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A mutation in the *expansin-like A2* gene enhances resistance to necrotrophic fungi and hypersensitivity to abiotic stress in *Arabidopsis thaliana*

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SUMMARY

Expansins are cell wall loosening agents, known for their endogenous function in cell wall extensibility. The *Arabidopsis* e*xpansin-like A2* (*EXLA2*) gene was identified by its down-regulation in response to infection by the necrotrophic pathogen *Botrytis cinerea*, and by the reduced susceptibility of an *exla2* mutant to the same pathogen. The *exla2* mutant was equally susceptible to *Pseudomonas syringae* pv. *tomato*, but was more resistant to the necrotrophic fungus *Alternaria brassicicola*, when compared with the wild-type or with transgenic, ectopic *EXLA2*-overexpressing lines. The *exla2* mutants also enhanced tolerance to the phytoprostane-A_1. This suggests that the absence or down-regulation of *EXLA2* leads to increased resistance to *B. cinerea* in a CORONATINE INSENSITIVE 1 (COI1)-dependent manner, and this down-regulation can be achieved by phytoprostane-A_1 treatment. *EXLA2* is induced significantly by salinity and cold, and by the exogenous application of abscisic acid. The *exla2* mutant also showed hypersensitivity towards increased salt and cold, and this hypersensitivity required a functional abscisic acid pathway. The differential temporal expression of *EXLA2* and the phenotypes in transgenic plants with altered expression of *EXLA2* indicate that plant cell wall structure is an important player during *Arabidopsis* developmental stages. Our results indicate that *EXLA2* appears to be important in response to various biotic and abiotic stresses, particularly in the pathogenesis of necrotrophic pathogens and in the tolerance to abiotic stress.

INTRODUCTION

The plant cell wall (CW) forms a barrier against pathogen attack and interconnects cells. It plays a wide array of distinct, sometimes opposite, roles. For example, the CW determines cell structure, provides resistance to mechanical stress and protects against pathogens. Yet, it must be permeable to signal molecule trafficking (Levy *et al*., 2002). Expansins are plant CW-remodelling proteins that are mainly involved in the pH-dependent extension of plant CWs, so-called acid growth (McQueen-Mason *et al*., 1992). This is determined by the activity of an H^+-ATPase localized in the plasma membrane to pump protons into the CW, making the pH 4.5–5.5, and causing the CW to relax (Cosgrove, 2000). Expansins are also involved in cell enlargement and CW modifications induced by plant hormones, including abscisic acid (ABA) (Zhao *et al*., 2012), auxin (McQueen-Mason *et al*., 1992), brassinosteroids (BLs; Sun *et al*., 2005), gibberellins (GAs; Cho and Kende, 1997), cytokinins (CKs; Downes and Crowell, 1998) and the classical defence hormones salicylate (SA), jasmonate (JA) and ethylene (ET; Cho and Cosgrove, 2002). Expansins are multigene families found in all phyla of the kingdom Plantae (Lee *et al*., 2001). Expansin genes are classified into four families: α- and β-*expansins* (*EXPA* and *EXPB*, respectively), *expansin-like* (*EXLA*) and *expansin-like related* (*EXLB*). In the *Arabidopsis* genome, 26 α- and six β-expansin genes were identified (Cosgrove, 2000; Kim *et al*., 2009). Three *expansin-like* genes were found in *Arabidopsis*: *EXLA1*, *EXLA2* and *EXLA3* (Cosgrove, 2000; Kende *et al*., 2004). The mode of action of expansins, causing loosening and extension of the CW, is still not clear. It has been hypothesized that they might disrupt noncovalent bonding between cellulose microfibrils and matrix glucans on the CW; others have proposed that they might be involved in the disturbance of hydrogen bonding between wall polysaccharides (Cosgrove, 2000). No hydrolytic or enzymatic activity has been found on the plant CW.

Recently, ABA has been reported as a regulator of plant defence against necrotrophic pathogens (Adie *et al*., 2007; Audenaert *et al*., 2002; Laluk *et al*., 2011). Despite the high susceptibility to the oomycete *Pythium irregulare* and the fungus *A. brassicicola*, ABA-impaired mutants show increased resistance to *Botrytis cinerea* (Adie *et al*., 2007). The transcriptome data also confirm that ABA affects JA biosynthesis and JA-dependent genes in response to *P. irregulare*. JA is a hormone which regulates plant maturation and pollen development, and is involved in the response to a variety of stresses. JA is produced from the polyunsaturated fatty acid α-linolenic acid after a series of enzymatic conversions leading to 12-oxo-phytodienoic acid (OPDA) (Mueller, 1997). OPDA undergoes three cycles of β-oxidation by OPDA reductase3 (OPR3) yielding JA. On stress, OPDA and JA regulate

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downstream gene expression via the CORONATINE INSENSITIVE 1 (COI1)-dependent pathway. The cyclopentenone OPDA also acts independently of the JA/COI1 pathway to alter gene expression (Ribot *et al*., 2008). Although the JA-insensitive mutant *coi1* is highly susceptible to necrotrophic pathogens, the *opr3* mutant is highly resistant (AbuQamar *et al*., 2006; Chehab *et al*., 2011; Stintzi *et al*., 2001). The cyclopentenone phytoprostane (PP) is also derived from α-linolenic acid, but formed via the nonenzymatic, free radical-catalysed pathway (Mueller, 1997). OPDA and PP are structurally similar to JA, but contain a reactive unsaturated carbonyl structure in the cyclo-ring. The phytoprostane-A_1 (PPA_1) induces glutathione-*S*-transferase (GST), increases phytoalexin biosynthesis and triggers the expression of the genes involved in primary and secondary metabolism (Thoma *et al*., 2003). *Arabidopsis* seedlings treated with PPA_1 result in root growth inhibition that is mediated through COI1, but independent of JA (Mueller *et al*., 2008; Stotz *et al*., 2013). However, the regulation of PPA_1-responsive genes is dependent on TGA transcription factors.

Microarray-based and functional analyses demonstrate an involvement of expansins in the early stages of symbiosis of the arbuscular mycorrhizal fungus *Glomus intraradices* on tomato (*Solanum lycopersicum*; Dermatsev *et al*., 2010). In addition, the legume sweetclover (*Melilotus alba*) expansin gene, *MaEXP1*, is up-regulated during the development of nitrogen-fixing nodules (Giordano and Hirsch, 2004). Expansins are also induced under drought. The temperature-tolerant grass and *Agrostis scabra* induce the *expansin-like* gene, *AsEXPL1*, after exposure to heat (Xu *et al*., 2007). Similarly, the resurrection plant, *Craterostigma plantagineum*, expresses α-expansin in leaves during dehydration (Jones and McQueen-Mason, 2004). In *Arabidopsis*, the accumulation of ABA and the expression of *EXPA3*, *EXPA4*, *EXPA8*, EXPA10 and *EXLB1* genes correlate with drought acclimation (Harb *et al*., 2010). ABA and indole acetic acid (IAA) induce expansin activity to enhance coleoptile growth in wheat on drought stress (Zhao *et al*., 2012). In response to cold stress, all three *AtEXLA* genes are up-regulated (Lee *et al*., 2005), which confirms that expansins are potentially involved in the programming of plant development under stress.

The role of *Arabidopsis expansin-like* genes in defence has not been studied in any detail. We show here that EXLA2 is involved in defence against necrotrophic fungi and in tolerance to abiotic stresses. The induction of *EXLA2* is dependent on ABA responses, but its expression is suppressed by exogenous PPA_1 application (Mueller *et al*., 2008). Mutations in *EXLA2* show increased resistance to *B. cinerea* and *Alternaria brassicicola*, elevated levels of tolerance to the cyclopentenone PPA_1 and reduced tolerance to increased salt or cold. *EXLA2* influences plant growth and development, including flowering time, morphology and plant size. Overall, this is the first report of an *expansin-like* gene in *Arabidopsis* that links plant development and defence.

RESULTS

EXLA2 gene encodes for an expansin-like A2 protein

The analyses of *EXLA2* cDNA (798 bp; Fig. S1, see Supporting Information) predicted an open reading frame of 265 amino acids corresponding to the coding sequence (Fig. S2, see Supporting Information). BLAST searches against the National Center for Biotechnology Information (NCBI) and The *Arabidopsis* Information Resource (TAIR) databases using the EXLA2 sequence predicted this protein to be a member of the expansin-like family (Fig. S2; Kende *et al*., 2004). EXLA2 encodes a 28.6-kDa protein (isoelectric point, 8.25).

Consistent with other expansins, the sequence of the EXLA2 protein has two conserved domains: (i) a glycoside hydrolase-like family 45; and (ii) a group-2 grass pollen allergen, preceded by a 21-amino-acid signal peptide (Fig. S2). We aligned our EXLA2 amino acid sequence with expansin-like proteins in other plant species. Protein sequences contain conserved cysteine (C) residues in the N-terminal region and conserved tryptophan (W) residues in the C-terminal region, and an ambiguous signal peptide (Fig. S2). In addition, all expansin-like proteins have one to three NXT/S motifs, which may be N-linked glycosylation sites (Lee and Kende, 2002). Expansin-like proteins have an additional tryptophan residue in the C-terminal region and two additional cysteine residues in the N- and C-terminal regions. All expansin-like proteins have a unique conserved motif (CDRC) at the N-terminus of domain 1, and an extension of 17 amino acids in domain 2, unlike expansin proteins (Fig. S2; Lee *et al*., 2001; Sampedro and Cosgrove, 2005). Expansins loosen CWs via a nonenzymatic mechanism by inducing the slippage of cellulose microfibrils in the plant CW (Cosgrove, 2000); EXLA2 may have a different mechanism of action from that of other expansins.

