intensity 100 μmol photons $m^{-2}\,s^{-1}$ and a relative humidity of 85%. Plants were placed under a plastic dome to maintain high humidity for 2 d before the measurement of SA contents as described by Chen *et al.* (2009) or for 3 d for the bacterial growth assays, following the published protocol (Yuan *et al.*, 2017).

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Stomatal aperture assay

Abaxial epidermis was removed from the newly fully expanded leaves of 4–5-wk-old plants and floated, cuticle-side up, on 10 mM MES/KOH (pH 6.2) in 5 cm deep Petri dishes (Sterilin, Cambrige, UK) at 22°C for 30 min in the dark to encourage stomata to close completely before peels were transferred to fresh Petri dishes and incubated for 2.5 h in the light under a PPD of 150 μmol $m^{-2} s^{-1}$ in 50 mM KCl, 10 mM MES/KOH (pH 6.15) at 22°C with either bacterial suspensions at a final concentration of 10^8 CFU ml^{-1} for 1 h or 100 ng μl^{-1} lipopolysaccharide (LPS, from *P. aeruginosa*; Sigma) for 3 h before measurements of stomatal apertures were conducted. Forty stomatal pores were measured per treatment in three separate replicated tests (total stomata measured = 120; *n* = 3). Experiments were conducted double blinded and repeated independently three times.

Insect defense assay

The two-choice tests for *S. exigua* larvae and aphids (*M. persicae*) were performed as described (Hu *et al.*, 2013). Each Petri dish (150 mm) containing 1% phytagel had two plants with two comparable genotypes (WT vs *big-1* or WT vs *doc1-1*). In total, either 60 larvae of *S. exigua* or adult aphids were placed equidistant from the two plants, and the numbers of larvae or aphids on each plant were recorded after 24 h incubation.

JA, SA quantitation

OPDA, JA, JA-Ile, and SA were quantitated according to Chen *et al.* (2011) with slight modifications. Briefly, Arabidopsis leaf tissue (0.1 g) was ground to fine powder in liquid nitrogen, and extracted with 1.25 ml 80% (v/v) methanol at 4°C for 12 h and corresponding standards were added to plant samples as the internal control before grinding. The extract was centrifuged (30 000 ***g***, 4°C, 20 min) and supernatant was collected and passed through a C-18 SPE-cartridge. The elution was dried under nitrogen gas stream and reconstituted in 100 μl of H_2O. The solution was acidified with 12 μl of 0.1 M hydrochloric acid and extracted with ethyl ether (three times, 0.5 ml). The next steps are in accordance with the above-mentioned reference. Finally, the dissolved sample was injected into an HPLC-ESI-Q-TOF-MS system (Bruker Daltonik GmbH, Bremen, Germany) for analysis. Separations were performed on a reversed-phase/SCX column (C18-SCX, 4 : 1, 150 × 2.1 mm, 5 μm, Shiseido) at a flow rate of 0.2 ml min^{-1} at 30°C, eluting with 40 mM ammonium acetate and acetonitrile (18 : 82, v/v).

Ethylene measurement

The ethylene contents of the plants grown in the gas chromatography (GC) vials (B7990-6A; National Scientific Co., Rockwood, TN, USA) were determined by GC as described (Du *et al.*, 2014). In brief, the GC vials containing Arabidopsis seedlings were immediately capped after treatment. At the indicated time points, the gas sample (1 ml) were introduced into a gas chromatograph equipped with a flame ionization detector column packed with activated aluminum at 100°C. ET was detected by an ionization detector and recorded by an integrator. The sample injected temperature is 80°C, whereas the column is 150°C.

RNA extraction and Q-PCR analysis

Total RNA was extracted from materials using Trizol® reagent (Invitrogen). RNA was treated with RNase-free DNase I, (Thermo Fisher Scientific, Waltham, MA, USA) to remove amounts of DNA. The absence of genomic DNA was confirmed by PCR using RNA as template without reverse transcription. Then the first strand cDNA was synthesized using a first strand cDNA synthesis kit (Thermo Fisher Scientific). cDNA corresponding to 20 ng of total RNA and 300 nM of each primer were used in Q-PCR reactions. Q-PCR was carried out on an ECO® real-time PCR thermal cycler (Illumina, San Diego, CA, USA) in a total reaction volume of 10 μl using the SYBR green dye PCR Master Mix (Toyobo, Tokyo, Japan), using these conditions: 95°C for 5 min, 40 two-step cycles at 95°C for 15 s and 60°C for 40 s, followed by dissociation melting curve analysis to determine the PCR specificity. The gene-specific primers used were designed using the online server of MWG biotech (http://www.mwg-biotech.com/). The relative RNA levels were calculated from cycle threshold (C_T) values according to the ΔC_T method, and relative target mRNA levels were normalized to *Actin3* (*At3g53750*) mRNA levels. For each target gene, the reactions were carried out in triplicate. The relative expression values were calculated from three biological replicates.

Anthocyanin and chlorophyll content measurement

Anthocyanin content was measured as described (Deikman & Hammer, 1995). Arabidopsis seedlings (50 mg) were placed into 1 ml extract buffer (propanol : HCl : H_2O, 18 : 1 : 81, v/v/v). Then the sample was boiled for 3 min and then incubated overnight at room temperature. Absorbance values (A_{535} and A_{650}) of the extraction solution were measured using spectrophotometer. The anthocyanin content is presented as (A_{535}–A_{650})/g fresh weight. Experiments were repeated three times. Chlorophyll of detached leaves was extracted from 80% acetone and determined at 663 and 645 nm according to Lichtenthaler (1987). Experiments were repeated independently three times.

Protochlorophyllide determination

Protochlorophyllide content was measured as previously described (Zhong *et al.*, 2014). Protochlorophyllide of etiolated seedlings was extracted in 90% (v/v) acetone containing 0.1% NH_3 in the dark at room temperature for 24 h. Then, the sample was centrifuged. The fluorescence of supernatant was then measured with a fluorescence microplate reader using excitation wavelength at 440 nm and fluorescence emission spectra at 634 nm.

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GUS staining

Arabidopsis seedlings (5 d old) were incubated in GUS staining solution containing 0.5 mg ml^{-1} 5-bromo-4-chloro-3-indolyl-β-D-glucuronide cyclohexylamine salt (X-Gluc), 50 mM sodium phosphate buffer (pH 7.0), 10 mM EDTA (pH 8.0), 0.1% (v/v) Trion X-100. The sample was vacuum-infiltrated for 5 min and incubated at 37°C to develop blue colour. Then the sample was transferred to 70% ethanol to be de-stained and subsequently photographed.

Statistical analysis

Each experiment was carried out at least three independent times with similar results. Statistical significances based on unpaired two sample Student's *t*-test were determined with SIGMAPLOT 10 (Systat Software Inc., San Jose, CA, USA).

