The ERECTA Receptor-Like Kinase Regulates Cell Wall–Mediated Resistance to Pathogens in Arabidopsis thaliana

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Some receptor-like kinases (RLK) control plant development while others regulate immunity. The Arabidopsis ERECTA (ER) RLK regulates both biological processes. To discover specific components of ER-mediated immunity, a genetic screen was conducted to identify suppressors of erecta (ser) susceptibility to Plectosphaerella cucumerina fungus. The ser1 and ser2 mutations restored disease resistance to this pathogen to wild-type levels in the er-1 background but failed to suppress er-1-associated developmental phenotypes. The deposition of callose upon inoculation, which was impaired in the er-1 plants, was also restored to near wild-type levels in the ser er-1 mutants. Analyses of er cell walls revealed that total neutral sugars were reduced and uronic acids increased relative to those of wild-type walls. Interestingly, in the ser er-1 walls, neutral sugars were elevated and uronic acids were reduced relative to both er-1 and wild-type plants. The cell-wall changes found in er-1 and the ser er-1 mutants are unlikely to contribute to their developmental alterations. However, they may influence disease resistance, as a positive correlation was found between uronic acids content and resistance to P. cucumerina. We propose a specific function for ER in regulating cell wall–mediated disease resistance that is distinct from its role in development.

The activation of plant and animal innate immunity systems involves a specific detection of microbe-associated molecular patterns (MAMPs) by different sets of host pattern-recognition receptors (PRR) (Ausubel 2005; Bittel and Robatzek 2007; Dangl and Jones 2001; Nürnberg and Brunner 2002). Several members of the plant leucine-rich repeat (LRR) receptor-like kinase (RLK) family, such as FLS2 and EFR, have been found to function as PRR (Gomez-Gomez et al. 2001; Shiu et al. 2004; Song et al. 1995; Zipfel et al. 2006). The immune responses mediated by these receptors can be modulated by additional RLK. For example, BAK1 (BRI1-associated kinase 1) regulates FLS2 function through the formation of MAMP-induced RLK complexes (Chinchilla et al. 2007). Additional RLK play relevant roles in plant immunity, although their exact modes of action have not been elucidated. For example, the ERECTA (ER) LRR RLK is required for resistance in Arabidopsis to the soilborne bacterium Ralstonia solanacearum 14–25, the necrotrophic fungus Plectosphaerella cucumerina, and the damping-off oomycete Phytophthora irregulare, as er mutant alleles (e.g., er-1) are more susceptible to these pathogens than wild-type plants (Adie et al. 2007; Godiard et al. 2003; Llorente et al. 2005). Also, Arabidopsis BAK1 has been shown to regulate resistance to necrotrophic fungi by negatively controlling cell-death progression upon pathogen infection (He et al. 2007; Kemmerling et al. 2007).

In addition to their functions in immunity, LRR RLK control different plant cell growth and developmental processes (Becraft 2002; Hématy and Höfte 2008). For instance, BAK1 participates in brassinosteroid-mediated signaling by forming heterodimeric complexes with BR11, a LRR RLK for the plant hormone brassinosteroid (Li et al. 2002). The ER protein regulates inflorescence architecture, lateral organ shape, ovule development, stomatal patterning, and transpiration efficiency through its genetic interaction with two closely related paralogs (ERL1 and ERL2) and the genes TMM and EPF1 encoding, respectively, a receptor-like protein (RLP) and a peptide (Hara et al. 2007; Masle et al. 2005; Pillitteri et al. 2007; Shpak et al. 2003, 2004, 2005; Torii et al. 1996). The inflorescence of er mutants forms flattened floral bud clusters due to a reduction of internodal and pedicel elongation, which produces shorter siliques than in wild-type plants (Shpak et al. 2004, 2005; Torii et al. 1996). Also, genetic interactions between ER and genes controlling different developmental and hormone-mediated pathways have been described (Fridborg et al. 2001; Mele et al. 2003). The molecular bases of this double functionality in immunity and development processes of some RLK such as BAK1 and ER remains elusive.

In contrast to the well-characterized ER-mediated developmental signaling pathway, relatively few genetic elements of ER-mediated immunity to necrotrophic and soil-borne patho-

* The e-Xtra logo stands for “electronic extra” and indicates that one supplementary table and five supplementary figures are published online.
gens have been identified (Adie et al. 2007; Godiard et al. 2003; Llorente et al. 2005). Resistance to these types of pathogens is often genetically complex in Arabidopsis and depends on the precise regulation of the ethylene (ET), jasmonic acid (JA), salicylic acid (SA) and abscisic acid (ABA) signaling pathways (Adie et al. 2007; Berrocal-Lobo et al. 2002; Hernández-Blanco et al. 2007; Thomma et al. 1999). Recent genetic analyses also showed that variation in plant wall composition results in altered disease resistance responses in a number of plant-pathogen combinations. Thus, the irregular xylem (irx) mutants impaired in cellulose synthase (CESA) subunits (AtCESA4 [IRX5], AtCESA7 [IRX3], and AtCESA8 [IRX1]) required for secondarily cell-wall formation, and the prcl (procluste 1) and irxl/ecl (isosaxen resistant; constitutive expression of VSP) mutants, which are defective, respectively, in the CESA6 and CESA3 subunits required for primary cell-wall formation, showed enhanced resistance to different necrotrophic, biotrophic, and soilborne pathogens (Ellis and Turner 2001; Hernández-Blanco et al. 2007). Interestingly, the the1 (theseus 1) mutant was isolated as a suppressor of the developmental alterations caused by the prcl and elil mutations, leading the authors to suggest that THE1 (a member of CrRLK1L family of RLK) may act as a cell-wall integrity sensor similar to those described in yeast (Hématy et al. 2007). Additional RLK from the CrRLK1L and the wall-associated kinase (WAK) families as well as the recently described FEI1 and FEI2 proteins may also serve as cell-wall sensors controlling cell growth and plant responses to pathogen-induced cell-wall alterations (Hématy and Höfte 2008; Xu et al. 2008).