Expression of *EXLA2* during development, defence and following hormone treatment

Because of its altered expression in *B. cinerea*-infected *Arabidopsis* plants (AbuQamar *et al*., 2006), we studied the role of the *EXLA2* gene (*At4g38400*) in plant defence. We showed that the *EXLA2* gene is expressed at low basal levels in healthy wild-type leaves (Fig. 1A). At 24 h post-inoculation (hpi) with *B. cinerea*, however, the levels of *EXLA2* transcript decreased by at least five-fold when compared with noninoculated wild-type plants. The transcripts of *EXLA2* were strongly reduced between 24 and 48 hpi with *B. cinerea* (Fig. 1A). In addition, none of the virulence or avirulence strains of *Pseudomonas syringae* pv. *tomato* (*Pst*) (*Pst*DC3000 or *Pst*DC3000*AvrRPM1*) used altered *EXLA2* gene expression compared with mock-inoculated leaves (Fig. S3A, see Supporting Information). This suggests that *EXLA2* may have a function in plant resistance specific to necrotrophic pathogens. We

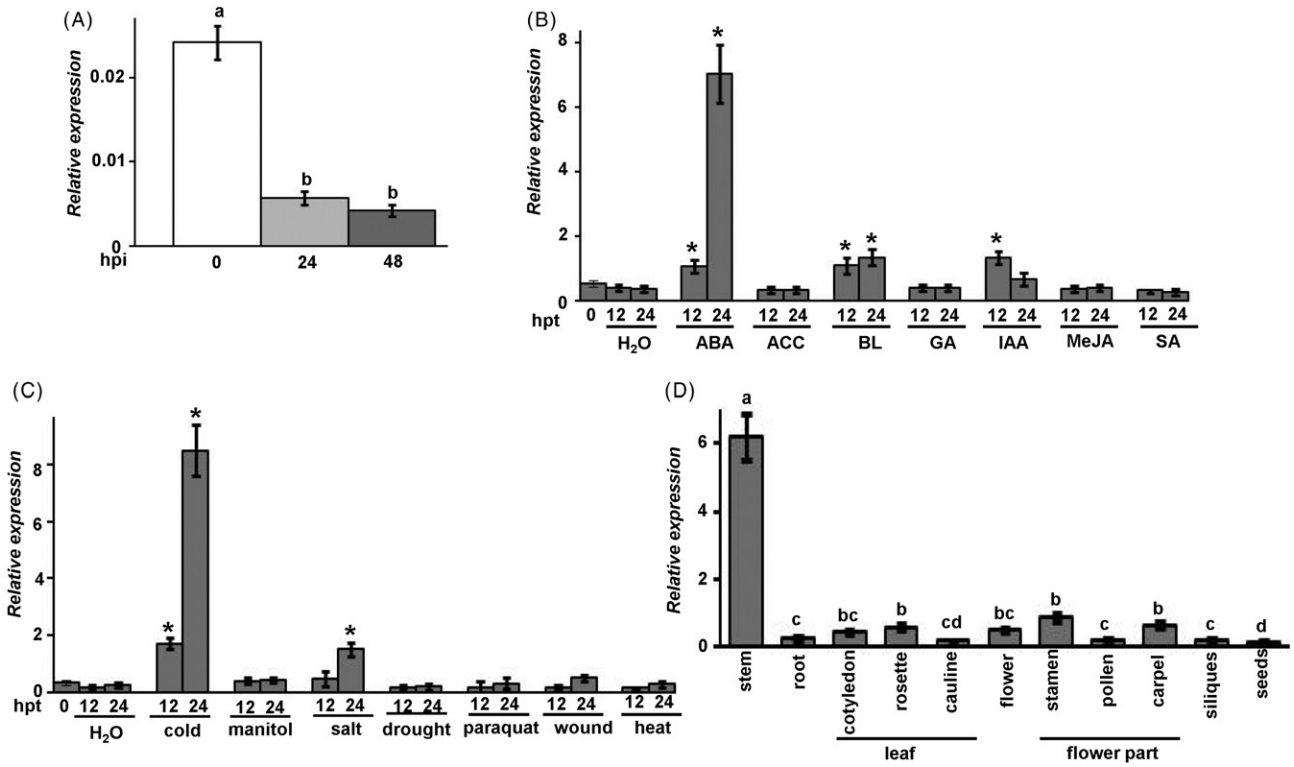

Fig. 1 *Expansin-like A2* (*EXLA2*) gene expression in response to stresses and development. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) amplification of *EXLA2* relative to *Arabidopsis Actin2* gene, in response to *Botrytis cinerea* (A), hormone treatments (ABA, abscisic acid; ACC, aminocyclopropane-1-carboxylic acid; BL, brassinosteroid; GA, gibberellin; IAA, indole acetic acid; MeJA, methyl jasmonate; SA, salicylic acid) (B), abiotic stresses (C) and plant tissues (D). hpi/hpt, hours post-inoculation/treatment.

also checked the expression of *EXLA2* in response to hormones, abiotic stresses and developmental stages (Fig. 1B–D).

Phytohormones play a major role in plant resistance to necrotrophic fungi (Robert-Seilaniantz *et al*., 2007). The exogenous application of ABA induced significantly the expression of *EXLA2*, and BL and IAA induced slight expression. The expression of *EXLA2* was unaltered on treatment with aminocyclopropane-1-carboxylic acid (ACC; a natural precursor of ET), GA, methyl jasmonate (MeJA) or SA at 12 h post-treatment (hpt; Fig. 1B). At 24 hpt with IAA or BL, *EXLA2* exhibited marginally basal or induced levels of *EXLA2* expression, whereas plants sprayed with the ABA hormone consistently expressed *EXLA2*, suggesting that *EXLA2* is highly induced on treatment with ABA. Because ABA is also a major regulator of abiotic stress responses (Fujita *et al*., 2006), we determined the expression of *EXLA2* in response to cold, osmotic, salt, drought, oxidative, wounding and heating stresses. *EXLA2* was up-regulated to both cold and salt (Fig. 1C), which indicates that plant responses to pathogen infection and cold/salinity stress can regulate antagonistically *EXLA2* expression.

We also studied the tissue-specific expression of *EXLA2* during different stages of plant development. In comparison with all other tissues, *EXLA2* transcripts were more abundant in the stem than in other tissues (Fig. 1D). Root, leaf and flower tissues showed moderate to low levels of *EXLA2* expression. The lowest level of basal expression was found in seeds (Fig. 1D). The predominant expression of *EXLA2* in stem tissues suggests a potential function of EXLA2 during plant development.

Mutations in *EXLA2* enhance resistance to necrotrophic fungi

To correlate the expression of *EXLA2* with its function in plant defence, the responses of *EXLA2* mutants, overexpression lines and wild-type plants to different plant pathogens were examined. Only one T-DNA insertion allele of the *EXLA2* gene (*SALK_147678; exla2*) was available (Fig. 2A, B; Sessions *et al*., 2002). We generated a reduced *EXLA2* gene expression line (*EXLA2* RNAi #4; Fig. 2B) using the 3′-*EXLA2* gene-specific region lacking sequences shared with the paralogues (Fig. S1). We also selected two *Arabidopsis* overexpression (35S:*EXLA2*) lines #2 and #5 that constitutively express *EXLA2* at a higher level (Fig. S3B).

The *exla2* and *EXLA2* RNAi #4 mutant lines and the transgenic 35S:*EXLA2* plants were tested for susceptibility to *B. cinerea* as described previously (AbuQamar *et al*., 2006). At 7 dpi of spray-inoculated *exla2* and *EXLA2* RNAi mutant plants, *B. cinerea* infection showed no expansion, with only 3%–6% of the plants