Results

Stomatal immunity and SA induction are compromised by mutations in *BIG* gene

The *BIG* gene encodes a calossin-like protein required for auxin transport (Ruegger *et al.*, 1997; Gil *et al.*, 2001; Yamaguchi *et al.*, 2007). Recently, we reported that *BIG* is also involved in elevated CO_2-induced stomatal closure (He *et al.*, 2018). In addition to regulating the uptake of CO_2 from the atmosphere and the loss of water vapor from the plant, stomata are also a major point of entry for many microbial pathogens entering plants. As a defence against pathogen attack, plants close their stomata thereby reducing infection (Melotto *et al.*, 2006; Kim *et al.*, 2010; He & Liang, 2018). Given the newly discovered role of *BIG* in the control of stomatal aperture we decided to investigate whether it is also involved in the mechanism underlying stomatal immunity. First, we monitored stomatal closure in responding to virulent live cells of *P. syringae* pv *tomato* (*Pst*) DC3000. At a *Pst* DC3000 concentration sufficient to induce reductions in stomatal aperture in WT, stomata of two mutant alleles of the *BIG* gene (*doc1-1* and *big-1*, as described by Gil *et al.* (2001) and Kasajima *et al.* (2007)) remained open (Fig. 1a,b). We found a similar result when we infected plants with *Pst* DC3000 (avrB), which is an avirulent isolate of *Pst* DC3000 carrying the effector protein AvrB (He *et al.*, 2004; Yan *et al.*, 2009; Cui *et al.*, 2010) (Fig. 1c). Similarly, *big* mutant stomata failed to close when plants were challenged with lipopolysaccharides (LPS), key components of the outer membrane of Gram-negative bacteria that are known to trigger immune responses in plants and animals (Melotto *et al.*, 2006; Shang-Guan *et al.*, 2018) (Supporting Information Fig. S1a,b).

We next inoculated 4-wk-old plants with *Pst* DC3000 and *Pst* DC3000 (avrB), respectively, by spraying. This mimics the natural infection process in which bacteria actively enter plant leaves through stomata before spreading and multiplying to high population densities in intercellular spaces. We found that while initial bacterial loadings were similar (see Fig. 1d, 0 d post inoculation), *big* mutants supported 10-fold and 1000-fold higher *Pst* DC3000 and *Pst* DC3000 (avrB) proliferation respectively than WT at 3 d post inoculation (dpi) (Fig. 1d,e). To determine whether BIG deficiency only affects stomata-mediated immunity, we performed bacterial infiltration assays and found that there was no significant difference in bacteria growth on leaves of WT and *big* mutants (Fig. S1c). This result suggests that BIG is a positive regulator of basal resistance against bacteria. As the proliferation response in *big* mutants was much greater after challenging with *Pst* DC3000 (avrB), all our subsequent experiments were primarily conducted using this pathogenic strain.

Given the SA-regulated innate immune system is an integral part of plant defense through stomatal closure (Melotto *et al.*, 2006), we studied the effect of *BIG* mutations on the transcription of SA pathway marker genes *PR1* and *PR5*. Quantitative real-time PCR (Q-PCR) analysis shows that the induction of both genes by *Pst* DC3000 (avrB) infection was significantly inhibited in *big* mutants (Fig. 1f, g). Next we determined SA concentration after infection in WT and *big* mutants. The data in Fig. 1(h) show that both mutants accumulated much less SA than WT at 48 h post inoculation. We further examined the expression of SA biosynthetic gene *ICS1* and found that the induction of *ICS1* was compromised in *big* mutants (Fig. S1d). These data suggest that BIG is involved in the control of SA biosynthesis during infection. Together, these results indicate that *BIG* is required for full resistance against both virulent and avirulent bacteria in Arabidopsis.

The expression of *BIG* is strongly downregulated by JA, which in turn regulates JA production and responses

We used Arabidopsis expressing a *BIG* promoter-driven β-glucuronidase (GUS) reporter (p*BIG*:GUS) (Guo *et al.*, 2013) to show that *BIG* promoter activity is detectable in most plant organs and tissues in line with previous work (Gil *et al.*, 2001; Yamaguchi *et al.*, 2007; Guo *et al.*, 2013). Interestingly, we found that the expression of the *BIG* gene was strongly downregulated by exogenous MeJA (Fig. 2a). By contrast, SA and auxin (IAA) triggered no appreciable change of *BIG* gene expression consistent with existing reports (Ruegger *et al.*, 1997; Gil *et al.*, 2001; Guo *et al.*, 2013). Q-PCR analysis revealed that *BIG* was rapidly downregulated and remained low after spraying with 100 μM MeJA. The reduction in *BIG* transcription induced by MeJA was abolished in the JA co-receptor mutant *coi1-1* mutant (Fig. 2b). Infection with *B. cinerea* (*Bc*) and mechanical injury, both expected to result in increased JA (Wasternack & Kombrink, 2009), brought about reductions in *BIG* expression (Fig. 2c,d). *BIG* downregulation could clearly be detected as early as 10 min (the earliest time point we examined) after wounding treatment. Transcription was reduced to a lowest point at 1 h post treatment, but returned to baseline at the 2 h time point, consistent with observations of Koo *et al.* (2009). Upon close inspection, the induced expression patterns of *JAV1*, an experimental control and an early response gene in JA signaling (Hu *et al.*, 2013) are opposite to *BIG* (Fig. 2e), suggesting *BIG* and *JAV1* are likely to be co-regulated. Together, these data suggest that *BIG* expression can be significantly suppressed by JAs and the suppression requires a functional COI1.

