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The Elongator complex-associated protein DRL1 plays a positive role in immune responses against necrotrophic fungal pathogens in Arabidopsis

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SUMMARY

DEFORMED ROOT AND LEAVES1 (DRL1) is an Arabidopsis homologue of the yeast TOXIN TARGET4 (TOT4)/KILLER TOXIN-INSENSITIVE12 (KTI12) protein that is physically associated with the RNA polymerase II-interacting protein complex named Elongator. Mutations in DRL1 and Elongator lead to similar morphological and molecular phenotypes, suggesting that DRL1 and Elongator may functionally overlap in Arabidopsis. We have shown previously that Elongator plays an important role in both salicylic acid (SA)- and jasmonic acid (JA)/ethylene (ET)-mediated defence responses. Here, we tested whether DRL1 also plays a similar role as Elongator in plant immune responses. Our results show that, although DRL1 partially contributes to SA-induced cytotoxicity, it does not play a significant role in SA-mediated expression of *PATHOGENESIS-RELATED* genes and resistance to the virulent bacterial pathogen *Pseudomonas syringae* pv. *maculicola* ES4326. In contrast, DRL1 is required for JA/ET- and necrotrophic fungal pathogen *Botrytis cinerea*-induced defence gene expression and for resistance to *B. cinerea* and *Alternaria brassicicola*. Furthermore, unlike the *TOT4/KTI12* gene which, when overexpressed in yeast, confers zymocin resistance, a phenotype of the *tot4/kti12* mutant, overexpression of *DRL1* does not change *B. cinerea*-induced defence gene expression and resistance to this pathogen. Finally, DRL1 contains an N-terminal P-loop and a C-terminal calmodulin (CaM)-binding domain and is a CaM-binding protein. We demonstrate that both the P-loop and the CaM-binding domain are essential for the function of DRL1 in *B. cinerea*-induced expression of *PDF1.2* and *ORA59*, and in resistance to *B. cinerea*, suggesting that the function of DRL1 in plant immunity may be regulated by ATP/GTP and CaM binding.

Keywords: Arabidopsis, *Botrytis cinerea*, DRL1, Elongator, ethylene, jasmonic acid, salicylic acid.

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INTRODUCTION

As a result of their sessile nature, plants have to endure and withstand a wide variety of microbial pathogens with diverse modes of attack and, accordingly, have evolved a highly intricate immune system involving pathogen recognition, signal transduction and the activation of appropriate transcriptional changes. Depending on the lifestyle of the invading pathogen, different signal molecules are synthesized to activate respective defence signalling pathways that are the most effective for resisting the invader (Loake and Grant, 2007; van Loon *et al.*, 2006; Pozo *et al.*, 2005). Salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) are the primary defence signal molecules that play central roles in the regulation of plant immune responses (Pieterse *et al.*, 2009). SA-activated defence signalling is generally effective against biotrophic and hemibiotrophic pathogens, whereas JA/ET-mediated defence responses confer resistance to necrotrophic pathogens (Glazebrook, 2005; Thomma *et al.*, 2001). Although SA- and JA/ET-mediated defence pathways have been well characterized (Broekaert *et al.*, 2006; Kazan and Manners, 2008; Vlot *et al.*, 2009), the signalling components of these pathways still remain to be fully uncovered.

Elongator is an RNA polymerase II-interacting protein complex, which was first identified in yeast and was later purified from human and Arabidopsis cells (Hawkes *et al.*, 2002; Nelissen *et al.*, 2010; Otero *et al.*, 1999; Wittschieben *et al.*, 1999). This complex consists of six subunits and its structure is highly conserved among yeast, humans and Arabidopsis. The six Elongator (ELP) subunits in Arabidopsis were named ELONGATA2 (ELO2)/ELP1, ELP2, ELO3/ELP3, ELO1/ELP4, ELP5 and ELP6 (Nelissen *et al.*, 2010). It should be noted that the acronym 'ELP' has been used previously to describe 'EDM2-lik proteins' in Arabidopsis (Eulgem *et al.*, 2007). A large body of evidence in yeast and humans has shown that Elongator plays important roles in diverse cellular and molecular processes, including histone modification, tRNA modification, exocytosis, α-tubulin acetylation and zygotic paternal genome demethylation (Creppe *et al.*, 2009; Hawkes *et al.*, 2002;

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Huang *et al*., 2005; Okada *et al*., 2010; Rahl *et al*., 2005; Winkler *et al*., 2002). In plants, Elongator has been shown to function in meristem and leaf development, cell cycle progression and response to abiotic stresses (Chen *et al*., 2006; Nelissen *et al*., 2005; Xu *et al*., 2012; Zhou *et al*., 2009). Recently, we have demonstrated that Elongator also plays an important role in SA- and JA/ET-mediated plant defence responses (Defraia *et al*., 2010, 2013; Wang *et al*., 2015). Arabidopsis *elo/elp* mutants exhibit delayed and/or reduced induction of defence genes, including the SA pathway marker gene *PATHOGENESIS-RELATED GENE1* (*PR1*) and the JA/ET defence pathway marker gene *PLANT DEFENSIN1.2* (*PDF1.2*), and display enhanced suceptibility to the hemibiotrophic bacterial pathogen *Pseudomonas syringae* and the necrotrophic fungal pathogens *Botrytis cinerea* and *Alternaria brassicicola* (Defraia *et al*., 2010, 2013; Wang *et al*., 2015). Although the molecular mechanisms by which Elongator modulates plant immunity still require further investigation (Wang *et al*., 2013), these results indicate that Elongator is required for full activation of the plant immune system.

DEFORMED ROOT AND LEAVES1 (DRL1) is an Arabidopsis homologue of the yeast TOXIN TARGET4 (TOT4)/KILLER TOXIN-INSENSITIVE12 (KTI12) protein (Butler *et al*., 1994; Frohloff *et al*., 2001), which is physically associated with the Elongator complex (Petrakis *et al*., 2005). TOT4/KTI12 does not specify an Elongator subunit (Fichtner *et al*., 2002), but yeast cells lacking TOT4/KTI12 are similar to *elp* mutants in resistance to the *Kluyveromyces lactis* toxin zymocin and also share other general phenotypes with *elp* mutants (Petrakis *et al*., 2005), indicating a close functional relationship between TOT4/KTI12 and Elongator. DRL1 is also physically associated with Elongator, and the Arabidopsis *drl1* and *elo/elp* mutants display similar morphological phenotypes, including narrow leaves, disorganized shoot apical meristem and short roots, and molecular phenotypes, such as transcriptome changes (Nelissen *et al*., 2003, 2005; Xu *et al*., 2012), suggesting that DRL1 and Elongator may also functionally overlap in plants. Although the role of Elongator in SA- and JA/ET-mediated defence signalling pathways has been defined (Defraia *et al*., 2010, 2013; Wang *et al*., 2015), whether or not DRL1 also functions in these pathways has not been tested.

DRL1 contains several highly conserved regions present in adenosine triphosphate/guanosine triphosphate (ATP/GTP)-binding or -utilizing proteins (Kaziro *et al*., 1991; Nelissen *et al*., 2003). The first is a P-loop [G-X(4)-G-K-S/T] spanning amino acids 8 to 15 in the N-terminus (Nelissen *et al*., 2003). This P-loop is highly conserved among TOT4/KTI12 homologues (Fichtner *et al*., 2002). An allele in which this motif is deleted fails to complement the zymocin-resistant phenotype of the yeast *tot4/kti12* mutant, indicating that the P-loop is essential for the function of TOT4/KTI12 (Fichtner *et al*., 2002). Whether this motif is important for the function of DRL1 in plants is unknown. The second is the G region (amino acids 148–152, N-K/R-X-D) of GTP-binding proteins, which interacts directly with the guanine ring of GTP. The third is P-X(2)-A/S-T (amino acids 194–199), which has been found in many ATP/GTP-utilizing enzymes. However, DRL1 lacks other highly conserved regions found in GTP-binding proteins; it may therefore not be a bona fide GTP-bining protein and may instead exert its function on ATP/GTP binding or transfer ATP/GTP to other proteins (Nelissen *et al*., 2003). DRL1 is also a calmodulin (CaM)-binding protein. It has been shown that the C-terminal 100 amino acids of DRL1 bind CaM in a calcium-dependent manner (Nelissen *et al*., 2003). A motif L-X(3)-F-X(2)-L-X(5)-L (amino acids 260–273) within the C-terminal 100 amino acids was considered as the calmodulin-binding domain (CBD) (Nelissen *et al*., 2003). However, it is currently unclear whether the CBD is necessary for the function of DRL1.

Here, we show that DRL1 is required for JA/ET-mediated, but not SA-mediated, defence responses. Mutations in *DRL1* delay and/or decrease JA/ET- and *B. cinerea*-induced defence gene expression and compromise resistance to *B. cinerea* and *A. brassicicola*. We further demonstrate that both the N-terminal P-loop and the C-terminal CBD of DRL1 are essential for its function in plant immune responses.