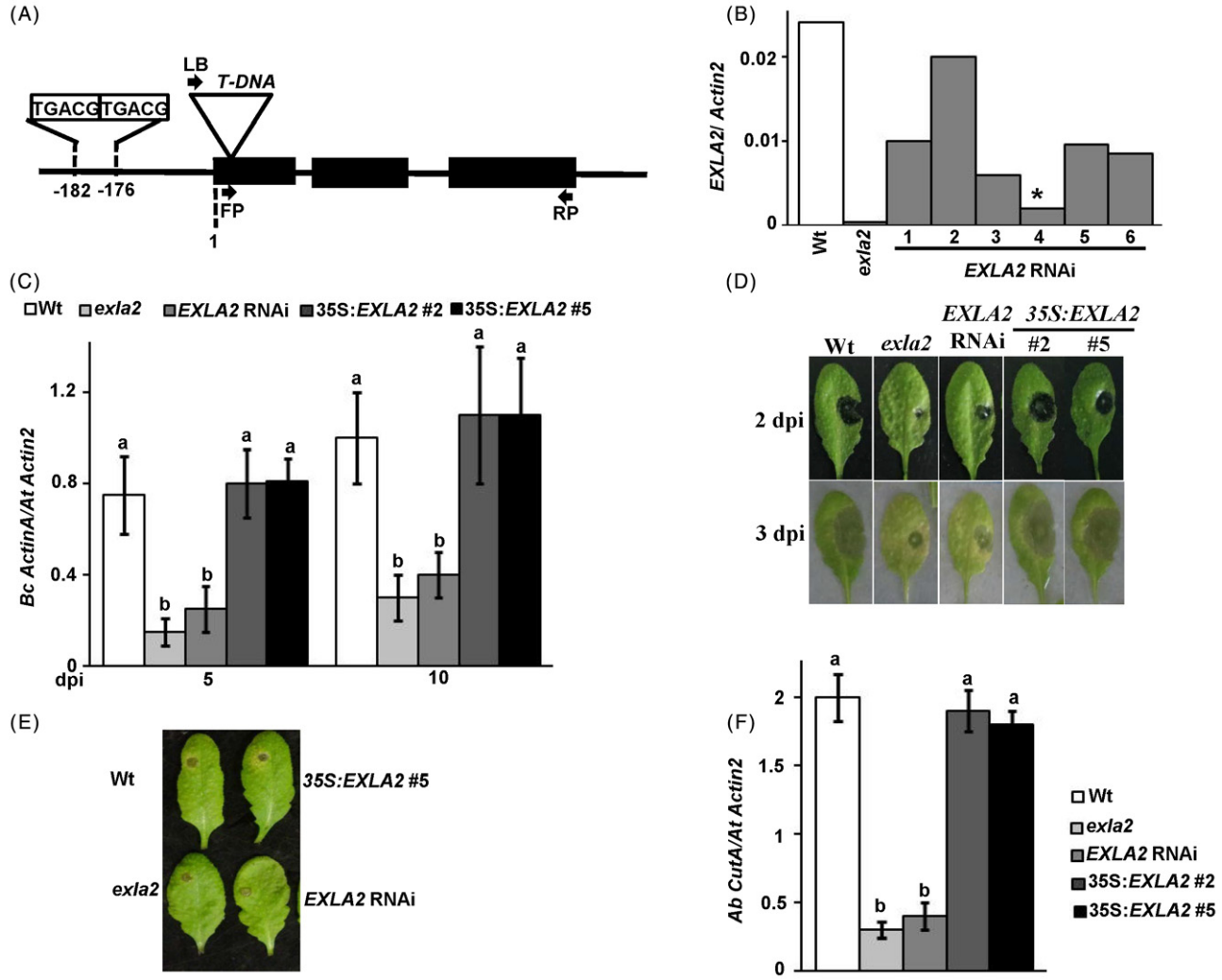

Fig. 2 Reduced *expansin-like A2* (*EXLA2*) transcript levels enhance resistance to necrotrophic pathogens. (A) Genomic organization of the *EXLA2* gene and the *exla2* insertion allele. (B) Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) of *exla2* T-DNA insertion and *EXLA2* RNAi mutant plants with lower constitutive *EXLA2* expression. (C) Fungal growth in plants after spray inoculation with *Botrytis cinerea*. (D) Disease symptoms in leaves after drop inoculation with *B. cinerea*. dpi, days post-inoculation. Disease symptoms (E) and fungal growth (F) in leaves after drop inoculation with *Alternaria brassicicola*. Wt, wild-type.

showing complete damage; however, almost 20% of the wild-type plants showed complete maceration (Fig. S4A, see Supporting Information). The *EXLA2*-overexpressing plants developed comparable disease symptoms and tissue damage to the wild-type plants. At 14 dpi with *B. cinerea*, the majority of the wild-type plants and overexpression lines had rotted off completely, whereas only approximately 30% of both mutant plants showed complete decay (Fig. S4A). These results were confirmed by determining the fungal growth in the wild-type and transgenic plants. At 5 and 10 dpi, wild-type plants exhibited more fungal biomass than the mutants, as assessed by accumulation on *B. cinerea* of *ActinA* relative to *AtActin2* (Benito *et al*., 1998; Fig. 2C). The fungal growth on the 35S:*EXLA2* #2 and #5 plants was indistinguishable from that on the wild-type at this level of inoculation. The same results were obtained when all genotypes were drop inoculated with a high concentration of *B. cinerea* spores (5 μL of 5×10^5 spores/mL). At 2 and 3 dpi, all leaves of different drop-inoculated genotypes showed relatively increased susceptibility towards *B. cinerea*, yet both *exla2* and *EXLA2* RNAi mutant leaves exhibited reduced susceptibility to this pathogen when compared with the wild-type or *EXLA2*-overexpressing transgenic lines (Fig. 2D). This can be seen by the lesser extent of necrotic spots, chlorosis and tissue maceration in both mutants.

We also inoculated plants with *A. brassicicola*, another necrotrophic fungal pathogen. We evaluated the disease symptoms on leaves that had been drop inoculated with *A. brassicicola* at 5 dpi. The inoculation of wild-type plants with fungal spores produced disease lesions that were surrounded by limited chlorosis around the inoculation site, indicating that the wild-type plants were resistant to the fungal pathogen (Fig. 2E; Zheng *et al*., 2006). In

both *exla2* mutant and *EXLA2* RNAi plants, inoculation of the fungal pathogen resulted in decreased disease symptoms characterized by no chlorotic lesions surrounding the inoculation spot (Fig. 2E). The *EXLA2*-overexpressing (line #5) plants showed the same level of chlorosis on their leaves surrounding the site of inoculation as that on wild-type leaves. We also measured fungal growth in inoculated plants using quantitative reverse transcription-polymerase chain reaction (qRT-PCR) of the *A. brassicicola cutinaseA* gene (Brouwer *et al*., 2003). The *exla2* and *EXLA2* RNAi mutant plants showed a clearly reduced *A. brassicicola cutinaseA* gene transcript level relative to wild-type plants, indicating reduced fungal growth in these mutants (Fig. 2F). Both symptom development and growth of the fungus confirmed that the *EXLA2* mutant plants were more resistant to *A. brassicicola*. Statistically, no significant increase in pathogen growth after *A. brassicicola* inoculation was observed in transgenic 35S:*EXLA2* relative to the wild-type plants, with disease lesions that did not expand significantly after 5 dpi (Fig. 2E, F). These results suggest a common host response strategy against these pathogens.

By contrast, there was no difference in the bacterial growth of *Pst* in any of the *EXLA2* mutants, 35S:*EXLA2* overexpression lines or wild-type plants when infiltrated with either the virulent (*Pst*DC3000) or avirulent (*Pst*DC3000*AvrRpm1*) strains of the bacterial pathogen (Fig. S4B, C). This suggests that there is limited or no role of *EXLA2* in plant defence to the bacterial infection used in this study.

Mutations in *EXLA2* enhance tolerance to PPA_1 and oxidative stress

We also tested the *EXLA2* genotypes with the cyclopentenones PPA_1 and OPDA (Mueller *et al*., 2008), and the oxidative stress inducer paraquat (AbuQamar *et al*., 2009; Mueller *et al*., 2008; Stotz *et al*., 2013; Taki *et al*., 2005). We first tested whether PPA_1 or OPDA affects the root growth of the wild-type and *exla2* mutant plants. On control medium without any of the oxylipins, OPDA or PPA_1, the roots of *exla2* mutants were relatively longer (123%) than wild-type roots (Fig. 3A, top, B). We compared the root growth inhibition of *exla2* T-DNA insertion and *EXLA2* RNAi mutants with wild-type seedlings on treatment with 75 μM PPA_1. It was clearly demonstrated that the inhibition of root growth in these mutants in response to PPA_1 treatment was less than that observed in wild-type roots, and was almost similar to that of the wild-type control grown on Murashige–Skoog (MS) medium alone (Fig. 3A, middle, B). Similarly, the *coi1* mutant did not show a significant inhibition in root length when treated with 25 μM PPA_1 (Stotz *et al*., 2013). The dosage differences of PPA_1 might be attributed to the slight inhibition in root growth in *exla2* in this study. The addition of 75 μM of OPDA inhibited strongly root growth in all seedlings. The inhibition of root growth in the *EXLA2* mutants in the presence of OPDA was similar to that observed in the wild-type (Fig. 3A, bottom, B). In response to these cyclopentenones, 35S:*EXLA2*-overexpressing transgenic plants showed a similar root growth inhibition response to the wild-type seedlings (Fig. 3B). Collectively, we found that root growth was inhibited by OPDA in all plants, but the *exla2* mutants were fairly tolerant to PPA_1 treatment. This indicates that mutations in *EXLA2* enhanced tolerance to PPA_1, but not to OPDA.

In addition, we found that the *exla2* mutant plants enhanced tolerance to paraquat (Fig. 3C). In general, the increased tolerance to oxidative stress and PPA_1, and resistance to *B. cinerea*, suggest that *EXLA2* regulates plant responses to oxidative stress, reactive oxylipins and defence against necrotrophic fungi.

Identification of the pathways regulating *EXLA2* expression

To further examine which pathways are involved in the reduced expression of *EXLA2* in *B. cinerea*-infected plants, the expression of *EXLA2* was quantified in mutants impaired in JA, ET, SA, ABA and phytoalexin responses. Therefore, defective mutants of various defence response pathways were tested, including the ET response (*ethylene-insensitive 2*, *ein2*), JA signalling (*coi1*), camalexin accumulation (*phytoalexin-deficient2*, *pad2*), ABA response (*ABA-insensitive 1-1*, *abi1-1*) and SA accumulation plants expressing the bacterial salicylate hydroxylase nahG gene, nahG, in response to *B. cinerea* (Fig. 4A, B). These mutants have been shown previously to exhibit increased susceptibility to *B. cinerea* (AbuQamar *et al*., 2006; Ferrari *et al*., 2003; Gosti *et al*., 1999; Thomma *et al*., 1999; van Wees *et al*., 2003). As in the wild-type plants, the basal levels of *EXLA2* transcripts in all impaired mutants were similar (Fig. 4A). Although only *coi1* mutant plants showed basal levels of *EXLA2* expression after inoculation with *B. cinerea*; the other infected mutants and wild-type plants exhibited reduced levels of *EXLA2* transcripts. This indicates that EXLA2 requires COI1 for its repressed expression during the disease response and that *EXLA2* is regulated via COI1.