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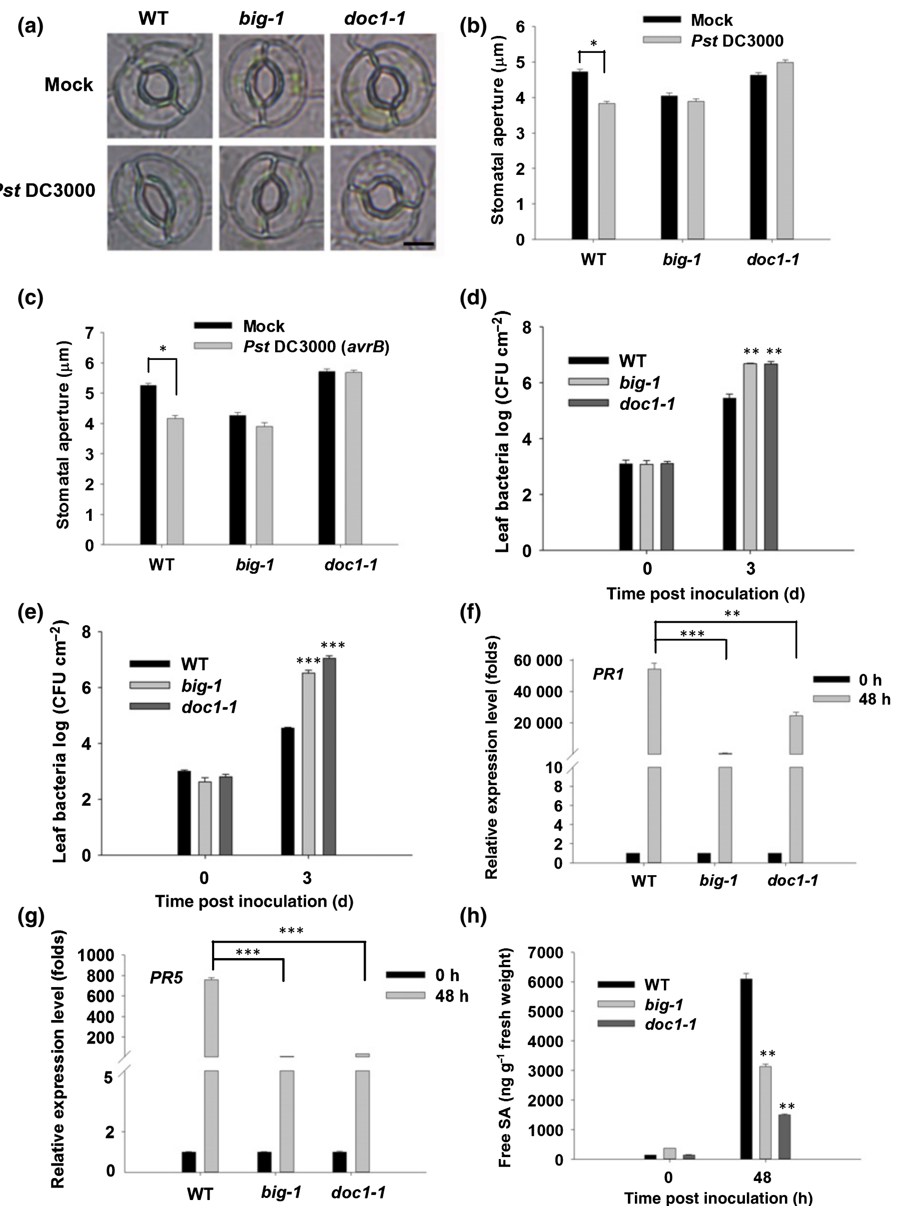

Fig. 1 Stomatal immunity and SA induction were compromised by mutations in Arabidopsis *BIG* gene. (a) Representative images showing stomata of *BIG* mutants and wild-type (WT) plants after incubation with *Pst* DC3000. Images were taken on 2.5 h light-preincubated epidermal peels, incubated for 1 h with bacterial suspensions *Pst* DC3000 (10^8 CFU ml^{-1}). Scale bar, 5 μm. (b-c) *Pst*-induced stomatal closure is corrupted in *BIG* mutants. Stomatal apertures were measured on 2.5 h light-preincubated epidermal peels, incubated for 1 h with bacterial suspensions *Pst* DC3000 or *Pst* DC3000 (*avrB*) (10^8 CFU ml^{-1}). Values are mean ± SD ($n = 120$). (d) *BIG* mutants support more *Pst* DC3000 growth. Five-week-old plants were sprayed with a bacterial suspension containing 5×10^8 CFU ml^{-1} in 10 mM $MgCl_2$ with 0.02% Silwet L-77. Bacterial growth was assessed 3 d after inoculation. (e) *BIG* mutants are more susceptible to *Pst* DC3000 (*avrB*) infection. Five-week-old plants are sprayed with a bacterial suspension containing 5×10^8 CFU ml^{-1} in 10 mM $MgCl_2$ with 0.02% Silwet L-77. Bacterial growth was assessed 3 d after inoculation. (f,g) Mutations in *BIG* gene inhibit *PR1* and *PR5* gene induction in response to *Pst* DC3000 (*avrB*). Treatment was performed as in (e), and the relative transcript levels of *PR1* and *PR5* were measured in WT and *BIG* mutant plants by Q-PCR assay after 48 h inoculation using *ACT3* as the internal control. (h) The induction of SA accumulation by *Pst* DC3000 (*avrB*) infection in *BIG* mutants is compromised compared with WT. Treatment was performed as in (e), and SA content was measured in WT and *BIG* mutant plants after 48 h infection. All experiments were repeated at least three times. The shown result was a representative of three independent biological experiments. Data are shown as means ± SD. Asterisks represent Student's *t*-test significance (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$), and the asterisks in (d,e) and (h) are shown when compared with the wild-type.

Our data suggest that mutations in *BIG* result in reduced SA production. We next investigated whether JA synthesis was also affected in *big* mutants. We found that multiple genes for JA synthesis were expressed at higher levels in *big-1* than WT suggesting that JA production was increased in the mutant (Fig. S2a). This suggestion was borne out when the levels of JA were measured. As shown in Fig. 2(f,g), before *Bc* inoculation or mechanical wounding, the levels of JAs including OPDA, JA and JA-Ile in *big* mutants were slightly yet consistently higher than those in WT. However, JA production increased dramatically after *Bc*

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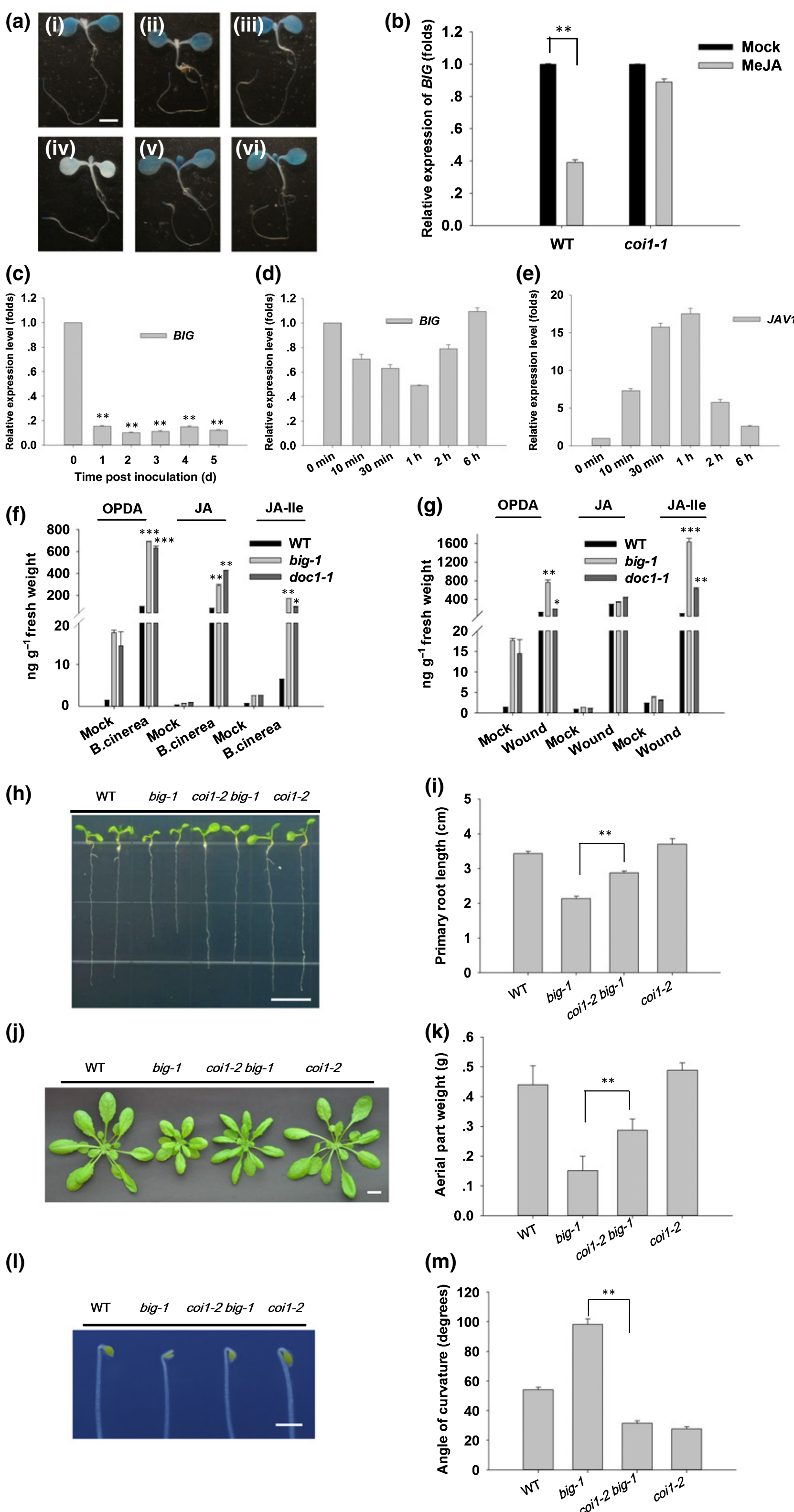