RESULTS

Mutations in *DRL1* partially restore SA tolerance to *npr1* and reduce JA/ET-induced, but not SA-induced, defence gene expression

NONEXPRESSOR OF PR GENES1 (NPR1) is a master transcription coactivator of the SA signalling pathway (Dong, 2004). Mutations in the *NPR1* gene not only block SA signalling, but also make the mutant plants hypersensitive to SA-induced cytotoxicity (Cao *et al*., 1994, 1997; Delaney *et al*., 1995; Kinkema *et al*., 2000; Shah *et al*., 1997). On half-strength Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) supplemented with high concentrations of SA, *npr1* seedlings are chlorotic and fail to develop beyond the cotyledon stage, whereas the wild-type exhibits tolerance to SA cytotoxicity. We have shown previously that mutations in the Elongator subunits ELP2 and ELO3/ELP3 restore SA tolerance to *npr1* (Defraia *et al*., 2010, 2013). As DRL1 is functionally associated with Elongator (Nelissen *et al*., 2003; Xu *et al*., 2012), mutations in the *DRL1* gene might also restore SA tolerance. To test this hypothesis, we identified two transfer DNA (T-DNA) insertion homozygous lines, SALK_056915 and SALK_140551, which exhibited significantly reduced expression of *DRL1* and displayed a morphology similar to that of the previously charaterized *elp2* mutant (Fig. S1a,b, see Supporting Information). As SALK lines are in the Col-0 genetic background (Alonso *et al*., 2003), SALK_056915 and SALK_140551 were named *drl1-C1* and *drl1-C2*, respectively. We generated double mutants *drl1-C1 npr1*

and *drl1-C2 npr1* by crossing with the *npr1-3* mutant and germinated the double mutant seeds on half-strength MS medium containing 0.3 mM of SA. As reported previously (Cao *et al.*, 1997; Kinkema *et al.*, 2000), the *npr1* seedlings turned chlorotic because of SA cytotoxicity, whereas the wild-type seedlings remained green (Fig. 1a). The *drl1-C1 npr1* and *drl1-C2 npr1* seedlings exhibited an intermediate phenotype (Fig. 1a), indicating partial restoration of SA tolerance in the double mutants. However, the *drl1-C1 npr1* and *drl1-C2 npr1* mutant plants were as susceptible as *npr1* to the hemibiotrophic bacterial pathogen *P. syringae* pv. *maculicola* (*Psm*) ES4326 (Fig. S2, see Supporting Information), suggesting that mutations in *DRL1* might not affect resistance to bacterial pathogens.

As mutations in Elongator genes inhibit SA- and JA/ET-mediated defence signalling (Defraia *et al.*, 2010, 2013; Wang *et al.*, 2015), we tested whether the *drl1* mutations affect SA- and JA/ET-induced defence gene expression. To this end, we treated the *drl1* mutants with SA or the JA derivative methyl jasmonate (MeJA) plus the ET precursor 1-aminocyclopropane-1-carboxylic acid (ACC) by soaking 2-week-old seedlings grown on half-strength MS plates with 0.5 mM SA or 0.1 mM MeJA plus 0.1 mM ACC. Twenty-four hours later, the induction of three SA pathway genes, *PR1*, *PR2* and *PR5*, as well as three JA/ET-inducible genes, *PDF1.2*, *BASIC CHITINASE* (*CHIB*) and *HEVEIN-LIKE* (*HEL*), was examined by real-time quantitative PCR (qPCR). As shown in Fig. 1b, expression of the three *PR* genes was induced to similar levels after SA treatment in the wild-type and the *drl1* mutants, suggesting that DRL1 is not essential for SA-induced *PR* gene expression. However, the *drl1* mutations inhibited JA/ET-induced expression of *PDF1.2*, *CHIB* and *HEL* (Fig. 1c), indicating that DRL1 is required for full induction of the three JA/ET-inducible defence genes.

Mutations in *DRL1* inhibit *B. cinerea*-induced, but not *Psm* ES4326-induced, defence gene expression

To test whether DRL1 is required for pathogen-induced defence gene expression, we inoculated 4-week-old soil-grown *drl1* and wild-type plants with *Psm* ES4326 or the necrotrophic fungal pathogen *B. cinerea* and monitored *Psm* ES4326-induced expression of *PR1*, *PR2* and *PR5*, as well as *B. cinerea*-induced expression of *PDF1.2*, *CHIB*, *HEL* and *OCTADECANOID-RESPONSIVE ARABIDOPSIS AP2/ERF59* (*ORA59*). The previously characterized *elp2* mutant was included as a control (Defraia *et al.*, 2010). Expression of these defence genes, except *ORA59*, was examined at 0 and 24 h post-inoculation (hpi). Expression of *ORA59* was examined at 0 and 6 hpi, as this gene is induced early after *B. cinerea* infection (Pré *et al.*, 2008). In the wild-type plants, the three *PR* genes were significantly induced by *Psm* ES4326, and *PDF1.2*, *CHIB*, *HEL* and *ORA59* were significantly induced by *B. cinerea* (Fig. 2a–g). In the *elp2* mutant, the induction of these defence genes was significantly reduced (Fig. 2a–g), confirming the previous results (Defraia *et al.*, 2010; Wang *et al.*, 2015). In contrast, compared with the wild-type, *Psm* ES4326-induced expression of *PR* genes was not significantly altered in the *drl1* mutants, whereas *B. cinerea*-induced expression of *PDF1.2*, *HEL* and *ORA59* was significantly reduced in these mutants (Fig. 2a–g). These results indicate that DRL1 is differentially required for *Psm* ES4326- and *B. cinerea*-induced defence gene expression.

We have shown previously that ELP2 suppresses the expression of wound-responsive genes (Wang *et al.*, 2015). During *B. cinerea* infection, expression of the wound-responsive genes *VEGETATIVE STORAGE PROTEIN1* (*VSP1*), *VSP2* and *JASMONATE RESPONSIVE1* (*JR1*) are significantly enhanced in the *elp2* mutant. To test whether DRL1 plays a similar role in the modulation of wound-responsive gene expression, we examined the expression levels of *VSP1* and *VSP2* in the samples used for the analysis of *ORA59* expression. As reported previously (Wang *et al.*, 2015), *VSP1* and *VSP2* were significantly up-regulated in the *elp2* mutant (Fig. 2h, i). *VSP1* was also significantly up-regulated in the *drl1* mutants, but the expression levels of *VSP1* and *VSP2* were significantly lower in the *drl1* mutants than in the *elp2* mutant (Fig. 2h, i). These results suggest that DRL1 plays a less important role than ELP2 in suppressing the expression of wound-responsive genes during *B. cinerea* infection.

Mutations in *DRL1* compromise resistance to the necrotrophic fungal pathogens *B. cinerea* and *A. brassicicola*, but not the hemibiotrophic bacterial pathogen *Psm* ES4326

We have shown previously that mutations in Elongator genes compromise resistance to the hemibiotrophic bacterial pathogen *Psm* ES4326 and the necrotrophic fungal pathogens *B. cinerea* and *A. brassicicola* (Defraia *et al.*, 2010; Wang *et al.*, 2015). To test whether DRL1 plays a similar role in the mediation of disease resistance, we tested the susceptibility of *drl1-C1* and *drl1-C2* to *Psm* ES4326, *B. cinerea* and *A. brassicicola*. The *elp2* mutant was again included in the experiment as a control. As reported previously (Defraia *et al.*, 2010; Wang *et al.*, 2015), the *elp2* mutant was significantly more susceptible than the wild-type to all three pathogens (Fig. 3). In contrast, the *drl1* mutants were as susceptible as the wild-type to the bacterial pathogen *Psm* ES4326 (Fig. 3a), but were more susceptible than the wild-type to the two necrotrophic fungal pathogens (Fig. 3b–e). Although the susceptibility of the *drl1* mutants to *B. cinerea* and *A. brassicicola* was not as strong as that of the *elp2* mutant (Fig. 3b–e), these results demonstrate that DRL1 is also an important player in the mediation of resistance to necrotrophic fungal pathogens.