Down-regulation of *EXLA2* alters the expression of cyclopentenone-regulated genes in response to *B. cinerea*

JA, OPDA and PPA_1 accumulate on pathogen infection (Mueller *et al*., 2008; Stotz *et al*., 2013; Thoma *et al*., 2003). The role of JA in plant resistance to *B. cinerea* via the COI1-dependent pathway has been well documented (Block *et al*., 2005; Kazan and Manners, 2008; Méndez-Bravo *et al*., 2011; Yan *et al*., 2009). In contrast, the role of the electrophilic oxylipins, OPDA and PPA_1, in plant defence is still poorly known.

In order to link *EXLA2* function in defence to specific pathway(s), we assessed the response of *B. cinerea*-infected tissues to molecular markers of different signalling pathways. The increased

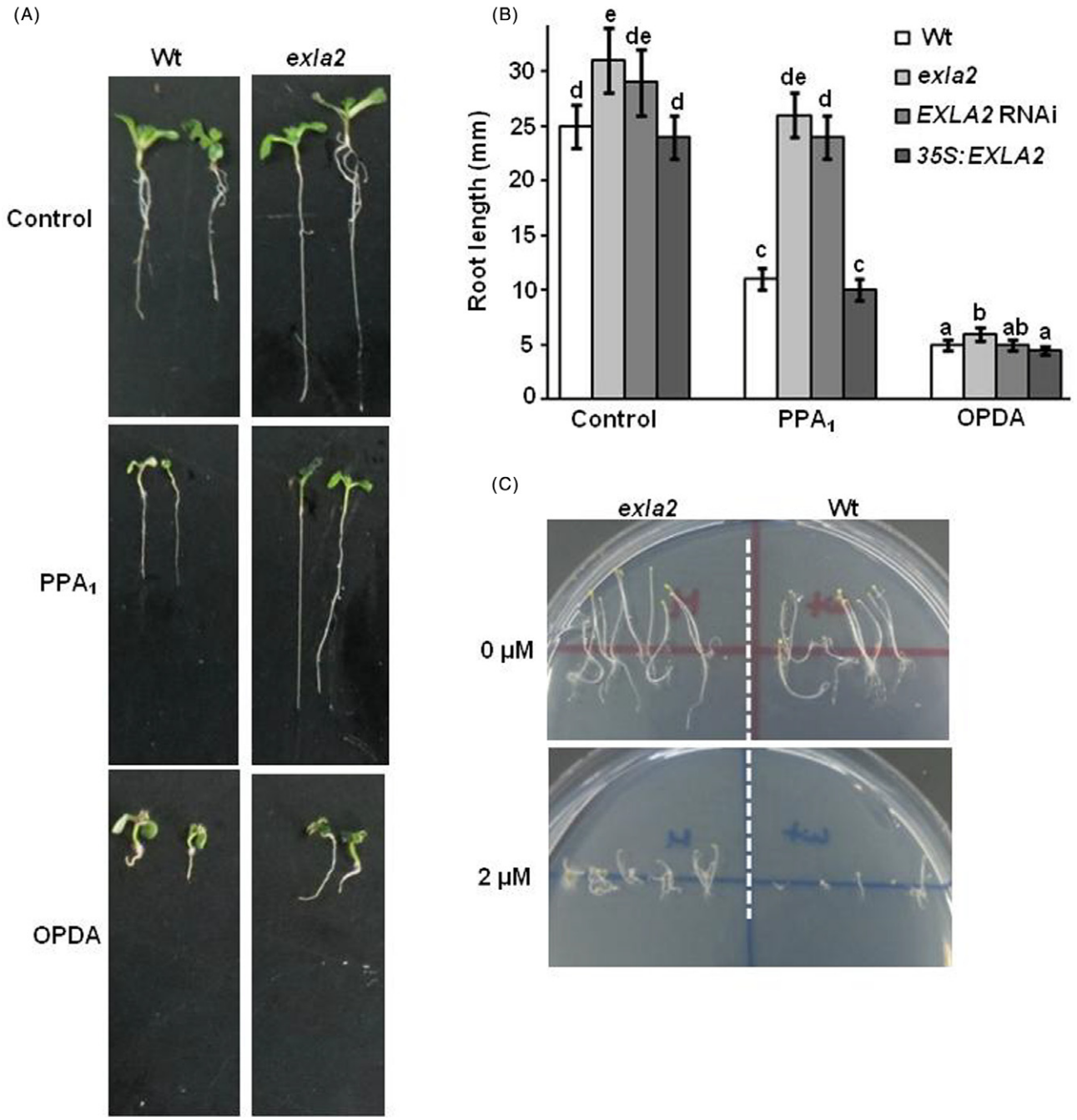

Fig. 3 Mutations in *expansin-like A2* (*EXLA2*) enhance tolerance to phytoprostane-A_1 (PPA_1) and oxidative stress. Germination of seeds and survival (A) and root growth of seedlings (B) grown on 12-oxo-phytodienoic acid (OPDA)- or PPA_1-containing medium (75 µM). (C) Germination and seedling survival of seeds plated on paraquat-containing medium. Wt, wild-type.

transcript levels of *EXLA2* in *coi1* and the responses of *exla2* to *B. cinerea* suggest that *EXLA2* may function through COI1. Therefore, we tested the expression of JA/COI1-regulated genes in both the wild-type and *exla2* mutant plants infected with *B. cinerea*. At 24 hpi with *B. cinerea*, the transcript levels of *MBP* (myrosinase binding protein), *HPL* (*hydroperoxide lyase*) and *PDF1.2* (*plant defensin 1.2*) were induced significantly in both wild-type and *exla2* mutant plants (Fig. S5A–C, see Supporting Information). These *B. cinerea*-induced genes are COI1 dependent and activated by JA and/or OPDA (Stintzi *et al*., 2001; Taki *et al*., 2005). This suggests that the down-regulation of *EXLA2* does not require these oxylipins, and hence *coi1* disrupts this repression after pathogen infection.

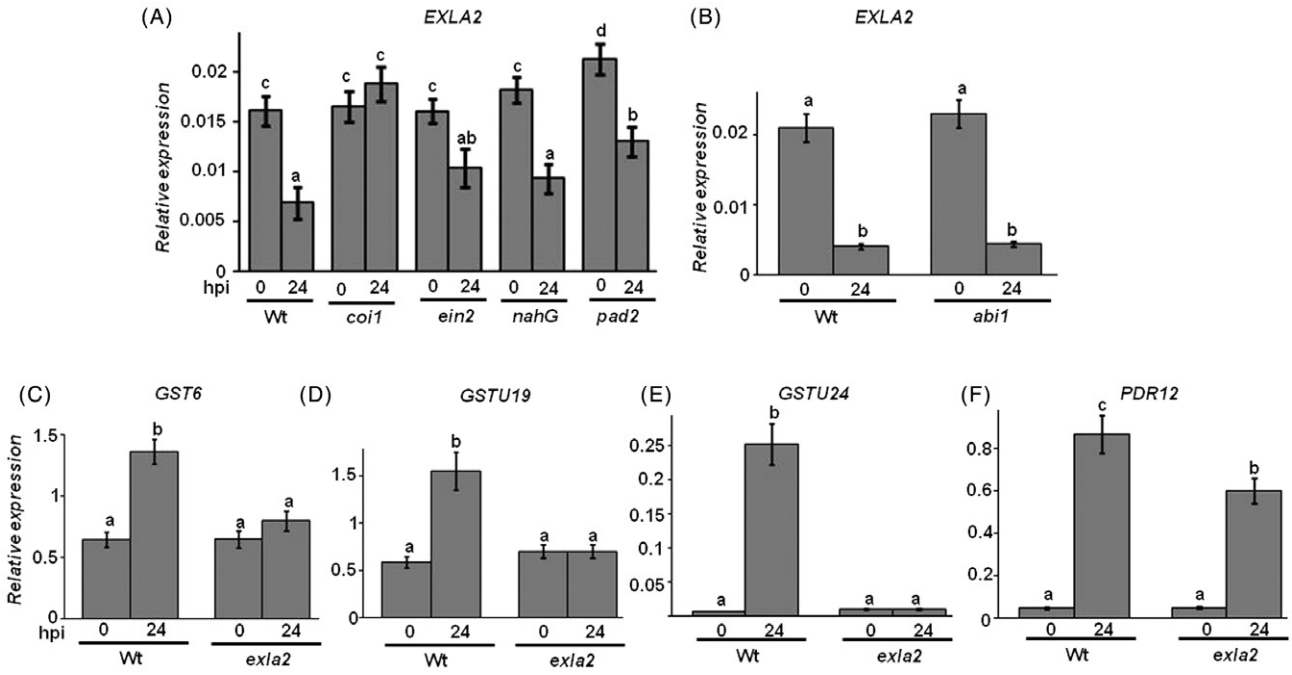

Fig. 4 Expression of *expansin-like A2* (*EXLA2*) in defence response pathways and of oxylipin-regulated genes during *Botrytis cinerea* infection. *Botrytis cinerea* suppressed *EXLA2* expression in *Arabidopsis coi1*, *ein2*, *nahG* and *pad2* mutants (A) and the *abi1* mutant (B). Expression of oxylipin-regulated genes *GST6* (C), *GSTU19* (D) *GSTU24* (E) and *PDR12* (F) in *exla2* mutant plants. Gene definitions are given in the text. Wt, wild-type.