inoculation and the increase was statistically greater in *big* mutants (Fig. 2f). Likewise, *big* mutants accumulated more OPDA and JA-Ile than WT in response to mechanical wounding (Fig. 2g), suggesting BIG functions as a negative regulator of JAs synthesis. This conclusion was reinforced by the elevated accumulation of JA and JA-Ile observed in *big* mutants after challenged with *Pst* DC3000 (avrB) (Fig. S2c). Intriguingly, expression levels of selected JA-responsive genes including several

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Fig. 2 The expression of *BIG* in Arabidopsis is strongly downregulated by JA which in turn regulates JA production and responses. (a) MeJA treatment suppresses GUS activity in *pBIG*:GUS transgenic plants. GUS staining of 5-d-old seedlings without any supplement as a control (I) or with 50 μM ABA (II), 10 μM ACC (III), 20 μM MeJA (IV), 25 nM IAA (V), and 50 mM Ca^{2+} (VI) for 24 h. Scale bar, 2 mm. (b) Jasmonate suppresses *BIG* expression in a COI1-dependent manner. Four-week-old Col-0 and *coi1-1* plants grown in soil were sprayed with 100 μM MeJA, and *BIG* transcript levels were analyzed at 24 h after MeJA treatment using Q-PCR assays. (c) *B. cinerea* infection significantly downregulates *BIG* gene expression. Four-week-old soil-grown Col-0 plants were inoculated with an 8-μl droplet of *B. cinerea* spore suspension at a concentration of 1.5×10^6 spores ml^{-1}, and *BIG* transcript levels in response to *B. cinerea* infection at the indicated times were analyzed using Q-PCR assays. (d) Mechanical injury significantly downregulates *BIG* expression. Q-PCR analysis of *BIG* transcript levels after wounding treatment at the indicated times. (e) Mechanical wounding significantly induces *JAV1* expression. Q-PCR analysis of *JAV1* transcript levels in response to wounding treatment at the indicated times. (f) *BIG* mutation promotes induction of JA production by *B. cinerea* infection. Four-week-old Col-0, *big-1*, *doc1-1* mutant plants were droplet inoculated at a concentration of 1.5×10^6 spores ml^{-1}, and OPDA, JA, JA-Ile contents in rosette leaves of Col-0 and *BIG* mutants were analyzed at 48 h inoculation. (g) *BIG* mutation promotes induction of JA production by mechanical wounding. OPDA, JA, and JA-Ile contents in rosette leaves of Col-0 and *BIG* mutants were measured at 1 h after wounding treatment. (h,i) Introduction of the *coi1-2* mutation in *big-1* mutant partly restores primary root length. Root growth and length of 7-d-old seedlings Col-0, *big-1*, *coi1-2 big-1*, *coi1-2* grown on ½MS medium plates. Scale bar, 1 cm. (j,k) Introduction of the *coi1-2* mutation in *big-1* mutant partly restores plant growth and biomass production. The aerial part of 5-wk-old Col-0, *big-1*, *coi1-2 big-1*, *coi1-2* were subjected to the fresh weight measurement. Scale bar, 1 cm. (l,m) Introduction of the *coi1-2* mutation in *big-1* mutant partly restores apical hook formation. The apical hook curvature of 4-d-old etiolated Arabidopsis seedlings including Col-0, *big-1*, *coi1-2 big-1*, *coi1-2* grown in the dark on ½MS medium was measured using ImageJ. Scale bar, 2 mm. All experiments were repeated at least three times. The shown result was a representative of three independent biological experiments. Data are shown as means ± SD. Asterisks represent Student's *t*-test significance (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$), and the asterisks in (c) are shown when compared with 0 d, the asterisks in (f) and (g) are shown when compared with the wild-type.

members of *JAZ* gene family (*JAZ1*, *JAZ5* and *JAZ10*), the general repressor *TOPLESS*, and the *CYP94B3* gene which encodes a JA-Ile 12-hydroxylase that catalyzes the conversion of JA-Ile into the biologically inactive 12-hydroxy-JA-Ile (Chini *et al.*, 2007; Thines *et al.*, 2007; Yan *et al.*, 2007; Szemenyei *et al.*, 2008; Kitaoka *et al.*, 2011) in *big* mutants were significantly higher than those in the WT (Fig. S2b). These negative regulators of JA responses are likely activated to fine-tune the JA pathway and protect *big* mutants from the hyper-activated JA signaling (Chini *et al.*, 2007).

Mutations in *BIG* restrict overall plant growth and development, characterized by a reduction in organ elongation and lateral root number as well as a decrease in apical dominance. These defects are well known to be associated with auxin and, as a result, considerable attention has been placed on the links between *BIG* gene and auxin action, particularly, auxin transport (Ruegger *et al.*, 1997; Gil *et al.*, 2001; Paciorek *et al.*, 2005; Kazan & Manners, 2009; Qi *et al.*, 2012; Guo *et al.*, 2013). Given that JA and auxin pathways interact extensively (Kazan & Manners, 2009, 2012; Pérez & Goossens, 2013), and *BIG* mutations have elevated JA, we next asked whether the increased JAs contribute to the aforementioned defects in plant development. To test this, we generated the *big-1 coi1-2* double mutant by genetic crossing *big-1* with *coi1-2*, a leaky *coi1* mutant that is insensitive to JA (Xu *et al.*, 2002). As shown in Fig. 2(h–k) and Fig. S3(c,d), *coi1-2* allele significantly, although not fully, rescued the growth defects of *big-1* as *coi1-2 big-1* plants have longer primary roots, produce more shoot biomass and larger seeds. Likewise, the defect of apical hook development observed in *big* mutants was largely rescued by *coi1-2* as the hook curvature angles of the double mutant *coi1-2 big-1* was comparable with those of *coi1-2* seedlings (Fig. 2l,m). In conclusion, the developmental defects found in *big* mutant alleles could be partially accounted for by increased JA accumulation. Our work also reveals a role of BIG in regulating apical hook formation that plays an essential role in seedling soil emergence after seed germination (Zhong *et al.*, 2009, 2014), and hence is likely of agricultural importance.