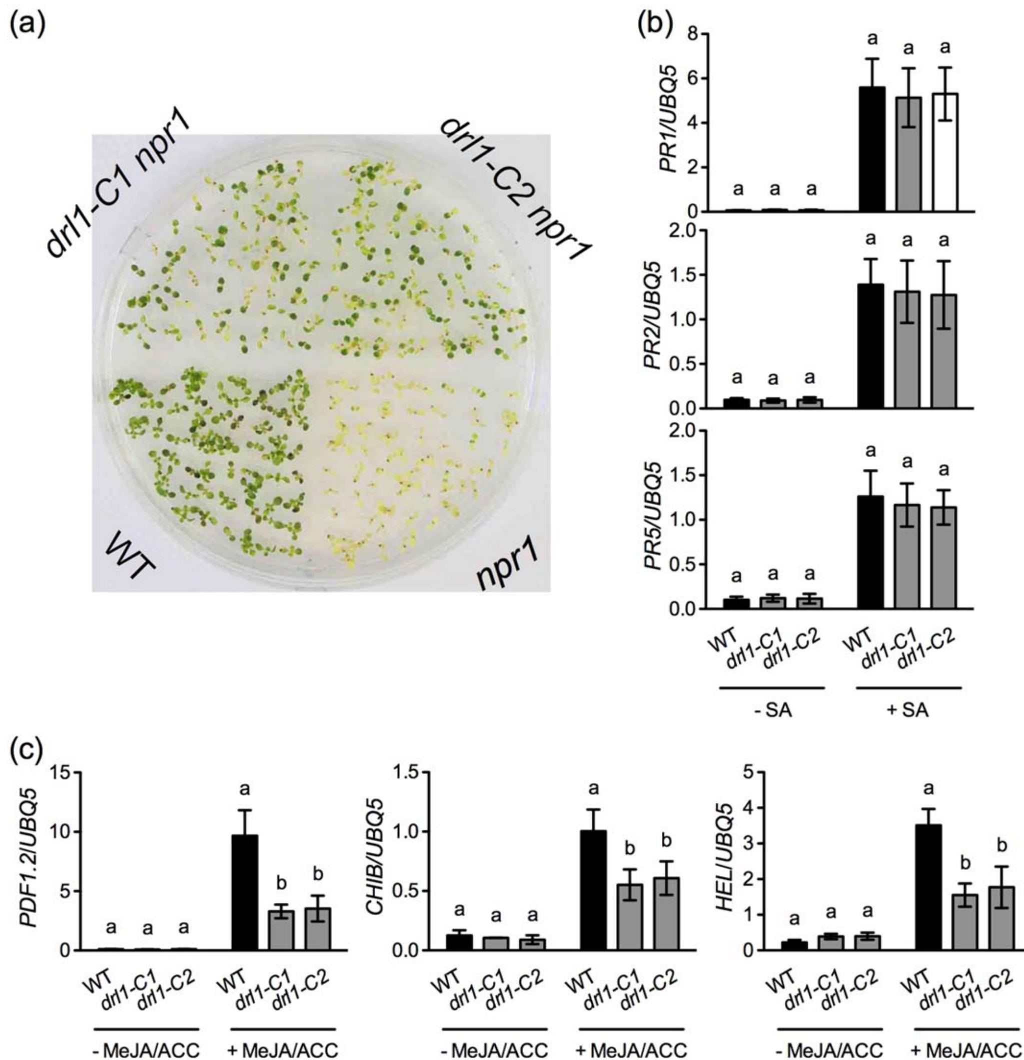

Fig. 1 Tolerance to salicylic acid (SA)-induced cytotoxicity in *drl1 npr1* double mutants, and SA- and jasmonic acid/ethylene (JA/ET)-induced defence gene expression in *drl1* mutants. (a) Seeds were plated on half-strength Murashige and Skoog (MS) agar medium containing 0.3 mM SA. After 3 days of stratification, the plate was transferred to a growth chamber and photographed 10 days later. WT, wild-type. (b, c) Two-week-old seedlings grown on half-strength MS medium were treated with or without 0.5 mM SA (b) and with or without 0.1 mM methyl jasmonate (MeJA) plus 0.1 mM 1-aminocyclopropane-1-carboxylic acid (ACC) (MeJA/ACC) (c). Total RNA was extracted from plant tissues, except roots, collected 24 h later and subjected to quantitative polymerase chain reaction (qPCR) analysis. Expression was normalized against the constitutively expressed *UBQ5*. Data represent the mean of three biological replicates with standard deviation (SD). Different letters above the bars indicate significant differences [$P < 0.05$, one-way analysis of variance (ANOVA)]. Comparison was made separately among the wild-type, *drl1-C1* and *drl1-C2* for each treatment. The experiments were repeated three times with similar trends.

Overexpression of *DRL1* does not affect defence gene expression and disease resistance

To further confirm that the defence phenotypes of the *drl1* mutants are caused by mutations in the *DRL1* gene, we generated transgenic *drl1-C1* plants expressing a wild-type *DRL1* driven by its native promoter or a modified, constitutive 35S promoter (Mindrinos *et al.*, 1994). Multiple transgenic lines were generated and analysed for both constructs (Fig. S3, see Supporting Information), and one representative transgenic line for each construct is shown in Fig. 4. Expression of *DRL1* was restored to wild-type levels in the *DRL1::DRL1 drl1-C1* transgenic plants (Fig. 4a). On *B. cinerea* infection, the expression levels of *DRL1* were not altered in the wild-type and *DRL1::DRL1 drl1-C1* plants (Fig. 4a), indicating that *DRL1* may not be regulated at the transcriptional level. Compared with the wild-type, *B. cinerea*-induced expression of the defence genes *ORA59* and *PDF1.2* was significantly inhibited in *drl1-C1*, but was induced to wild-type levels in *DRL1::DRL1 drl1-C1* plants (Fig. 4b, c). Furthermore, although *drl1-C1* was

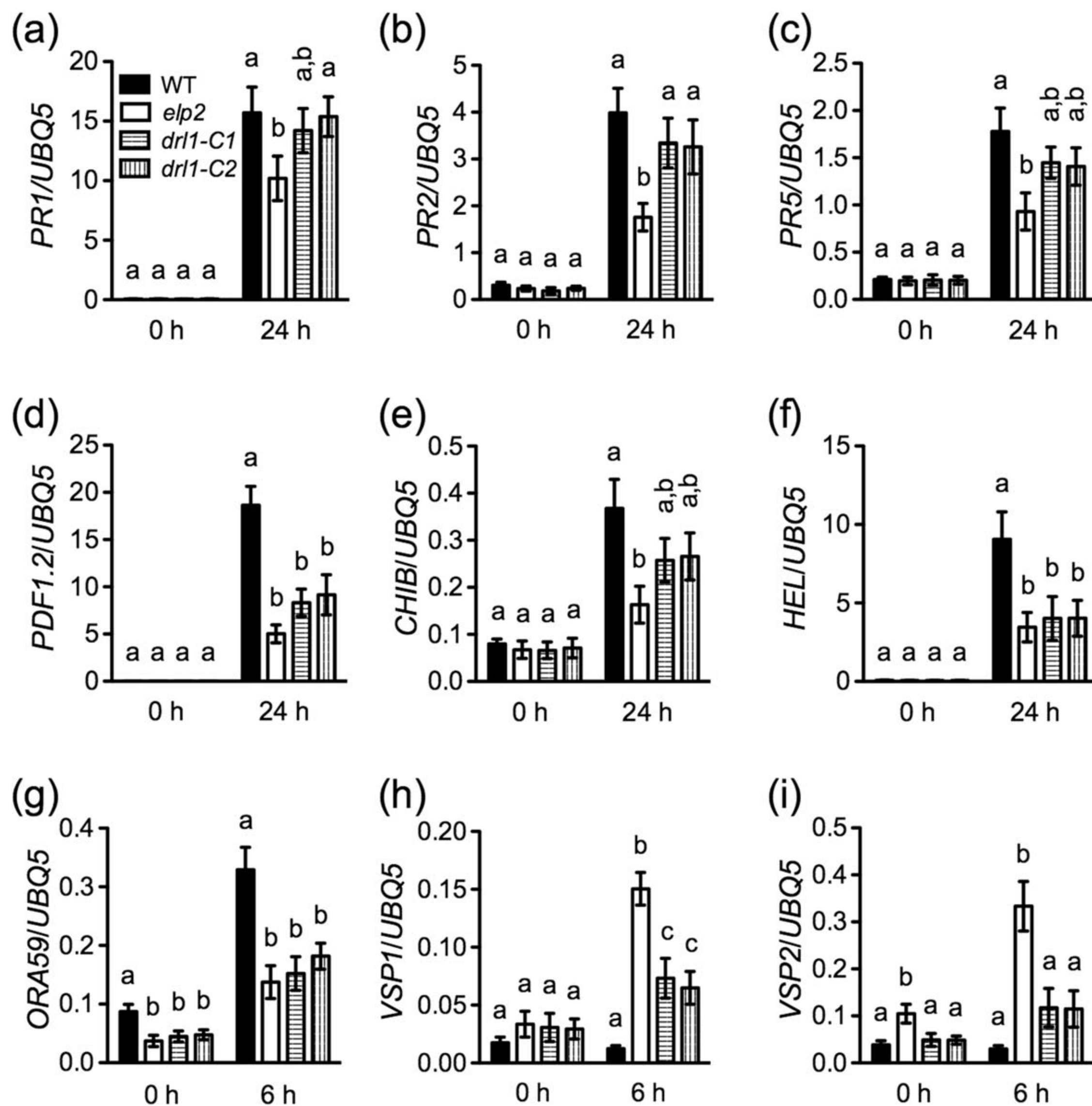

Fig. 2 Pathogen-induced defence gene expression in *drl1* mutants. (a–c) Wild-type (WT), *elp2*, *drl1-C1* and *drl1-C2* plants were inoculated with *Pseudomonas syringae* pv. *maculicola* (*Psm*) ES4326 [optical density at 600 nm (OD_{600}) = 0.001]. Total RNA was extracted from the inoculated leaves collected at the indicated time points and analysed for the expression of *PR1* (a), *PR2* (b) and *PR5* (c) using quantitative polymerase chain reaction (qPCR). (d–i) Wild-type, *elp2*, *drl1-C1* and *drl1-C2* plants were inoculated with *Botrytis cinerea* spores. Total RNA was extracted from the inoculated leaves collected at the indicated time points and analysed for the expression of *PDF1.2* (d), *CHIB* (e), *HEL* (f), *ORA59* (g), *VSP1* (h) and *VSP2* (i) using qPCR. Expression was normalized against the constitutively expressed *UBQ5*. Data represent the mean of three biological replicates with standard deviation (SD). Different letters above the bars indicate significant differences [$P < 0.05$, one-way analysis of variance (ANOVA)]. The comparison was made separately among the genotypes for each time point. All experiments were repeated three times with similar trends.