To verify which of the oxylipins modulates *B. cinerea*-regulated gene expression, we assessed the expression profile of oxylipin-regulated genes to *B. cinerea* in the *exla2* mutant (AbuQamar *et al*., 2006; Mueller *et al*., 2008; Stotz *et al*., 2013; Windram *et al*., 2012). Using qRT-PCR, we analysed the expression of a number of genes that were responsive or known to be up-regulated by PPs or OPDA. Among these are genes related to detoxification, such as GSTs (*GST6*, *GSTU19* and *GSTU24*) (Mueller *et al*., 2008; Stotz *et al*., 2013). All three genes were up-regulated in response to *B. cinerea* in the wild-type (Fig. 4C–E). No induction of *GST6*, *GSTU19* or *GST24* was observed in the *exla2* mutant after infection, suggesting that the regulation of these genes is affected by the down-regulation of *EXLA2* to the necrotrophic fungus *B. cinerea*. *PDR12* (*pleiotropic drug resistance 12*) is also known for its strong up-regulation in response to PPs; yet, its induction was significantly lower in *exla2* relative to the wild-type. Previously, *GST6*, *GSTU19* and *PDR12* exhibited a strong induction by PPs (Mueller *et al*., 2008). However, UDP-glucuronyl/glycosyltransferase (*UGT73B2*), ABC transporter (*multidrug resistance-associated protein 1*, *MRP1*) and *TOLB-like* (*TOLB protein-related*) were induced in response to *B. cinerea* in both the mutant and wild-type seedlings (Fig. S5D, E). Previous reports have shown that electrophilic oxylipins accumulate in plants during pathogen infection (Block *et al*., 2005; Thoma *et al*., 2003). All the tested genes were either responsive or up-regulated by PPs or OPDA, but only slightly or not induced by JA (Mueller *et al*., 2008; Stotz *et al*., 2013), which indicates that *B. cinerea* affects the cyclopentenones. Overall, our data suggest that there is a common regulation between electrophilic oxylipins and *B. cinerea*, and that *EXLA2* plays a major role in this regulation.

Arabidopsis PR-1 (*pathogenesis related-1*) and *PR-4* are markers of the SA and ET signalling pathways, respectively. As expected, the transcript levels of *PR-1* and *PR-4* were induced significantly at 24 hpi with *B. cinerea* in both wild-type and *exla2* mutant plants (Fig. S5C). Together, the lack of alteration in the repression of *EXLA2* expression in *nahG* and *ein2* mutants or in the induction of *PR-1* and *PR-4* in the wild-type or *exla2* mutant suggests that neither the SA nor the ET signalling pathway modulates *EXLA2* expression in response to *B. cinerea*.

EXLA2 mutation causes hypersensitivity to NaCl and cold, mediated by ABA

To study the function of the *EXLA2* gene, plants of all *EXLA2* genotypes were assayed for their responses to hormones and abiotic stress. We tested the germination responses of the *exla2* mutant on medium supplemented with ABA. Although, *exla2* plants showed enhanced growth on MS medium lacking ABA relative to the wild-type, the germination of seedlings of this mutant showed hypersensitivity to medium supplemented with ABA (Fig. 5A). This was measured by a reduction in root growth in the *exla2* mutant, when compared with the wild-type plants, as

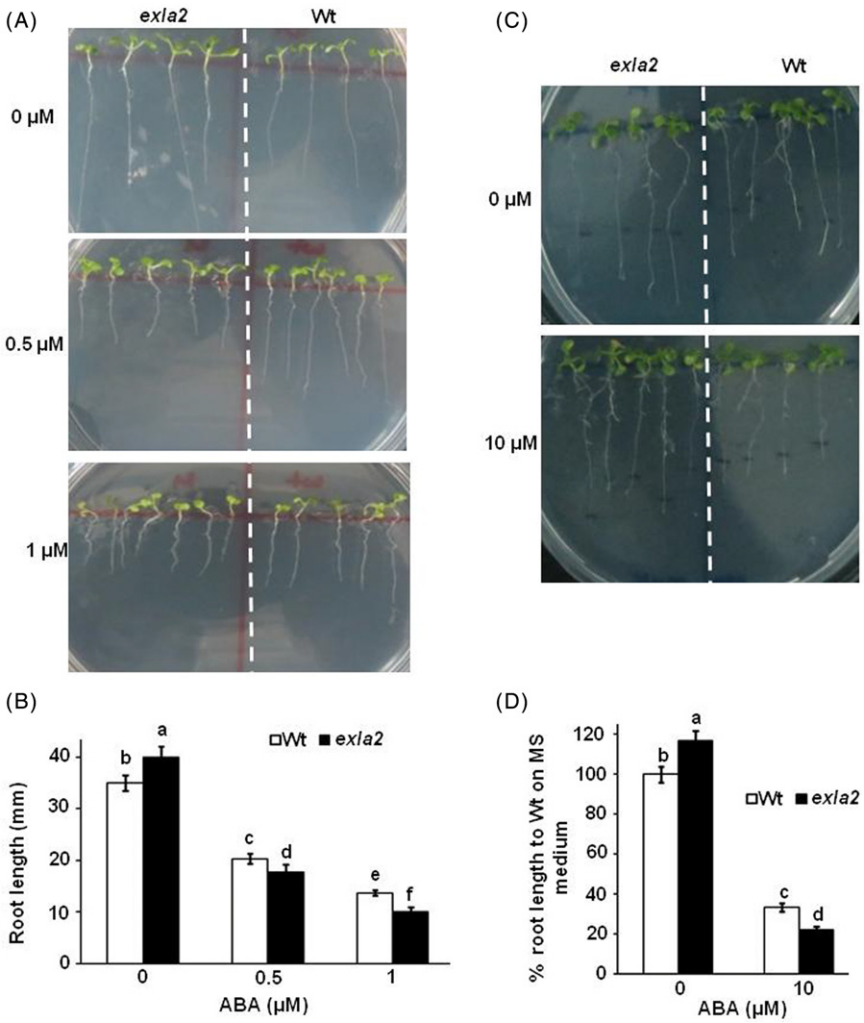

Fig. 5 Abscisic acid (ABA) responses of *expansin-like A2* (*exla2*) mutant and wild-type (Wt) seedlings. (A) Germination and seedling survival of seeds plated on ABA-containing medium. (B) Root length measurements of seedlings growing on ABA. (C) Sensitivity of root growth in seedlings after transfer to ABA-containing medium. (D) Percentage of reduction in root growth after transfer to ABA-containing medium.

the concentration of ABA increased (Fig. 5B). Not only was seed germination altered in *exla2*, but also the roots of *exla2* seedlings were sensitive to ABA (Fig. 5C, D). We also checked the germination of seeds using other hormones. The germination on medium supplemented with SA, MeJA, IAA, GA or ACC was not affected by the *exla2* mutation, limiting the role of *EXLA2* to particular hormone responses (Fig. S6, see Supporting Information).

The *exla2* mutant plants were also tested for the sensitivity of seed germination and seedling root growth to increased salinity. Mutant plants of *exla2* displayed significantly reduced seed germination and growth on medium containing high salt concentrations compared with the corresponding Col-0 wild-type plants (Fig. 6A, B). On normal MS medium, roots of *exla2* plants were longer and healthier than those of wild-type plants (Fig. 6A, B). However, on MS medium supplemented with 50 mM NaCl, both the wild-type and *exla2* plants germinated fully, but the *exla2* plants showed reduced radicle growth after germination. This retardation in root growth after germination continued at 100 mM NaCl; the germination of *exla2* seeds was mostly inhibited at 150 mM NaCl (Fig. 6A, B). To test seedling sensitivity to NaCl, seedlings were pre-germinated on MS medium and then transferred to medium containing 150 mM NaCl. The root growth of *exla2* was reduced dramatically relative to that of wild-type plants (Fig. S7A, B, see Supporting Information).