To further investigate the function of BIG in JA signalling, we analyzed anthocyanin accumulation and leaf senescence in response to JA treatment. Anthocyanin accumulation is a hallmark of JA-induced responses (Franceschi & Grimes, 1991; Feys *et al.*, 1994; Tamari *et al.*, 1995). As shown in Fig. S2(d), strong anthocyanin pigmentation developed in seedlings of *big* mutants. We quantified this difference revealing that anthocyanin contents in *big* mutants were significantly higher (*big-1*, 7.3-fold and *doc1-1*, 4.1-fold) than WT (Fig. S2e). Exogenous JAs promote leaf senescence in Arabidopsis (He *et al.*, 2002). When we incubated detached leaves from *big* mutants and WT plants in sterile water with or without 100 μM MeJA, as shown in Fig. S2(f,g), leaves from the mutants displayed a rapid yellowing suggestive of an accelerated senescence or earlier onset of senescence than WT. We also tested anthocyanin accumulation and leaf senescence in *coi1-2 big-1* and found that *COI1* mutation can alleviate the anthocyanin accumulation and leaf senescence of *big-1* in the presence of MeJA (Fig. S2h–k). In conclusion, BIG modulates a multitude of plant developmental processes through JA activity.

BIG functions in jasmonate-mediated plant defenses

JA signalling often conditions plant resistance to biotic stresses (Thomma *et al.*, 1998; Rowe *et al.*, 2010). Because BIG deficiency promotes JAs production, we expected the JA-mediated plant resistance to pathogens should be enhanced. However, when droplet inoculated with a 5 μl suspension of *Bc* spores, which induces JA-dependent defense responses (Glazebrook, 2005), much more severe necrosis phenotypes were observed in *big* mutants than in WT, as the average diameter of necrotic lesion in infected leaves of *big* mutants was much larger (Fig. 3a, b), suggesting that BIG positively regulates plant resistance against *Bc* infection. On the face of it, this is rather counterintuitive. To substantiate the outcome of this fungal inoculation, we evaluated the pathogen growth by trypan blue staining whereby hyphal structures and dead plant cells would be stained blue. We found that at 16 h post infection with *Bc*, hyphae growth in *big*

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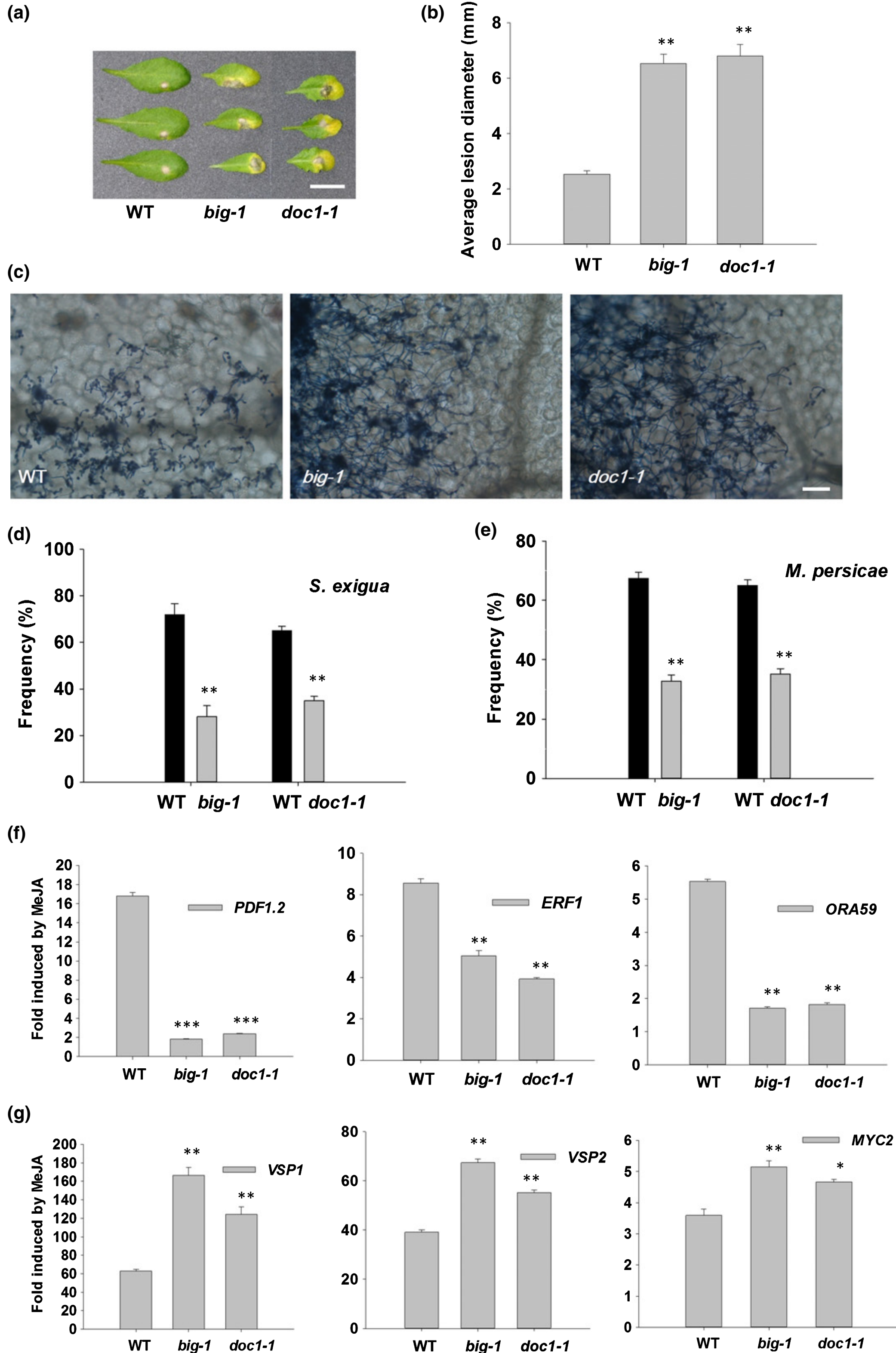

mutants were much more pronounced compared with WT (Fig. 3c). Together, these data indicate that *BIG* mutations dampen plant defenses against necrotrophic fungus, consistent with an early report on *A. brassicicola* (Qi *et al.*, 2012). Plants are frequently attacked by herbivores and are protected by JA-inducible defenses (Wasternack & Hause, 2013). We performed two-choice tests to determine whether *BIG* is required for plant defense against insect feeding, and found that mutant plants retained significantly fewer *S. exigua* larvae when larvae were given the choice between *big* mutant and WT plants (Fig. 3d). A