significantly more susceptible than the wild-type to *B. cinerea*, *DRL1::DRL1 drl1-C1* plants were as susceptible as the wild-type (Fig. 4d, e). These results demonstrate that the *DRL1::DRL1* transgene complements the defence phenotypes of the *drl1-C1* mutant.

In *35S::DRL1 drl1-C1* transgenic plants, expression of *DRL1* was significantly elevated (Figs 4a and S3a). However, *B. cinerea*-induced expression of *ORA59* and *PDF1.2* in *35S::DRL1 drl1-C1* plants was comparable with that in wild-type plants (Figs 4b, c and S3b). In line with the gene expression results, *35S::DRL1 drl1-C1* transgenic plants were as susceptible as the wild-type to *B. cinerea* (Figs 4d, e and S3c). These results indicate that overexpression of the *DRL1* gene does not change defence gene induction and *B. cinerea* resistance.

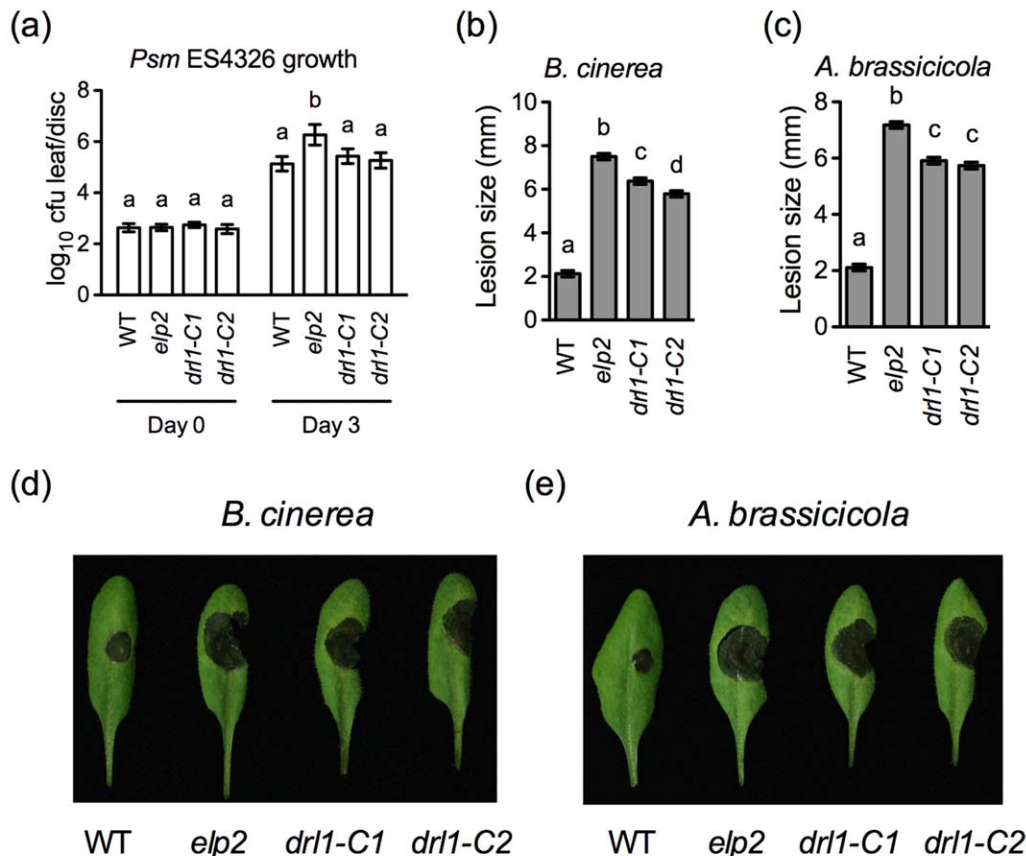

Fig. 3 Disease susceptibility of *drl1* mutants. (a) Leaves of 4-week-old wild-type (WT), *elp2*, *drl1-C1* and *drl1-C2* plants were inoculated with *Pseudomonas syringae* pv. *maculicola* (*Psm*) ES4326 [optical density at 600 nm (OD_{600}) = 0.0001]. The *in planta* bacterial titres were determined immediately and at 3 days post-inoculation. Data represent the mean of eight biological replicates with standard deviation (SD). Different letters above the bars indicate significant differences [$P < 0.05$, one-way analysis of variance (ANOVA)]. The comparison was made separately among the genotypes for each time point. The experiment was repeated three times with similar trends. cfu, colony-forming unit. (b, c) Sizes of the necrotic lesions formed on *Botrytis cinerea*- (b) and *Alternaria brassicicola*-infected (c) wild-type, *elp2*, *drl1-C1* and *drl1-C2* plants. Lesion sizes on 72 leaves measured in three independent experiments were combined and analysed as a one-way ANOVA, blocked by experiment. The resulting mean and standard error (SE) are presented. Different letters above the bars indicate significant differences ($P < 0.05$). (d, e) Symptoms of the necrotic lesions formed on *B. cinerea*- (d) and *A. brassicicola*-infected (e) wild-type, *elp2*, *drl1-C1* and *drl1-C2* plants. Photographs were taken at 4 days post-inoculation.

The *drl1* mutation alters *B. cinerea*-induced transcriptome reprogramming

To define how DRL1 modulates fungal pathogen-induced transcriptional changes at the genome level, we performed a microarray experiment on *B. cinerea*-infected *drl1-C1* and wild-type plants (NCBI GEO Series number GSE79961). Triplicate experiments were performed independently, and the data were analysed to identify genes that showed a two-fold or higher induction or suppression with a low *q* value ($\leq$0.05) in *drl1-C1* and the wild-type. Similar to multiple previous microarray analyses (AbuQamar *et al*., 2006; Rowe *et al*., 2010; Wang *et al*., 2015; Windram *et al*., 2012), dramatic transcriptome changes were detected in the wild-type plants after *B. cinerea* infection. The numbers of genes that were up- and down-regulated by two-fold or more at 6 hpi (2238 and 1887, respectively) were close to those (1812 and 1531, respectively) reported previously (Wang *et al*., 2015), confirming the transcriptional changes induced by *B. cinerea* infection. Interestingly, although profound transcriptional changes also occurred in the *drl1-C1* mutant, the numbers of genes that were up- and down-regulated by two-fold or more (1400 and 998, respectively) were much lower than those (2238 and 1887, respectively) in the wild-type (Fig. 5a). The *drl1-C1* mutation also shifted the transcriptome profiles of the mutant plants. Although most of the genes up- or down-regulated two-fold or more in *drl1-C1* were also found in the wild-type, approximately 10.6% and 11.6% of the genes that were up- and down-regulated in *drl1-C1*, respectively, were not found in the wild-type (Fig. 5a). Taken together, these results indicate that the *drl1* mutation has a significant impact on *B. cinerea* infection-induced transcriptome reprogramming.