In order to investigate whether impaired gene expression of *EXLA2* also extended to cold stress, 2-week-old, soil-grown *exla2* mutant and wild-type plants were exposed to cold stress at 4 °C in the light. In contrast with the wild-type, the growth of *exla2* mutant plants was reduced dramatically (Fig. 6C, top panel); moreover, the *exla2* mutant accumulated anthocyanin in leaves after 1 week of cold treatment, but less accumulation was observed in wild-type leaves (Fig. 6D). In all experiments, the 35S:*EXLA2*-overexpressing transgenic lines showed similar phenotypes to the wild-type plants, indicating that *EXLA2* is required, but not sufficient, for *Arabidopsis* tolerance to abiotic stress. Because ABA is known to regulate abiotic stress, we determined the expression of ABA-induced *EXLA2* in the mutant of the negative regulator of ABA signalling, *ABI1* (Gosti *et al*., 1999). The

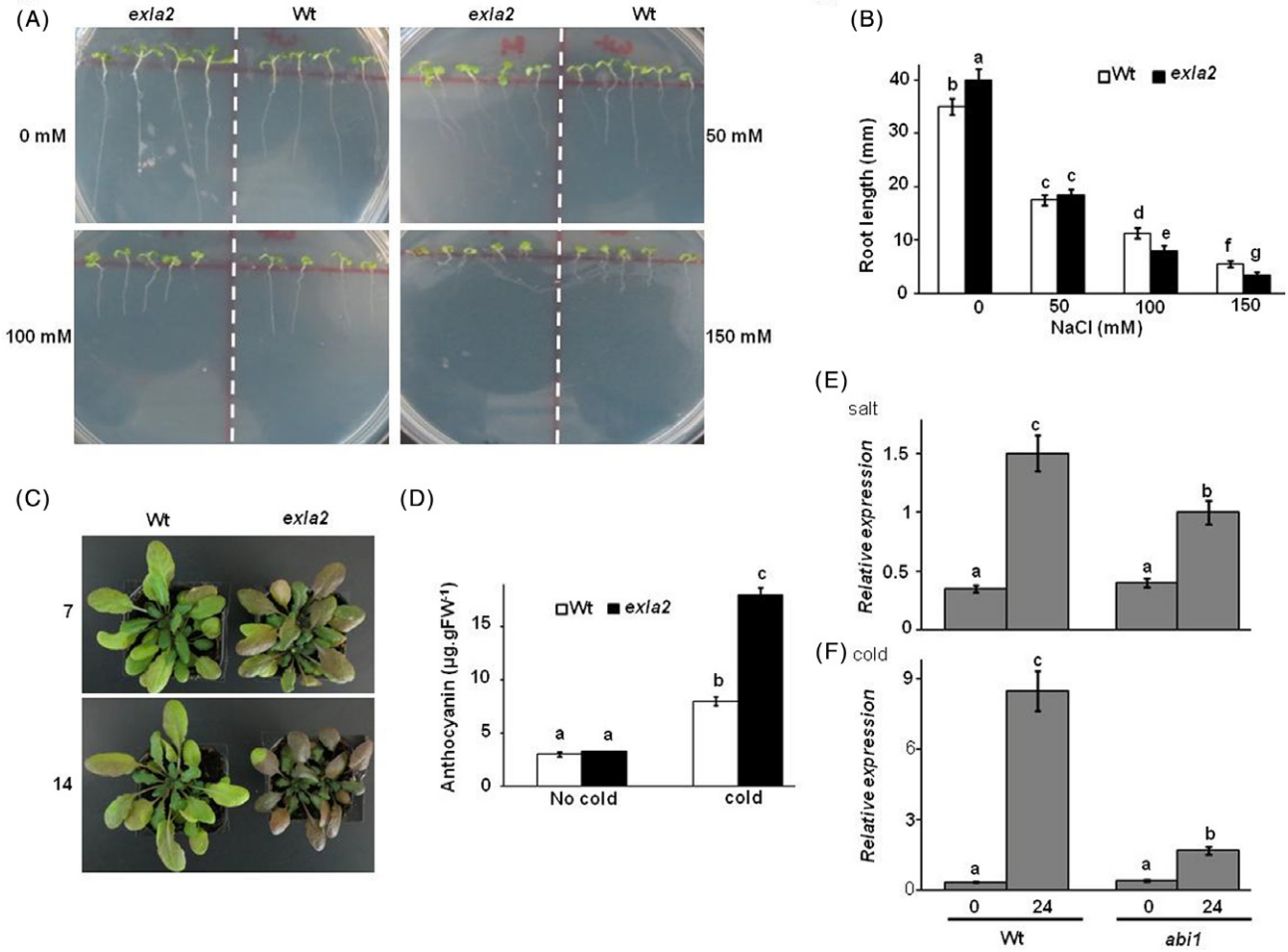

Fig. 6 *Expansin-like A2* (*EXLA2*) mutation is sensitive to salt and cold. Seed germination and seedling survival (A) and seedling root length measurements (B) on NaCl-supplemented medium. Seedling sensitivity (C) and anthocyanin content in leaves at 7 days post-treatment (dpt) (D) with cold stress. *EXLA2* expression in *abi1* mutant in response to salt (E) and cold (F). Wt, wild-type.

ABA-insensitive *abi1-1* showed reduced induction of *EXLA2* expression in response to salt and cold relative to wild-type plants (Fig. 6E, F). The induction of *EXLA2* gene was weaker after cold stress than after salt stress in *abi1-1* mutants. In summary, ABA positively regulates *EXLA2* expression to abiotic stress.

EXLA2 is essential for normal plant growth

The expression of expansins correlates with plant growth and development (Cho and Kende, 1997; Wu *et al*., 1996). Our expression profiling data confirmed the constitutive expression of *EXLA2* at different developmental stages of *Arabidopsis* (Fig. 1D). In order to determine the effects of the transgenic *EXLA2* lines, the phenotypes of all genotypes of *EXLA2* were monitored from germination to seed maturation. Indeed, we did not distinguish any abnormal phenotype in the leaf shape in *EXLA2* mutant plants; however, altered vegetative growth under normal growth conditions was observed. During the stages of vegetative growth, we noticed larger and greater numbers of rosette leaves in *exla2* and *EXLA2* RNAi plants relative to the wild-type (Fig. 7A; Fig. S9A, see Supporting Information). Interestingly, transgenic plants overexpressing *EXLA2* were smaller in size (Fig. 7A), but showed no difference in the total number of leaves when compared with the wild-type (Fig. S9A). Clearly, *exla2* showed the tallest plants, and the plant height was reduced gradually in *EXLA2* RNAi, followed by the wild-type and, finally, 35S:*EXLA2* transgenic plants, which were the shortest of all (Fig. 7C). Therefore, plant height is strongly associated with the levels of expression of *EXLA2* (Fig. 2B; Fig. S1B).

We also checked whether altered levels of *EXLA2* transcript showed differences in the time of flowering (Yang and Karlson, 2012). Except for *exla2*, all plants with different *EXLA2* expression levels initiated primary fluorescence at 24 days post-germination (dpg), which was similar to that in the wild-type; however, seedlings of *exla2* mutant alleles started to flower 3 days earlier (Table 1). This suggests that the down-regulation of *EXLA2* has an effect on the early time of flowering.

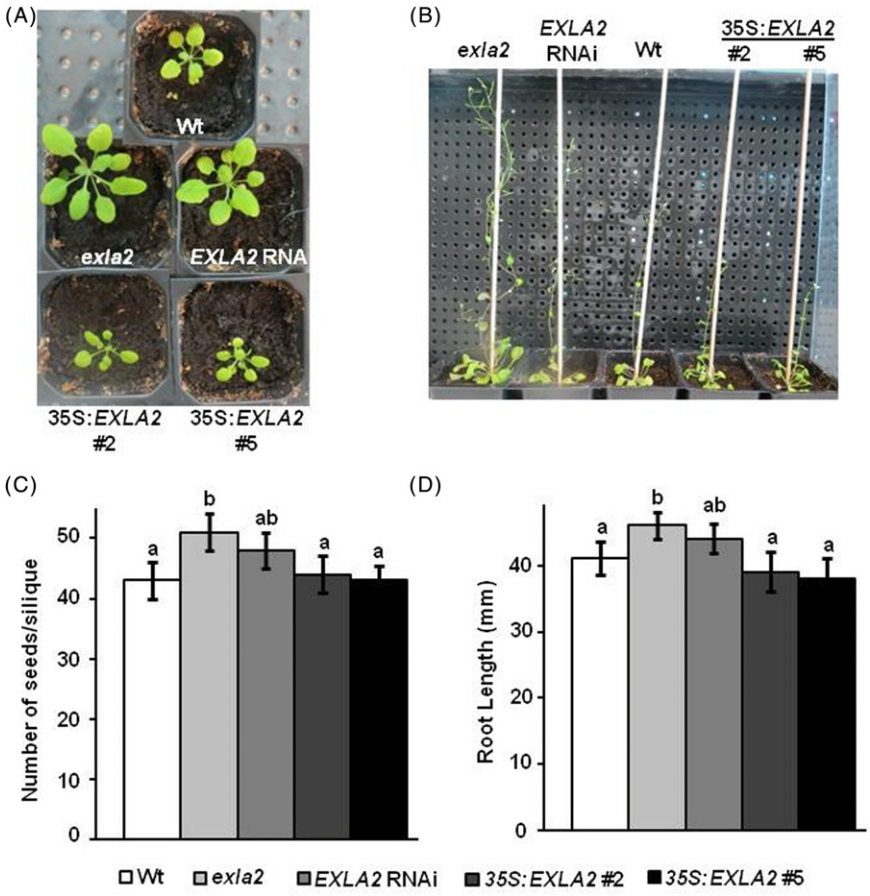

Fig. 7 Phenotypic analysis of *expansin-like A2* (*EXLA2*) mutant and overexpressing transgenic plants. Vegetative growth at 14 days post-germination (dpg) (A), plant size at 40 dpg (B), seed number per silique (C) and root elongation measurement (D). Wt, wild-type.

Table 1 Analysis of flowering time in *EXLA2* genotypes.

Genotype	Flowering time (dpg)	Sample size (*n*)
Wt	23.8 ± 1.01	12
exla2	21.2 ± 0.90	18
EXLA2 RNAi	22.6 ± 1.0	14
35S:EXLA2 #2	24.3 ± 0.82	15
35S:EXLA2 #5	24.5 ± 0.62	15

dpg, days post-germination; Wt, wild-type.