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Fig. 3 BIG functions in jasmonate-mediated plant defenses in Arabidopsis. (a) *BIG* mutants are more susceptible to *B. cinerea* infection. Four-week-old Col-0 (wild-type, WT), and *big-1, doc1-1* mutant plants were droplet inoculated at a concentration of 1.5×10^6 spores ml^{-1}, symptoms on rosette leaves were analyzed 4 d after inoculation. Scale bar, 1 cm. (b) Average diameter of lesion from Col-0 and *BIG* mutants 4 d following inoculation was analyzed from (a). (c) *BIG* mutants are more susceptible to *B. cinerea* infection. Treatment was performed as in (a), and trypan blue staining indicating fungal growth on leaves of Col-0 and *BIG* mutants was performed at 16 h inoculation. Scale bar represents 20 μm. (d) *BIG* mutation inhibits the preference of larvae of *S. exigua* in the two-choice tests. In total, 60 larvae of *S. exigua* were placed equidistant from the two plants (WT vs *big-1* or WT vs *doc1-1*), and the numbers of larvae on each plant were recorded after 24 h incubation. (e) *BIG* mutation inhibits the preference of aphids in the two-choice test. Treatment was performed as in (d), and the numbers of aphids on each plant were recorded after 24 h incubation. (f) Loss of BIG inhibits ERF1-branch of JA pathway. Four-week-old Col-0 and *BIG* mutant plants grown in soil were sprayed with 100 μM MeJA, and fold change of JA-responsive genes *ERF1*, *ORA59*, and *PDF1.2* dependent on JA and ET in WT and *BIG* mutants 6 h following MeJA treatment was analyzed by Q-PCR assay using *ACT3* as the internal control. (g) Dysregulated *BIG* enhances MYC2-branch of JA pathway. Treatment was performed as in (f), and fold changes of JA-responsive genes *VSP1*, *VSP2*, and *MYC2* in WT and *BIG* mutant plants 6 h following MeJA treatment were analyzed by Q-PCR assays. *ACT3* was used as the internal control. All experiments were repeated at least three times. The shown result was a representative of three independent biological experiments. Data are shown as means ± SD. Asterisks represent Student's *t*-test significance (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$) when compared with the wild-type.

similar feeding repelling effect of *BIG* mutation was observed when aphids (*M. persicae*) were tested (Fig. 3e).

On the basis of our discovery that BIG positively and negatively regulates plant resistance to necrotrophic *Bc* and phytophagous insects, respectively, we hypothesized that BIG might orchestrate the antagonism between two parallel MYC2- and ERF1/ORA59-branches in the JA pathway that determine resistance to pests and pathogens. To test this hypothesis, we first examined the expression of *VSP2* and *PDF1.2*, marker genes of these two branches, and found that in the presence of 100 μM MeJA, the transcripts of *PDF1.2* as well as *ERF1* and *ORA59* were substantially increased at 6 h post MeJA treatment, but the induced increments were much less in *big* mutants than in WT (Fig. 3f), implicating impaired ERF1-branch in *big* mutants. However, in marked contrast, the induced dosage of JA-responsive genes *VSP1*, *VSP2* and *MYC2* in *big* mutants by MeJA treatment was significantly higher than those in WT (Fig. 3g), suggesting that the MYC2 branch was potentiated by *BIG* deficiency. Notably, the overexpression of *THIONIN2.1* (*THI2.1*, Fig. S3a) in *big* mutants might account for the enhanced resistance to *Fusarium* wilt disease caused by the root-infecting fungal pathogen, *F. oxysporum* (Kazan & Manners, 2009; Thatcher *et al.*, 2012). To further ascertain the upregulation of the MYC2 branch, we next investigated whether BIG affects MYC2 protein level. For this purpose, we generated p35S:MYC2-4Myc/*big-1* by genetic crossing the transgenic plants harboring the p35S:MYC2-4Myc construct (Zhai *et al.*, 2013) with *big-1* plants. The result presented in Fig. S3(b) indicates that MYC2 protein abundance was higher in p35S:MYC2-4Myc *big-1* compared with p35S:MYC2-4Myc transgenic plants, suggesting MYC2 turnover is perturbed by *BIG* deficiency. Taken together, these results suggest that fully functional BIG is required for tightly regulating MYC2 activity and JA signaled plant resistance to pests and pathogens.

Disruption of *BIG* gene suppresses ethylene accumulation

ET accumulation can be rapidly induced to modulate the function of JA pathway (von Dahl *et al.*, 2007). Plant immune responses against necrotrophs are co-controlled by JA and ET as well exemplified in mutants *coi1-1* (JA insensitive) and *ein2-1* (ET insensitive), which both are more susceptible to fungal infection than WT Arabidopsis (Penninckx *et al.*, 1998; Thomma *et al.*, 1998, 1999; Zhu *et al.*, 2011; Wasternack, 2014). The observation that *big* mutants accumulate more JA, but are less resistant to *Bc* invasion, prompted us to investigate whether the ET pathway was compromised by *BIG* disruption. ET synthesis genes displayed much lower expression levels in *big* mutants than WT (Fig. S4a). ET accumulation induced by either mechanical injury or *Bc* inoculation in *big* mutants is less pronounced than that in WT (Fig. 4a,b). This finding is compatible with the reported hyposensitivity of *big* mutants such as *umb1*, *tir3-1* and *asa1* to ET (Kanyuka *et al.*, 2003). Consistently, resistance to *B. cinerea* in *big* mutant lines was restored when treated with the ethylene precursor 1-aminocyclopropane-carboxylic acid (ACC) (Fig. 4c,d). These results suggest that reduced resistance against *Bc* infection observed in *big* mutants can be ascribed to the ET deficiency.

Previous studies have shown that photo-oxidative damage is associated with increased levels of protochlorophyllide, a phototoxic intermediate in chlorophyll synthesis (Reinbothe *et al.*, 1996; Huq *et al.*, 2004; Zhong *et al.*, 2009, 2014). ET facilitates cotyledon greening of etiolated seedlings upon light irradiation by activating EIN3/EIL1-induced expression of protochlorophyllide oxidoreductase A and B (PORA/B) (Zhong *et al.*, 2009), therefore, we next examined whether *BIG* is required in this process. Figure 4(e) shows that *BIG* mutations caused a significant reduction in the rate of cotyledon greening with respect to WT seedlings. The levels of protochlorophyllide in *big* mutant seedlings were significantly higher than WT (Fig. 4f). The expression levels of *PORA/B* gene were significantly reduced in *big* mutants (Fig. S4b). Given that *BIG* mutations also impair the formation of apical hook, it is tempting to speculate that BIG function is required for seedling soil emergence and survival from photooxidation, which are two key adaptive traits of great importance in agriculture.