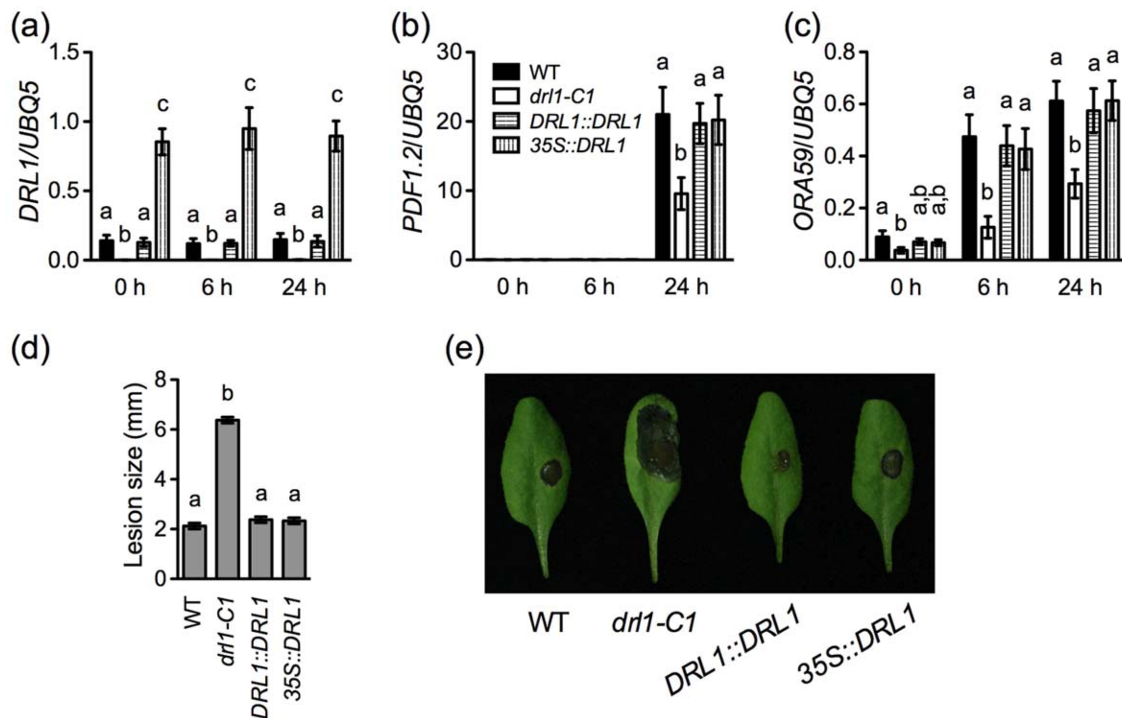

Fig. 4 Transgenic expression of *DRL1* in *drl1* plants. (a–c) Wild-type (WT), *drl1-C1*, *DRL1::DRL1* and *35S::DRL1* plants were inoculated with *Botrytis cinerea* spores. Total RNA was extracted from the inoculated leaves collected at the indicated time points and analysed for the expression of *DRL1* (a), *PDF1.2* (b) and *ORA59* (c) using quantitative polymerase chain reaction (qPCR). Expression was normalized against the constitutively expressed *UBQ5*. Data represent the mean of three biological replicates with standard deviation (SD). Different letters above the bars indicate significant differences [$P < 0.05$, one-way analysis of variance (ANOVA)]. The comparison was made separately among the genotypes for each time point. The experiments were repeated three times with similar trends. (d) Sizes of the necrotic lesions formed on *B. cinerea*-infected wild-type, *drl1-C1*, *DRL1::DRL1* and *35S::DRL1* plants. Lesion sizes on 72 leaves measured in three independent experiments were combined and analysed as a one-way ANOVA, blocked by experiment. The resulting mean and standard error (SE) are presented. Different letters above the bars indicate significant differences ($P < 0.05$). (e) Symptoms of the necrotic lesions formed on *B. cinerea*-infected wild-type, *drl1-C1*, *DRL1::DRL1* and *35S::DRL1* plants. Photographs were taken at 4 days post-inoculation.

To identify genes whose expression is modulated by DRL1, we analysed the genes that were differentially expressed between *drl1-C1* and the wild-type. Genes that showed a two-fold or larger difference in their expression levels with a low *q* value ($\leq$0.05) were chosen for further analysis. A total of 354 (115 up- and 239 down-regulated) and 803 (404 up- and 399 down-regulated) genes were differentially expressed between *drl1-C1* and the wild-type at 0 and 6 hpi, respectively. Gene ontology (GO) analysis of the genes differentially expressed at 6 hpi revealed that genes involved in plant defence, such as response to chitin, defence response and innate immune response, were significantly enriched in the down-regulated genes (Fig. S4a, see Supporting Information), whereas genes involved in metabolic reactions, such as sulfur compound biosynthesis, photosynthesis and amino acid metabolism, were significantly enriched in the up-regulated genes (Fig. S4b). A total of 200 defence response genes, which were differentially expressed between *drl1-C1* and the wild-type at 6 hpi, are displayed as a heat map (Fig. 5b and Table S3, see Supporting Information). These genes include 20 ET-responsive genes, 23 JA/ET-responsive genes, 50 JA-responsive genes and 107 other defence genes. All ET- and JA/ET-responsive genes and the majority of JA-responsive and other defence genes were down-regulated in the *drl1-C1* mutant. Among the down-regulated genes are a group of well-characterized defence genes, including *ORA59*, *ETHYLENE RESPONSE FACTOR1* (*ERF1*), *WRKY DNA BINDING PROTEIN 33* (*WRKY33*), *SIGMA FACTOR BINDING PROTEIN 1* (*SIB1*), *PHYTOALEXIN DEFICIENT 3* (*PAD3*), *GDSL LIPASE-LIKE 1* (*GLIP1*) and *CYP79B2* (Table 1), which have been demonstrated to function in resistance to necrotrophic fungal pathogens (Ferrari *et al*., 2007; Kliebenstein *et al*., 2005; Lai *et al*., 2011; Lorenzo *et al*., 2003; Oh *et al*., 2005; Pré *et al*., 2008; Zheng *et al*., 2006). Therefore, DLR1 may contribute to *B. cinerea* resistance by modulating the expression of these defence genes.

Both the N-terminal P-loop and the C-terminal CBD of DRL1 are required for its function in plant immunity

DRL1 contains an N-terminal P-loop and a C-terminal CBD (Nelissen *et al*., 2003). The P-loop is required for the function of TOT4/

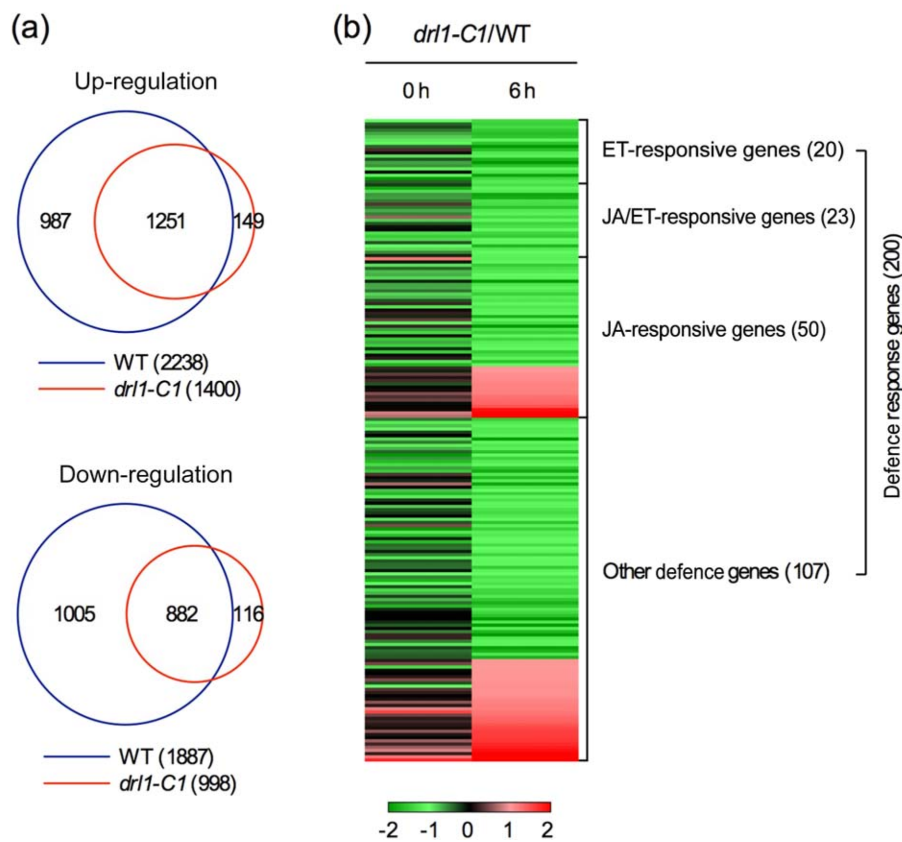

Fig. 5 *Botrytis cinerea*-induced transcriptome changes in *drl1* plants. (a) Overlaps among the genes that were up- or down-regulated at 6 h post-inoculation (hpi) in *drl1-C1* and the wild-type (WT). (b) Heatmap analysis of the 200 defence genes that were differentially expressed between *drl1-C1* and the wild-type at 6 hpi. A clear down-regulation (green) of the majority of the 200 genes was observed.