Siliques of *exla2* allele mutant plants were longer than those of the wild-type (Fig. S9B, C) and showed an increased seed number when compared with the wild-type or any other transgenic plants (Fig. 7C). We also measured the root length under aseptic conditions. Ten days after planting the seeds, an increase in root length was observed on MS plates in the mutant allele, whereas the *EXLA2* RNAi root system did not increase significantly (Fig. 7D); this was correlated with the relatively increased levels of *EXLA2* gene expression in the RNAi line (Fig. 2B). The 35S:*EXLA2* seedlings did not show an alteration in root elongation (Fig. 7D).

It should also be mentioned that *EXLA2* expression correlates significantly with the expression of several genes associated with plant development (Fig. S8, see Supporting Information). As a result, *EXLA2* is required for normal plant growth throughout the plant developmental stages.

DISCUSSION

Here, we have described the molecular and genetic function of the *EXLA2* gene in plant defence against necrotrophic pathogens and abiotic stress. Expansin and expansin-like (collectively expansins) are CW-loosening proteins that increase the extensibility of the CW (McQueen-Mason *et al*., 1992). With no known enzymatic activity, expansins regulate germination, fruit ripening and pollination, and may mediate defence responses *in planta*. Several genetic studies have shown that structures of the CW play an important role in plant morphology and growth, tolerance to abiotic stresses and pathogenesis to necrotrophs, including *B. cinerea*. We have identified mutations in *EXLA2* that enhance disease resistance to *B. cinerea* and *A. brassicicola*. Mutations altered in plant CW or cutin/cuticle structure, e.g. *att1* (*aberrant induction of type III genes*), *bdg* (*bodyguard*), *lcr* (*lacerata*), *rwa2* (*reduced wall acetylation 2*) and *sma4* (*symptoms to multiple avr genotypes 4*), exhibit high resistance to *B. cinerea* (Kurdyukov *et al*., 2006; Manabe *et al*., 2011; Tang *et al*., 2007; Wellesen *et al*., 2001; Xiao *et al*., 2004). In tomato, double suppression of

both *polygalacturonase* and *LeEXP1* decreases susceptibility to *B. cinerea* in ripening fruit (Cantu *et al*., 2008). The down-regulation of *EXLA2* protects *Arabidopsis* by limiting pathogen invasion. Therefore, *EXLA2* may function in defence against necrotrophic fungi. We hypothesize that the greater the repression of *EXLA2*, the greater the resistance towards necrotrophic pathogens; yet, the mode of action in the *Arabidopsis–B. cinerea* interaction is unknown.

In plants, OPDA and PP are cyclopentenone signalling molecules that are synthesized enzymatically and nonenzymatically from α-linolenic acid (Creelman and Mullet, 1997; Durand *et al*., 2009; Mosblech *et al*., 2009; Mueller, 1997). The cyclopentenone oxylipins, OPDA and phytoprostane (i.e. PPA_1), are biologically active via the action of reactive oxygen species (ROS) on pathogen infection (Sattler *et al*., 2006). In addition, PP activates the expression of stress response genes, leading to enhanced protection from subsequent oxidative stress (Loeffler *et al*., 2005; Thoma *et al*., 2003). In our study, mutants impaired in *EXLA2* expression were tolerant to oxidative stress and PPA_1 (Fig. 3). Yet, little is known about OPDA and/or PPA_1 as electrophilic oxylipins in response to *B. cinerea*. We examined whether these cyclopentenones altered the expression of *EXLA2*. Microarray studies have revealed that the down-regulation of *EXLA2* is associated with PPA_1 (Mueller *et al*., 2008). We hypothesize that the accumulation of cyclopentenones on *B. cinerea* infection leads to the repression of *EXLA2*. In tomato (*Solanum lycopersicum*), OPDA and PPA_1 accumulate on infection with pathogens, including *B. cinerea* (Thoma *et al*., 2003). Notably, *EXLA2* contains a duplication of the specific binding sequence of the TGA motif in its promoter (Fig. 2A). We speculate that there might be a transcriptional regulator that binds to this motif, and thereby regulates the expression of the *EXLA2* gene during *B. cinerea* infection. Thus, further investigation is required to test this hypothesis. We confirmed that the major responses of *exla2* seedlings are dependent (at least partly) on cyclopentenones by analysing the expression of oxylipin-regulated genes (AbuQamar *et al*., 2006; Mueller *et al*., 2008; Stotz *et al*., 2013) in response to *B. cinerea*. It has been reported that 38% of the genes are co-regulated by the cyclopentenone PPA_1 and *B. cinerea* (Mueller *et al*., 2008). The representative set of genes related to detoxification, *GST6*, *GSTU19* and *GSTU24*, and the *PDR12* gene encoding the ABC transporter protein (Mueller *et al*., 2008; Stotz *et al*., 2013), were strongly up-regulated in response to *B. cinerea*; yet their induction was altered in the *exla2* mutant. Although the regulation of *UGT73B2*, *MRP1* and *TOLB-like* was not affected by *B. cinerea* infection in the *EXLA2*-impaired mutant, all of the mentioned genes were also up-regulated by the cyclopentenones OPDA and PPA_1, but not by the cyclopentanone JA (Fig. 4; Mueller *et al*., 2008). Cyclopentenone oxylipins accumulate in plants during pathogen infection (Block *et al*., 2005; Thoma *et al*., 2003). Our data illustrate that these genes regulated by cyclopentenones are not properly expressed after *B. cinerea* infection in *exla2*. Altogether, the overlap of the responses of the *exla2* mutant to *B. cinerea*, oxidative stress and the cyclopentenone PPA_1 leads to common regulation between electrophilic oxylipins and *B. cinerea* that is associated with *EXLA2*.

We also demonstrated that *EXLA2* was regulated via COI1. Our finding of a basal level of *EXLA2* expression in the *coi1* mutant, compared with the wild-type (Fig. 4A), on exposure to *B. cinerea* confirms that *EXLA2* also requires *COI1* for repressed expression during the disease response. Both the *coi1* and *exla2* mutants showed increased tolerance to PPA_1 treatment (Fig. 3; Stotz *et al*., 2013), but opposite responses to *B. cinerea* (Fig. 2; AbuQamar *et al*., 2006). The differences in disease response might be a result of the regulation of JA and/or its conjugates in the *coi1* mutant, but not in *exla2*; however, further investigation is required. Mutant plants defective in *OPR3* and *EXLA2* displayed increased resistance to *B. cinerea* and *A. brassicicola* (Stintzi *et al*., 2001; Fig. 2), suggesting that electrophilic oxylipins regulate *COI1*-dependent gene expression in response to necrotrophic pathogens (Chehab *et al*., 2011; Ribot *et al*., 2008; Stintzi *et al*., 2001). In general, our findings confirm that cyclopentenone oxylipins mediate resistance to *B. cinerea* through the JA-independent COI1 signalling pathway. In addition, the *EXLA2* repressed expression in *nahG* and *ein2* mutants, similar to the wild-type, and the induction of *PR-1* and *PR-4* in both wild-type and *exla2*, on exposure to *B. cinerea*, illustrate that *EXLA2* expression is not modulated by SA or ET signalling pathways in response to *B. cinerea*. Altogether, the down-regulation of *EXLA2* and its specificity to cyclopentenone and *B. cinerea* suggest that JA-independent COI1 signalling regulates the plant response to *B. cinerea*.

Phytohormones act individually or may crosstalk on pathogen infection. In response to plant necrotrophic fungi, ABA may act as a positive or negative regulator. In tomato, ABA signalling mutants, *abi1*, are resistant to necrotrophic pathogens (Audenaert *et al*., 2002), but susceptible to these pathogens in *Arabidopsis* (Gosti *et al*., 1999). *Arabidopsis* mutants impaired in ABA biosynthesis and insensitive to ABA show an increased susceptibility to *P. irregulare* and *A. brassicicola* (Mauch-Mani and Mauch, 2005), but are more resistant to *B. cinerea* (Adie *et al*., 2007). We conclude a limited effect of ABA in disease resistance mediated by *EXLA2*.

We argue that the *EXLA2* gene has a discrete function in plant development and response. The induction of *EXLA2* and the sensitivity of its mutants to cold and salt confirm other data of the up-regulation of *AtEXLA2* in *Arabidopsis* and *Populus* species on exposure to abiotic stress (Janz *et al*., 2012; Lee *et al*., 2005). In addition, the high basal expression of *EXLA2* in stem tissues (Fig. 1), the salt-induced *EXLA2* homologue in developing xylem (Janz *et al*., 2012) and its up-regulation during CW regeneration of cotton protoplasts (Yang *et al*., 2008) display an involvement of *EXLA2* in CW modification and metabolism. Altogether, this shows the importance of *EXLA2* in adaptive responses to abiotic stresses,

and that *expansin-like* genes may play a role in plant growth and the stress response (Lee *et al*., 2005). In most cases, the silencing of expansin genes leads to growth inhibition, whereas excessive ectopic expression leads to abnormal growth (Quiroz-Castañeda and Folch-Mallol, 2011). *AtEXP10* antisense lines were smaller in plant size and rosettes, whereas overexpressing lines were larger (Cho and Cosgrove, 2000). By contrast, the mutation of *EXLA2* showed larger plants, and transgenic *EXLA2*-overexpressing plants were smaller (Fig. 7). Although *exla2* plants exhibited early flowering, long siliques and an increased number of seeds per silique, *EXLA2* RNAi plants were not significantly different from those of the wild-type or ectopic *EXLA2*-overexpressing transgenic lines. We believe that the 'leaky' *EXLA2* expression of the RNAi lines indicates that a null expression of *EXLA2* is required for these phenotypes.