Discussion

Plants have evolved an intricate system to restrict JA overactivation under stress-free growth conditions, however they also have the capacity to rapidly produce JA under stress (Yan *et al.*, 2018). Here we show that BIG is a regulator of the JA pathway and plays an integral role in the concomitant activation of JAs

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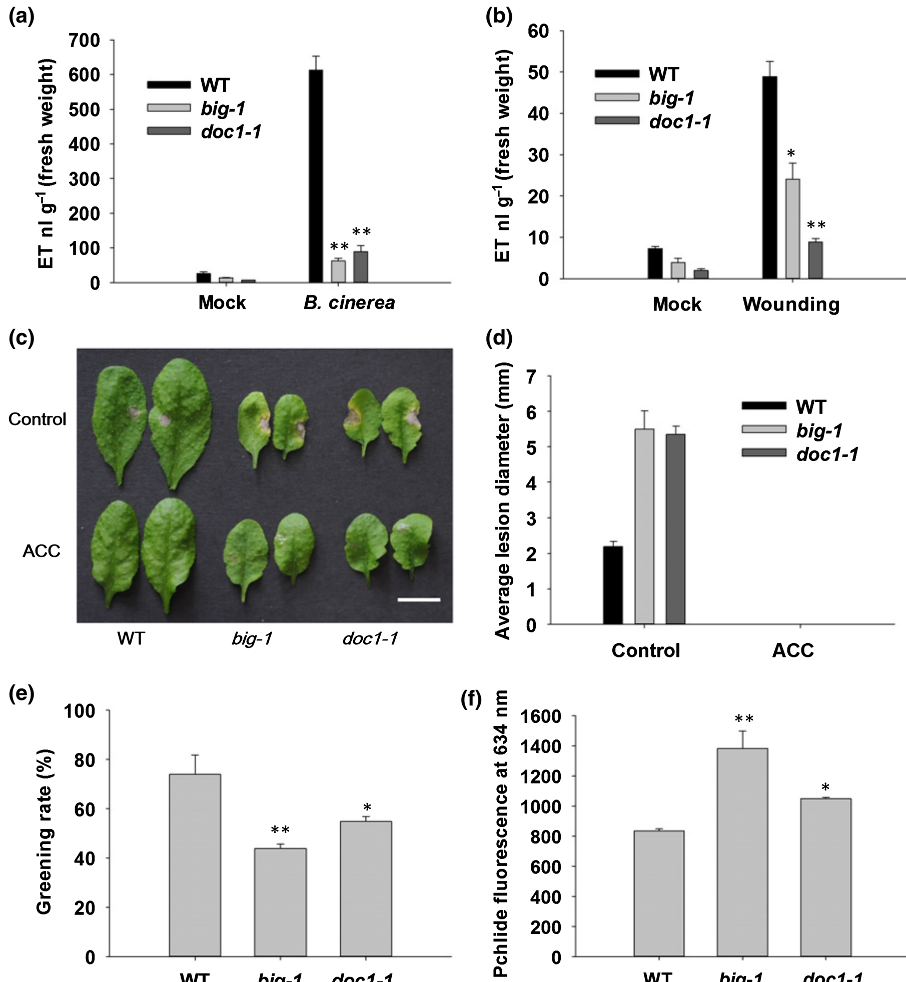

Fig. 4 Disruption of *BIG* gene suppresses ethylene accumulation in Arabidopsis. (a) *BIG* mutation inhibits ethylene accumulation in response to *B. cinerea* infection. Two-week-old seedlings grown in 40 ml GC vials were inoculated with *B. cinerea* spore suspension at a concentration of 4.0×10^5 spores ml^{-1}, and ET content in the headspace of the GC vials was measured at 48 h inoculation by gas chromatography. (b) *BIG* mutation inhibits ethylene accumulation induced by wounding treatment. Two-week-old seedlings grown in 40 ml GC vials were injured with forceps, and ET production in the headspace of the GC vials was determined at 24 h after wounding treatment. (c) Application of ACC can restore the resistance to *B. cinerea* in *big* mutants. Four-week-old Col-0, *big-1, doc1-1* mutant plants were treated for 48 h with spraying 0.5 mM of the ethylene precursor 1-aminocyclopropane-carboxylic acid (ACC) or with water (the solvent) as the mock control. Then the ACC treated and control plants were droplet inoculated at a concentration of 1.5×10^6 spores ml^{-1}, symptoms on rosette leaves were analyzed 4 d after inoculation. Scale bar, 1 cm. (d) Average diameter of lesion from Col-0 and *BIG* mutants 4 d following inoculation was analyzed from (c). (e) *BIG* mutants showed reduction of cotyledon greening compared to wilddowntype (WT) plants. Greening rate of 6-d-old etiolated seedlings followed by 2 d of light exposure was analyzed. (f) *BIG* mutants displayed higher protochlorophyllide fluorescence. The protochlorophyllide fluorescence at 634 nm of 6-d-old etiolated seedlings was determined. All experiments were repeated at least three times. The shown result was a representative of three independent biological experiments. Data are shown as means ± SD. Asterisks represent Student's *t*-test significance (*, $P < 0.05$; **, $P < 0.01$) when compared with the wild-type.

and ET synthesis in response to mechanical and biotic injury. BIG deficiency results in an increase of endogenous JAs and enhanced JAs sensitivity, which not only disturbed the antagonism of JAs and SA actions but tips the balance between the two parallel MYC2 and ERF1 branches in the JA pathway, making *big* mutants more susceptible to fungal pathogen *B. cinerea* and bacterium *P. syringae* but less attractive to herbivorous insects. The suppressive effect on auxin transport widely observed in various *big* mutants appears to be brought about by the JA pathway, as JAs negatively modulate the intracellular trafficking and turnover of PIN proteins and thus the auxin transport capacities (Paciorek *et al.*, 2005; Pan *et al.*, 2009; Sun *et al.*, 2009, 2011). A recent study lends further support to this idea, as *A. brassicicola* infection that activates JAs accumulation also reduces protein levels of the PIN family and therefore reduces auxin transport rate (Qi *et al.*, 2012). *max4* mutant could restore the auxin transport of *tir3-1* (an independent allele of *big* mutant) (Prusinkiewicza *et al.*, 2009) but not the developmental defects including dwarfism, over-branching and delayed flowering that are also characteristics of several JA-sensitive mutants such as *cev1*, *cex1*, *cet1*, and *joe2* that were all characterized by elevated JA contents (Kunkel & Brooks, 2002; Bonaventure *et al.*, 2007; Qi *et al.*, 2012). Furthermore, the upregulation of *FLC* transcription and delayed flowering in *big* mutants (Kanyuka *et al.*, 2003;

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Ebine *et al.*, 2012) correspond well to the mechanistic insights into the mode of action of two transcription factors TOE1 and TOE2, which regulate flowering time through JA pathway (Zhai *et al.*, 2015).