Table 1 Partial list of the defence genes that were differentially expressed between *drl1-C1* and the wild-type during *Botrytis cinerea* infection.

		drl1-C1/WT				
		0 h		6 h		
AGI locus	Gene name	$\log_2$(FC)	*q* value	$\log_2$(FC)	*q* value	AGI description
Defence-response genes						
At1g06160	*ORA59*	−1.818	0	−1.091	0	OCTADECANOID-RESPONSIVE ARABIDOPSIS AP2/ERF 59
At3g23240	*ERF1*			−1.249	0	ETHYLENE-RESPONSIVE TRANSCRIPTION FACTOR 1
At2g38470	*WRKY33*	−1.081	0.000012			WRKY DNA-BINDING PROTEIN 33
At3g56710	*SIB1*			−1.056	0.000862	SIGMA FACTOR BINDING PROTEIN 1
At3g26830	*PAD3*	−1.252	0.000001	−1.375	0.000005	BIFUNCTIONAL DIHYDROCAMALEXATE SYNTHASE/CAMALEXIN SYNTHASE
At5g40990	*GLIP*			−1.222	0	GDSL LIPASE 1
At4g39950	*CYP79B2*			−1.735	0	CYTOCHROME P450, FAMILY 79, SUBFAMILY B, POLYPEPTIDE 2
At2g26020	*PDF1.2b*			−1.514	0	PLANT DEFENSIN 1.2b
At5g44430	*PDF1.2c*			−1.692	0	PLANT DEFENSIN 1.2c
At3g04720	*HEL*	−1.052	0.00026	−1.53	0.00003	HEVEIN-LIKE
Wound response genes						
At3g16470	*JR1*			1.198	0.000043	JA-RESPONSIVE PROTEIN 1
At5g24780	*VSP1*			1.082	0.00033	VEGETATIVE STORAGE PROTEIN 1
At5g24770	*VSP2*			3.368	0	VEGETATIVE STORAGE PROTEIN 2

KTI12 in yeast (Fichtner *et al.*, 2002). To test whether the P-loop and the CBD of DRL1 are required for the function of DRL1 in plant immunity, we introduced point mutations into the *DRL1* cDNA to generate a P-loop mutant, in which a conserved lysine residue (K14) was changed to arginine, and a CBD mutant, in which two conserved amino acid residues, a phenylalanine and a leucine (F264 and L267), were changed to aspartate and glutamate, respectively (Fig. 6a). In addition, a valine residue (V265)

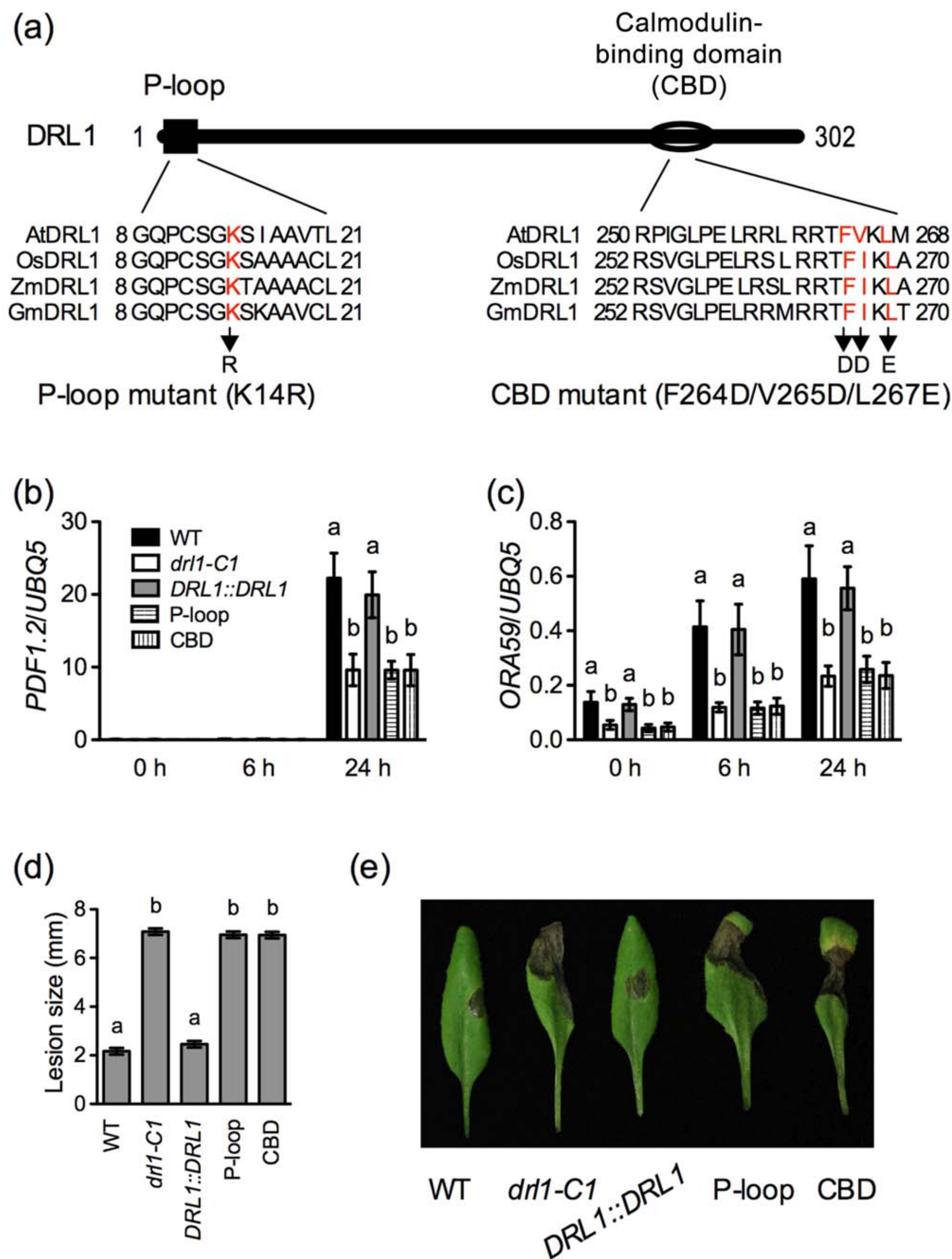

Fig. 6 Characterization of the P-loop and C-terminal calmodulin-binding domain (CBD) mutants of DRL1. (a) Schematic representation of the P-loop and CBD mutations. The P-loop and the C-terminal CBD of Arabidopsis DRL1 (AtDRL1) are aligned with those of rice (OsDRL1), maize (ZmDRL1) and soybean (GmDRL1) DRL1. Only sequences that are part of the alignment are shown. The mutated amino acid residues are labelled in red. Arrows indicate the mutations created in the P-loop and CBD mutants. (b, c) Wild-type (WT), *drl1-C1*, *DRL1::DRL1*, P-loop mutant and CBD mutant plants were inoculated with *Botrytis cinerea* spores. Total RNA was extracted from the inoculated leaves collected at the indicated time points and analysed for the expression of *PDF1.2* (b) and *ORA59* (c) using quantitative polymerase chain reaction (qPCR). Expression was normalized against the constitutively expressed *UBQ5*. Data represent the mean of three biological replicates with standard deviation (SD). Different letters above the bars indicate significant differences [$P < 0.05$, one-way analysis of variance (ANOVA)]. The comparison was made separately among the genotypes for each time point. The experiments were repeated three times with similar trends. (d) Sizes of the necrotic lesions formed on *B. cinerea*-infected wild-type, *drl1-C1*, *DRL1::DRL1*, P-loop mutant and CBD mutant plants. Lesion sizes on 72 leaves measured in three independent experiments were combined and analysed as a one-way ANOVA, blocked by experiment. The resulting mean and standard error (SE) are presented. Different letters above the bars indicate significant differences ($P < 0.05$). (e) Symptoms of the necrotic lesions formed on *B cinerea*-infected wild-type, *drl1-C1*, *DRL1::DRL1*, P-loop mutant and CBD mutant plants. Photographs were taken at 4 days post-inoculation.

was also changed to aspartate to further modify the net charge of the domain (Fig. 6a). Mutated *drl1* cDNAs driven by the *DRL1* native promoter were transformed into *drl1-C1* to test the functionality of the P-loop and the CBD mutant. Multiple transgenic lines were produced and characterized for both constructs (Fig. S5, see Supporting Information) and one representative transgenic line for each construct is shown in Fig. 6. Neither the P-loop nor the CBD mutant complemented the defence phenotypes of *drl1-C1*, including decreased induction of the defence genes *PDF1.2* and *ORA59* by *B. cinerea* infection and enhanced susceptibility to *B. cinerea* (Fig. 6b–e). These results clearly indicate that both the P-loop and the CBD of DRL1 are important for its function in the plant immune response.

DISCUSSION

In this study, we analysed the function of DRL1 in plant immune responses. Our results show that: (i) DRL1 makes a partial contribution to SA-induced cytotoxicity, but does not play a significant role in SA-mediated *PR* gene expression and *Psm* ES4326 resistance; (ii) DRL1 is required for JA/ET-induced expression of *PDF1.2*, *CHIB* and *HEL*, *B. cinerea*-induced expression of a large group of defence genes, and resistance to *B. cinerea* and *A. brassicicola*; (iii) overexpression of the *DRL1* gene does not alter the basal expression and induction of *PDF1.2* and *ORA59* and resistance to *B. cinerea*; and (iv) both the N-terminal P-loop and the C-terminal CBD of DRL1 are critical for the basal expression and induction of *PDF1.2* and *ORA59* and for resistance to *B. cinerea*.