Overall, *EXLA2* has the potential to serve in plant development and defence through the regulation of endogenous signal molecules and/or pathogen-derived effectors. Future investigations into the identification of pathogen-suppressed *EXLA2* gene expression and the relationship with membrane-associated microbe pattern (MAMP)-triggered defence will help to explain the functions of EXLA2 in innate immunity against *B. cinerea*.

EXPERIMENTAL PROCEDURES

Plant growth, pathogen cultures and disease assays

The *Arabidopsis* wild-type plants, mutants and transgenic overexpression lines used were in the Columbia background. Plants were grown in soil under fluorescent light (150 μE/m^2/s; 12 h light/12 h dark) at 23 ± 2 °C and 60% relative humidity. The plant growth conditions and assays have been described previously (AbuQamar *et al*., 2006).

The culture of *B. cinerea* strain *BO5-10* and *A. brassicicola* strain MUCL20297, spore collection, plant inoculation and disease assays were performed on whole plants or detached leaves as described previously (AbuQamar *et al*., 2006; Zheng *et al*., 2006). Bacterial culture and disease assays were performed as described by Zheng *et al*. (2006).

Determination of fungal growth in inoculated plants

Botrytis cinerea and *A. brassicicola* growth in inoculated plants was determined on the basis of the levels of expression of *B. cinerea ActinA* and *A. brassicicola cutinaseA*, respectively, using qRT-PCR (Benito *et al*., 1998; van Wees *et al*., 2003). The relative expression of *B. cinerea ActinA* and *A. brassicicola cutinaseA* to that of *AtActin2* expression was determined as described previously (Bluhm and Woloshuk, 2005). Three technical replicates of the qRT-PCR assay were used for each sample from three biological replicates.

Generation of transgenic lines and identification of the *EXLA2* mutant allele

We used BLAST searches against the NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and TAIR (http://www.arabidopsis.org/) databases to predict the protein sequence of EXLA2. *exla2* (*SALK_147678;* stock number N647678) was obtained from the Nottingham Arabidopsis Stock Centre (NASC, Nottingham, UK) (Sessions *et al*., 2002). The T-DNA insertion in the *exla2* mutant was confirmed by PCR using a T-DNA-specific primer (LBa1, 5′-TGGTTCACGTAGTGGGCCATCG-3′) and an *EXLA2*-specific primer (RP, 5′-AGAAACTCAAAACCAAAAATCAGTG-3′). Homozygous *exla2* mutant plants were identified by PCR using a pair of primers corresponding to sequences flanking the T-DNA insertion (LP, 5′-GGCTATCAGTACAGCATGTTATGTG-3′; RP).

The *EXLA2* cDNA (clone name RAFL04-09-M02; accession number AF378855) was obtained from the RIKEN BioResource Center (Ibaraki, Japan) (Seki *et al*., 1998, 2002). To generate overexpression plants, full-length *EXLA2* cDNA was cloned after the cauliflower mosaic virus 35S promoter into a modified version of the binary vector pCAMBIA 1200, transformed into *Agrobacterium* strain GV3101, and transformed into plants by *Arabidopsis* floral dip transformation (Clough and Bent, 1998). Transgenic plants were selected on MS medium supplemented with hygromycin, and overexpressing lines were identified by qRT-PCR using the full-length *EXLA2* cDNA.

To generate an *EXLA2* RNAi construct, 250 bp from the 3′ end (3 bp) and the 3′ untranslated region (247 bp) of *EXLA2* were amplified by PCR with the primers *EXLA2* RNAi-LP (5′-GC*ACTAGTCCATGG*GATCACATCTG GAACTGA-3′; *Spe*I and *Nco*I sites are shown in italic) and *EXLA2* RNAi-RP (5′-CG*GGATCCGGCGCGCC*CAAAAAAACTGTAATGTC-3′; *BamH*I and *Asc*I sites are shown in italic), and cloned into the RNAi vector pGSA1165 (http://www.chromdb.org/rnai/order_vectors.html).

Morphological analysis of transgenic lines

Plants were grown at 23 °C up to 60 dpg for morphological analyses. For statistical analyses, 20 different plants were measured and photographed. Root elongation tests and measurements were carried out according to Yang and Karlson (2012).

Sensitivity treatments

All seeds were surface sterilized and plated on MS medium with 2% (w/v) sucrose and 0.8% (w/v) agar, pH 5.7 (Zheng *et al*., 2006). Sensitivity assays were performed using MS medium supplemented with different concentrations of chemicals. Ten-day-old seedlings grown *in vitro* were treated with 75 μM OPDA or PPA_1 (Cayman Europe, Tallinn, Estonia), as described previously (Mueller *et al*., 2008); 50, 100 and 200 mM NaCl; 2 and 4 μM IAA; 0.5 and 1 μM ABA; 25, 50 and 100 μM MeJA, 10 μM GA; 25, 50 and 100 μM SA; or 2 μM paraquat. For paraquat, seedlings were grown in the dark. For cold treatment, seedlings were grown in soil for 2 weeks and then subjected to cold acclimation at 4 °C for another 14 days (Dong *et al*., 2006). Anthocyanin measurements were carried out and quantified photometrically by the absorbance at 535 nm (Teng *et al*., 2005).

Gene expression treatments

Gene expression analyses were determined as described previously (Laluk *et al*., 2011; Veronese *et al*., 2006; Zheng *et al*., 2006). Plants (4 weeks old) grown on soil were sprayed with 3×10^5 *B. cinerea* spores/mL,

100 μM SA, 100 μM MeJA, 100 μM ACC, 100 μM IAA, 100 μM ABA, 2 μM BL, 100 μM GA, 150 mM NaCl, 300 mM mannitol or 100 μM paraquat (methyl viologen).

Seedlings on soil were cold treated with a continuous temperature of 4 °C on crushed ice in a cold chamber. Wounding was performed by pressing approximately 60% of the leaf surface area using serrated forceps.

RNA extraction and expression analysis

RNA extraction, cDNA synthesis and qRT-PCR expression analyses were performed as described previously (Dhawan *et al*., 2009). RT-PCR and qRT-PCR were performed using gene-specific primers, with *Arabidopsis Actin2* as an endogenous reference for normalization. A minimum of three technical replicates was used for each sample with a minimum of two biological replicates for qRT-PCR. Expression levels were calculated by the comparative cycle threshold method, and normalization to the control was performed as described previously (Bluhm and Woloshuk, 2005). The primers used are listed in Table S1 (see Supporting Information).

Statistical analysis

All experiments were repeated at least three times with similar results. Analysis of variance and Duncan's multiple range test were performed to determine the statistical significance (SAS Institute, 1999). Mean values followed by asterisks or letters are significantly different from the corresponding control, or different from each other, respectively ($P = 0.05$).

Accession numbers

Sequence data from this article can be found in the GenBank/EMBL data libraries under the following accession numbers: *Actin2* (At3G18780), *PR-1* (At2g14610), *EXLA1* (At3g45970), *EXLA3* (At3g45960); *PR-4* (At3g04720); *ABI1* (At4G26080); *CYP81D11* (At3g28740), *UGT73B2* (At4g34135), *GST6* (At2g47730), *GSTU24* (At1g17170), *GSTU19* (At1g78380), *MRP1* (At1g30400), *PDR12* (At1g15520), *HSP70* (At3g12580), *HSP17.6* (At2g29500), *OPR1* (At1g76680), *OPR3* (At2g06050), *ELI3-2* (At4g37990), *AOX3* (At1g32350), *TOLB* (At4g01870), *MBP* (At3g16460), *HPL* (At4g15440), *PHO1;H10* (At1g69480), *GST6* (At2g47730).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1 Multiple sequence alignment of *expansin-like A2* (*EXLA2*) and closely related *Arabidopsis* cDNA.

Fig. S2 Multiple sequence alignment of expansin-like A2 (EXLA2) and closely related *Arabidopsis* and other EXLA2 proteins in plant species.

Fig. S3 Expression of *expansin-like A2* (*EXLA2*) on exposure to *Pseudomonas syringae* and identification of *AtEXLA2*-overexpressing plants.

Fig. S4 Plant responses of altered *expansin-like A2* (*EXLA2*) expression on exposure to *Botrytis cinerea* and *Pseudomonas syringae*.

Fig. S5 Expression of oxylipin-responsive and defence-related genes during *Botrytis cinerea* infection.

Fig. S6 Germination and growth responses of the *expansin-like A2* (*exla2*) mutant are not altered on exposure to salicylic acid (SA), methyl jasmonate (MeJA), indole acetic acid (IAA), gibberellin (GA) or aminocyclopropane-1-carboxylic acid (ACC).

Fig. S7 Sensitivity of *Arabidopsis expansin-like A2* (*EXLA2*) mutant to salt.

Fig. S8 *Expansin-like A2* (*EXLA2*) expression at different developmental stages in relation to other genes.

Fig. S9 Additional morphological analyses of *expansin-like A2* (*EXLA2*) mutant and overexpressing transgenic plants.

Table S1 List of primers (sequence 5′ to 3′) used in this study.