Importantly, mutations in the *BIG* gene recapitulate the links between JAs and other hormones as well as light pathways (De Wit *et al.*, 2013; Wasternack & Hause, 2013). Independent *big* alleles, *asa1* and *umb1* confer a significant reduction in sensitivity to cytokinin in root growth inhibition assays, whereas, *asa1*, *umb1* and *tir3-1* as well as *ga6* (Sponsel *et al.*, 1997; Kanyuka *et al.*, 2003) are affected in their response to gibberellin, which mutually antagonizes JAs in regulating seedling growth and resistance to pathogens via the interactions of JAZ and DELLA proteins (Hou *et al.*, 2010; Yang *et al.*, 2012). *doc1-1* and *tir3-1* have altered expression of light-regulated genes when grown in darkness (Gil *et al.*, 2001), while the *asa1* mutant suppresses the constitutive shade avoidance syndrome of *phyA phyB* mutant as well as the low R/FR light-induced shade avoidance syndrome in WT (Kanyuka *et al.*, 2003). Therefore, *BIG* provides an additional connection between light signalling and JA pathway in plant growth and defense responses. *BIG* warrants recognition as a key regulator of JA signal pathway.

The induction of ET by mechanical and biotic injury is compromised by BIG disruption, consistent with the reduced responsiveness of *tir3-1*, *asa1* and *umb1* to ET (Kanyuka *et al.*, 2003), suggesting that BIG acts as a signal integration point via which JA and ET pathways are coordinated. Currently, we still know little information about how ET abundance is affected by BIG deficiency. Given the indispensable role of reactive oxygen species (ROS) in the full activation of SA (Durrant & Dong, 2004; Torres *et al.*, 2006) and ET production (Wang *et al.*, 2002), as well as in bacteria- and LPS-induced stomatal closure (Shang-Guan *et al.*, 2018), which all are damaged by BIG deficiency. In particular, the plant resistance to avirulent bacteria that is dependent to ROS signalling is more dramatically affected by BIG disruption relative to virulent bacteria. In addition, *doc1-1* and *rao3*, two independent *big* mutant alleles, displayed misregulated peroxidases and AOX1a (Gil *et al.*, 2001; Ivanova *et al.*, 2014), suggesting that *BIG* might have a large effect on redox regulation and ROS scavenging capacity. Furthermore, a potential role for BIG in ROS signalling is supported by characterization of two independent mutants, *doc1-1* and *big-j588*. Each has a substitution in the conserved cysteine residues in the conserved UBR box motif (Gil *et al.*, 2001; Guo *et al.*, 2013; Parsons *et al.*, 2015), which is thought to provide a mechanism that senses and regulates the oxidative stress response in plants through the redox dependent thiol-disulphide exchange (Rhee *et al.*, 2000; Grek *et al.*, 2013; Song *et al.*, 2014b; Parsons *et al.*, 2015). It is worth a mention that JA has recently been reported to positively mediate elevated CO_2-induced stomatal closure (Geng *et al.*, 2016), and elevated CO_2 impacts on plants resistance via redox signaling to activate JA pathway (Mhamdi & Noctor, 2016; Williams *et al.*, 2018). Whether the regulatory function of BIG in CO_2-induced stomatal closure as we have recently demonstrated (He *et al.*, 2018) is mediated by a similar JA/SA-redox signaling mechanism awaits further study. Another possibility is that BIG contains many conserved domains that may contribute to the control of ET synthesis through protein–protein interactions (Gil *et al.*, 2001). Nonetheless, additional work is required to better understand the possible role of BIG in regulating not only the cellular ROS homeostasis but also the orchestrated production of JA and ET in response to biotic and mechanical injury.

BIG is ubiquitously expressed in most organs at varied levels, but is rapidly decreased in floral organs upon transition to the reproductive phase (Gil *et al.*, 2001; Yamaguchi *et al.*, 2007; Guo *et al.*, 2013). This observed reduction of *BIG* expression during the development of flowers is compatible with the observation that JAs accumulate to high levels for floral development and defense (Li *et al.*, 2017). As a negative regulator of JAs synthesis, the expression of *BIG* is controlled at the transcriptional level by JAs, suggesting that there might exist a threshold level of JAs that is needed to trigger the downregulation of *BIG* and abrogate the inhibitory effects of BIG on the JA production, which in turn promotes the accumulation of JAs. This idea fits well with the observed Ca^{2+}/CaM-dependent, but JA-signaling-independent 'initiation activation' of JA biosynthesis, which elevates JA content to a moderate level before further triggering JA production to a sufficiently high level through the 'feedback regulation' mechanisms to cause a JA burst (Yan *et al.*, 2018).

A wide range of JAs-regulated responses both developmental and defensive is altered in *big* mutants in a COI1-dependent manner, suggesting that BIG acts relatively upstream in the JA signalling pathway. This view is compatible with the finding that genes *BIG* and *JAV1* are co-regulated and suggests, as for JAV1, that BIG is an early component in the JA pathway. Therefore, we propose that, at resting state, a ubiquitous and relatively highly expressed BIG, together with JAZs, the JJW complex and CYP94B3 constitute a multi-layer of tight control system that helps the plant restrict the production of JAs and therefore to minimize their inhibitory effect on plant growth and development. However, in response to certain development cues and environmental stresses, plants activate JAs synthesis and once the accumulation of JAs reaches to a threshold level, *BIG* expression will be downregulated, and the repression effect of BIG on JA production is hence removed. This removal results in a rapid JA increase that further downregulates BIG expression, and which in turn amplifies the JA increase, thereby forming a feedback loop to accelerate JA buildup and hence mount a fast, strong stress adaptive response. Another notable function of BIG is its involvement in modulating hook development and *PORA/B* transcription. Hook formation plays an essential role in seedling soil emergence after seed germination, and the downregulation of PORA/B in *big* mutants will probably lead to a lack of POR activity, which is essential for chlorophyll synthesis. Therefore, the BIG mutation might not only hamper prompt seedling emergence from soil, and the conversion of protochlorophyllide to chlorophyll, but also prevent the timely scavenging of ROS upon light irradiation. As a consequence, this will cause photo-oxidative damage and even premature seedling death according to the reports by Zhong *et al.* (2009, 2014). Whether BIG function in Arabidopsis translates well to monocot crop plants such as rice merits further investigation. Taken together, we propose that

JA is likely to play a central role in BIG regulating plant defense responses and the hormonal balance between JA, ET, SA, and auxin as well as the plant adaptive response to various environmental changes such as light and CO_2 concentration to reconcile the growth and defense dilemma in plants.

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Author contributions

Y-KL and R-XZ designed research. R-XZ, SCG, JJH, SCL, YHH, HD, ZML and RC performed research. Y-KL, R-XZ, AMH, Y-QF, LZX and CYL analyzed data. Y-KL, R-XZ and AMH wrote the paper. R-XZ and SCG contributed equally to this work.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article:

Fig. S1 Stomatal immunity and induction of SA biosynthetic gene were compromised by BIG mutations in Arabidopsis.

Fig. S2 Loss of function of Arabidopsis *BIG* gene disturbed the jasmonate pathway.

Fig. S3 *BIG* gene is involved in regulating JA-responsive gene expression, protein accumulation and seed size in Arabidopsis.

Fig. S4 *BIG* mutation affects ET production and POR expression in Arabidopsis.

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