It has been shown that yeast cells lacking TOT4/KTI12 or an Elongator subunit display indistinguishable pehnotypes, including resistance to the *K. lactis* toxin zymocin and hypersensitivity to high temperature (above 38 °C) and drugs, such as caffeine and calcofluor white (Fichtner *et al.*, 2002; Frohloff *et al.*, 2001). Similarly, Arabidopsis *drl1* and *elo*/*elp* mutants exhibit almost identical morphological phenotypes, such as narrow leaves, disorganized shoot apical meristem, and short roots, and similar transcriptome changes (Nelissen *et al.*, 2003, 2005; Xu *et al.*, 2012). These results suggest significant functional overlap between TOT4/KTI12 or DRL1 and the Elongator complex. However, our results indicate that DRL1 and Elongator do not play largely overlapping roles in plant immune responses. Whilst Elongator is required for both SA- and JA/ET-mediated defence responses, DRL1 only contributes to JA/ET-mediated defence signalling (Figs 1–3), indicating that Elongator has a broader role than DRL1 in plant immune responses. Furthermore, although both *drl1* and *elo*/*elp* mutants are suspectible to *B. cinerea* and *A. brassicicola*, *drl1* mutants are significantly less susceptible than the *elp2* mutant (Fig. 3b, c), suggesting that DRL1 plays a less important function than Elongator, even in JA/ET-mediated resistance to necrotrophic fungal pathogens.

DRL1 is required for JA/ET-induced expression of the defence marker genes *PDF1.2*, *CHIB* and *HEL* (Fig. 1c), indicating that it positively contributes to JA/ET-mediated defence responses. Indeed, mutations in *DRL1* inhibit *B. cinerea*-induced expression of many JA/ET-responsive defence genes, including *ORA59* and *ERF1* (Fig. 5b and Table 1), which encode two well-documented AP2/ERF domain transcription factors that have been shown to directly regulate *PDF1.2* expression and *B. cinerea* resistance (Lorenzo *et al.*, 2003; Pré *et al.*, 2008). DRL1 is also required for transcription factor WRKY33-mediated defence signalling (Zheng *et al.*, 2006). In *drl1*, basal expression of the *WRKY33* gene and *B. cinerea*-induced expression of several WRKY33 target genes, including *PAD3* and *GLIP1*, are reduced (Table 1). Both *PAD3* and *GLIP1* have been demonstrated to play positive roles in resistance to *B. cinerea* (Ferrari *et al.*, 2007; Oh *et al.*, 2005). In addition, the induction of the *SIGMA FACTOR BINDING PROTEIN1* (*SIB1*) gene, which encodes a WRKY33-interacting protein required for *B. cinerea* resistance (Lai *et al.*, 2011), is also decreased (Table 1). Therefore, the reduced defence gene expression and enhanced susceptibility to *B. cinerea* in *drl1* are probably mainly attributed to the attenuated JA/ET- and WRKY33-mediated defence signalling.

In yeast, loss of TOT4/KTI12 function, elevation of *TOT4*/*KTI12* copy number and overexpression of *TOT4*/*KTI12* all lead to resistance to the *K. lactis* toxin zymocin (Fichtner *et al.*, 2002; Frohloff *et al.*, 2001), implying that the cellular level of TOT4/KTI12 may play an important role in the regulation of zymocin sensitivity. Interestingly, although ectopic expression of the *DRL1* gene rescues the growth retardation phenotype of yeast cells lacking TOT4/KTI12, overexpression of *DRL1* in wild-type yeast does not elicit zymocin resistance (Jun *et al.*, 2015). Similarly, overexpression of *DRL1* in Arabidopsis does not confer any detectable morphological and defence phenotypes (Figs S6, see Supporting Information, and 4). These results suggest that cellular DRL1 protein levels may not be vigorously regulated and that the basal level of DRL1 may be sufficient for its function in plant development and immunity. In support of these hypotheses, expression of the *DRL1* gene is not altered by *B. cinerea* infection (Fig. 4a), implying that *DRL1* may not be regulated at the transcriptional level.

The DRL1 protein contains two (P-loop and N-K/R-X-D box) of the five highly conserved sequence motifs that are required for GTP binding and hydrolysis in GTP-binding proteins (Kaziro *et al.*, 1991; Nelissen *et al.*, 2003). The missing motifs are critical for GTP hydrolysis (Kaziro *et al.*, 1991), suggesting that DRL1 might not be a bona fide GTP-binding protein. In addition, previous work has shown that deletion of the P-loop renders TOT4/KTI12 inactive (Fichtner *et al.*, 2002), and we found that substitution of an arginine for the conserved lysine residue (K14R) in the P-loop destroys the function of DRL1 (Fig. 6). These results clearly demonstrate that the P-loop is important for the function of TOT4/KTI12 and DRL1. As DRL1 contains another conserved motif (P-X-X-A/S-T), which

exists in many ATP/GTP-utilizing proteins, and the P-loop is also found in many nucleotide triphosphate-utilizing enzymes, it has been proposed that DRL1 may exert its function on ATP/GTP binding or transfer ATP/GTP to another protein (Nelissen *et al.*, 2003). However, the precise relationship between DRL1 and ATP/GTP requires further investigation.

It has been demonstrated that DRL1 is a CaM-binding protein. Nelissen *et al.* (2003) reported that the C-terminal 100 amino acids of DRL1 bind CaM in a calcium-dependent manner, and identified a motif L-X(3)-F-X(2)-L-X(5)-L (amino acids 260–273) within this region as the CBD. Very recently, uisng a bioinformatic approach, Jun *et al.* (2015) have identified another CBD in the N-terminus of the protein, but whether it binds CaM has not been tested. CaM-binding proteins have been implicated in plant immune responses (Poovaiah *et al.*, 2013). For instance, the CaM-binding transcription factor SIGNAL RESPONSIVE1 (SR1)/CALMODULIN BINDING TRANSCRIPTION ACTIVATOR3 (CAMAT3) interacts with the CGCG box motifs in the promoters of *ENHANCED DISEASE SUSCEPTIBILITY1* (*EDS1*) and *NON-RACE-SPECIFIC DISEASE RESISTANCE1* (*NDR1*), which encode two key componenets of the SA- and resistance gene-mediated defence pathways, to negatively regulate *EDS1* and *NDR1* expression (Du *et al.*, 2009; Nie *et al.*, 2012). Another CaM-binding transcription factor CALMODULIN-BINDING PROTEIN 60-LIKE.g (CBP60g) is recruited to the promoter of the SA biosynthesis gene *ISOCHORISMATE SYNTHASE1* (*ICS1*)/*SALICYLIC ACID INDUCTION DEFICIENT2* (*SID2*) to activate its transcription (Wang *et al.*, 2009). Here, we demonstrate that DRL1 is another CaM-binding protein functioning in plant immune responses. Substituions of three amino acids in the C-terminal CBD of DRL1 made the protein unable to complement the *drl1* defence phenotype (Fig. 6), suggesting that CaM binding at this motif may be important for the function of DRL1 in plant immunity. Whether the putative N-terminal CBD is also required for the function of DRL1 remains to be tested (Jun *et al.*, 2015). Neverthelesss, as it is well known that CaM binding is a highly conserved mechanism modulating target protein functions in eukaryotic cells (Poovaiah *et al.*, 2013), it will be interesting to determine how CaM binding affects the function of DRL1.

In yeast, deletion of *TOT4/KTI12* does not affect the structural integrity of the Elongator complex (Fichtner *et al.*, 2002), indicating that TOT4/KTI12 is not a stable structural component of the complex. However, TOT4/KTI12 and DRL1 are physically associated with the Elongator complex in yeast and Arabidopsis, respectively (Petrakis *et al.*, 2005; Xu *et al.*, 2012), suggesting that they may function through Elongator. Indeed, deletion of *TOT4/KTI12* leads to a decrease in histone acetylation levels in chromatin, a molecular phenotype also seen in yeast cells lacking ELP3 (Petrakis *et al.*, 2005). As TOT4/KTI12 does not have histone acetyltransferase (HAT) activity, it is probably required for the normal HAT activity of the Elongator complex. Whether TOT4/KTI12 or DRL1 is also required for Elongator's other molecular functions, such as tRNA modification and DNA demethylation/methylation, is not clear (Esberg *et al.*, 2006; Jia *et al.*, 2015; Okada *et al.*, 2010; Wang *et al.*, 2013). Nevertheless, so far, the available evidence suggests that TOT4/KTI12 and DRL1 are modifiers of Elongator in yeast and Arabidopsis, respectively (Nelissen *et al.*, 2003; Petrakis *et al.*, 2005). The results presented here indicate that DRL1 is involved in most, but not all, of Elongator's functions. As the N-terminal P-loop and the C-terminal CBD are required for the function of DRL1 in plant immunity, how ATP/GTP and/or CaM binding regulates the activities of DRL1 and Elongator deserves further investigation.

EXPERIMENTAL PROCEDURES

Plant materials and growth conditions

The wild-type used was the *Arabidopsis thaliana* (L.) Heynh. Columbia (Col-0) ecotype and the mutant alleles used were *Atelp2-5* (Defraia *et al.*, 2010), *drl1-C1* (SALK_056915) and *drl1-C2* (SALK_140551). The T-DNA insertion lines were obtained from the Arabidopsis Biological Resource Center at Ohio State University. Homozygous mutant plants of the T-DNA insertion lines were confirmed with a pair of primers flanking the T-DNA insertions (Table S1, see Supporting Information) and the left border primer LBa1 (Alonso *et al.*, 2003). Arabidopsis seeds were sown on autoclaved soil (Metro-Mix200, Grace-Sierra, http://www.sungro.com) and vernalized at 4 °C for 3 days. Plants were germinated and grown at approximately 23 °C under a 16-h light/8-h dark regime.

Pathogen infection

Inoculation of plants with *Psm* ES4326 was performed by pressure infiltration with a 1-mL needleless syringe, as described previously (Zhang *et al.*, 2012). After inoculation, eight infected leaves, one from each plant, were collected for each genotype or time point to examine the growth of the pathogen. The *B. cinerea* strain B05 and *A. brassicicola* strain MUCL 20297 were used in this study. *Botrytis cinerea* and *A. brassicicola* inoculation were performed as described previously (Wang *et al.*, 2013). Briefly, the fungal pathogens were grown on BD Difco Potato Dextrose Agar (Becton, Dickinson and Company, Sparks, MD, USA) for about 10 days at 24 °C. Spores were harvested, resuspended in BD Difco Potato Dextrose Broth (Becton, Dickinson and Company) at a density of (1–5) $\times 10^5$ spores/mL, and incubated for 2 h prior to inoculation. Five-microliter spore suspensions were dropped on the adaxial surface of rosette leaves (one drop on each leaf), where the leaves had been gently wounded with a needle. Symptoms were monitored for 3–4 days (4–5 days for *A. brassicicola*). The lengths and widths of the lesions were measured with a caliper before disease symptoms expanded beyond inoculated leaves and the average of the length and the width was used to represent the size of a lesion. In each experiment, 24 plants per genotype were used for three sub-experiments. Each sub-experiment was performed in the same flat under the same clear plastic dome. In each flat, plants from different genotypes (eight plants per genotype) were randomly arranged.

One leaf on each plant was inoculated and all leaves were inoculated with the same spore suspension. A total of 24 lesions (one on each plant) were measured and used for statistical analysis.

RNA analysis

RNA extraction, reverse transcription and real-time qPCR analysis were carried out as described by Defraia *et al.* (2010). In each experiment, three independent biological samples were collected at each time point per genotype and analysed. *UBQ5* was used as the reference gene for qPCR normalization, because it is one of the most stably expressed genes (Gutierrez *et al.*, 2008). The primers used for qPCR in this study have either been reported previously or are shown in Table S2 (see Supporting Information).

Microarray analysis

Four-week-old soil-grown plants were inoculated with the *B. cinerea* strain B05. Total RNA samples extracted from leaf tissues collected at the indicated time points after *B. cinerea* inoculation were subjected to microarray analysis as described previously (Wang *et al.*, 2013).

The mean signals obtained from Agilent Feature Extraction software were background corrected using the normexp + offset method, in which a small positive offset ($k = 50$) was added to move the corrected intensities away from zero (Ritchie *et al.*, 2007). The resulting data were log transformed (using 2 as the base) and normalized between individual samples by scaling the individual log-transformed signal intensities so that all datasets had comparable lower quartile, median and upper quartile values (Smyth, 2004). After normalization, the empirical Bayes moderated *t*-statistics, which are implemented in the limma Bioconductor package (Smyth, 2004), were performed for differential expression detection. In each comparison, a *P* value and fold change were computed for each gene locus. The gene expression fold changes were computed based on the normalized log-transformed signal intensity data. To control the false discovery rate and correct multiple hypothesis testing, a *q* value was calculated and used to assess the significance of each test employing the approach of Benjamini and Hochberg (1995). The comparison results were further explored to obtain numbers of overlapping genes between different comparisons.

Plasmid construction and plant transformation

A pair of primers (*SmaI-DRL1F*, 5′-TCCCCCGGGCTCTTGAACATCTTCAGCGTC-3′; *SacI-StrepII-DRL1R*, 5′-CGAGCTCATTTTTCAAATTGAGGATGAGACCATGCACTAGCGTTATTACCTCCAAACTC-3′) was used to amplify the promoter and coding regions of *DRL1* from genomic DNA. The PCR products were digested with *Sma*I and *Sac*I, and then ligated into the corresponding sites of the vector pBI101, resulting in the plasmid pBI101-DRL1::DRL1. Site-directed mutagenesis of the conserved amino acid residues in the P-loop and the CBD of DRL1 was performed in the pBI101-DRL1::DRL1 construct using a PCR-based QuickChange site-directed mutagenesis kit (Stratagene, LaJolla, CA, USA). The primers used for the site-directed mutagenesis of the P-loop were *DRL1(K14R)F* (5′-CCTTGTAGTGGTAGATCAATAGCTGCAG-3′) and *DRL1(K14R)R* (5′-CTGCAGCTATTGATCTACCACTACAAGG-3′), and, for the CBD mutagenesis, the primers were *DRL1(FVKL/DDKE)F* (5′-GAGGCTTCGAAGAACGGATGATAAAGAGATGGGTCAATCGAG-3′) and *DRL1(FVKL/DDKE)R* (5′-CTCGATTGACCCATCTCTTTATCATCCGTTCTTCGAAGCCTC-3′). The presence of the expected mutations was confirmed by DNA sequencing. For the overexpression of *DRL1*, a pair of primers (*SalI-DRL1F*, 5′-GCGTCGACATGGCGCTAGTTGTGATTTGTG-3′; *SacI-StrepII-DRL1R*) was used to amplify the coding region of *DRL1* from genomic DNA. The PCR products were digested with *Sal*I and *Sac*I, and then ligated into the corresponding sites of the vector pBI1.4T, resulting in the plasmid pBI1.4T-35S::DRL1. The plasmids were introduced into the *Agrobacterium tumefaciens* strain GV3101 (pMP90) by electroporation and transformed into the *drl1-C1* mutant following the floral dip method (Clough and Bent, 1998).

Chemical treatment

Two-week-old seedlings grown on half-strength MS medium were treated with 0.5 mM SA or a combination of 0.1 mM MeJA and 0.1 mM ACC. Seedlings for negative controls were treated with water. The aerial parts of the seedlings were collected and subjected to total RNA extraction.

Statistical methods

Except for those used in microarray analysis, statistical analyses were performed using the one-way analysis of variance (ANOVA) in Prism 5.0b (GraphPad Software, La Jolla, CA, USA). Lesion sizes measured in three independent experiments were combined and analysed as a one-way ANOVA, blocked by experiment, using JMP 11 (JMP Software, Cary, NC, USA). Other experiments were conducted three times with similar trends, and results from a representative experiment are presented.

Accession numbers

Sequence data from this article can be found in the Arabidopsis Genome Initiative or GenBank/EMBL databases under the following accession numbers: DRL1 (At1g13870); PR1 (At2g14610); PR2 (At3g57260); PR5 (At1g75040); PDF1.2 (At5g44420); CHIB (At3g12500); HEL (At3g04720); WRKY33 (At2g38470); ORA59 (At1g06160); VSP1 (At5g24780); VSP2 (At5g24770); UBQ5 (At3g62250); and NCBI Gene Expression Omnibus Series number GSE79961 (microarray data).

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website.

Fig. S1 Characterization of *drl1* T-DNA insertion mutants.

Fig. S2 Growth of *Pseudomonas syringae* pv. *maculicola* (*Psm*) ES4326 in *drl1 npr1* double mutants.

Fig. S3 Characterization of 35S::DRL1 drl1 transgenic plants.

Fig. S4 Gene ontology (GO) analysis of the genes differentially expressed between *drl1* and the wild-type.

Fig. S5 Characterization of multiple independent transgenic lines expressing the P-loop and C-terminal calmodulin-binding domain (CBD) mutants of DRL1.

Fig. S6 Morphology of the *DRL1::DRL1 drl1* and *35S::DRL1 drl1* transgenic plants.

Table S1 Primers used for the identification of homozygous T-DNA insertion mutant plants.

Table S2 Primers used for quantitative polymerase chain reaction (qPCR) analysis of gene expression.

Table S3 Defence genes that were differentially expressed between *drl1-C1* and the wild-type during *Botrytis cinerea* infection.