To identify new elements of ER-mediated immunity, a genetic screen was conducted in a mutagenized population of the highly susceptible er-1 mutant to find suppressors of erecta susceptibility (ser) to the necrotrophic fungus P. cucumerina. Two mutants, ser1 and ser2, were identified that restored the er-1 susceptibility levels to those found in wild-type plants but failed to restore the er-associated development phenotypes to wild-type morphology. A comparative analysis of the cell-wall structure and composition and defense responses in these genotypes reveal that composition of the host cell wall may be a determinant of fungal infection success. The results presented here also suggest that ER plays a role in regulating cell wall-mediated resistance to pathogens that is distinct from its role in plant development.

RESULTS

Isolation and characterization of ser mutants.

A suppressor screen was conducted to identify new elements of ER-mediated innate immunity. We inoculated about 10,000 M2 seedlings of the Landsberg er-1 (Ler) mutant with a spore suspension of P. cucumerina, and two recessive ser mutants, ser1 and ser2, were identified that, upon fungal inoculation, exhibited a reduction of fungus multiplication in planta, as demonstrated by quantification of P. cucumerina DNA by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) (Fig. 1A). The fungus multiplication in the ser plants was comparable to that of the wild-type plants (Landsberg, La-0) and lower than that observed in the highly susceptible er-1 parental plants and the SA-compromised NahG line (Fig. 1A). The macroscopic disease ratings of ser1 er-1 and ser2 er-1 mutants at latter timepoints of infection (e.g., 14 days postinoculation [dpi]) correlated with the fungal biomass determined at the initial stages of infection (Fig. 1B).

Mapping was used to place SER1 on chromosome 2 between markers TID16A and F12K2A, a 500-kb interval encompassing ERECTA, and SER2 on chromosome 5 between the markers nga151 and nga76 (data not shown). Genetic linkage analysis confirmed that SER1 and ER were tightly linked (\(\chi^2 = 456, P = 0.01\)). The ER coding region was sequenced in both er-1 and ser1 er-1 plants, but no differences were found (data not shown). Thus, the ser1 mutation was not an intragenic mutation in er-1. Also, the expression of ER gene was quantified by qRT-PCR in the er-1, ser1 er-1, and ser2 er-1 plants, but no differences were found (data not shown). The P. cucumerina multiplication in the ser2 single mutant was quantified by qRT-PCR at 3 dpi and was found to be slightly reduced compared with that of wild-type plants and the ser2 er-1 double mutant (Supplementary Fig. 1).

To determine the specificity of the disease resistance of the ser1 er-1 and ser2 er-1 mutants, we infected the double mutants with a broad range of pathogens, including fungi, bacteria, and oomycetes. The ser1 er-1 and ser2 er-1 mutants were as susceptible as the parental er-1 plants to most pathogens tested, with the exception of the biotrophic fungus Golovinomyces cichoracearum, which showed a slight reduction of growth on the ser1 er-1 mutant compared with the parental er-1 plants (Table 1; Supplementary Fig. 2). Interestingly, although ER is required for resistance to the soilborne bacterium R. solanacearum in Arabidopsis (Godiard et al. 2003), the disease symptoms caused by this pathogen on the ser1 er-1 and ser2 er-1 mutants was similar to those observed in er-1 plants (Table 1).
Genetic evidence indicates that the ET, JA, and SA signaling pathways are required for resistance to P. cucumerina in Arabidopsis and that the hyperactivation of any of these pathways is sufficient to increase plant resistance to this fungus (Berrocal-Lobo et al. 2002). We studied the activation status of these defense pathways in ser-er-1 plants by testing the expression of marker genes of these signaling pathways. The expression of the SA-responsive gene PDF1.2a or the ET and JA-associated gene PDF1.2a was not constitutively upregulated in uninfected serl er-1 and ser2 er-1 plants (Fig. 1C and D). Upon inoculation with P. cucumerina, the steady-state levels of these genes were lower in the ser er-1 mutants than in the er-1 plants (Fig. 1C and D). As described previously, in the NahG line blocked in SA-signaling (Berrocal-Lobo et al. 2002), the pathogen-induced expression of PDF1 and PDF1.2a was, respectively, lower and higher than in er-1 plants (Fig. 1C and D). These results suggest that the resistance mechanism in serl er-1 and ser2 er-1 plants to P. cucumerina may not be dependent on the constitutive activation of either the SA or JA and ET pathways.

The ser1 and ser2 mutants fail to suppress the er-associated developmental phenotypes.

The different er mutant alleles display developmental alterations such as round leaves with short petioles, compact flower buds, and blunt, short, wide siliques as compared with wild-type plants (Lease et al. 2001; Torii et al. 1996). To determine whether the ser mutations suppressed these er-associated developmental alterations, we compared the flower buds as well as the pedicel and silique lengths of ser er-1 mutants with those of er-1 plants. As shown in Figure 2, the ser mutations were unable to restore the er-1-associated developmental features to their wild-type morphology. By contrast, in the ser er-1 plants, the flower buds were more compact and pedicels and siliques were even shorter than in er-1 plants (Fig. 2A and C). Also, flowering time in the ser2 er-1 plants was delayed about 3 weeks relative to er-1 plants (data not shown). These data indicate that the SER1 and SER2 genes affected in a distinct manner the disease resistance mechanisms and the developmental processes regulated by ER.

er-1 displays changes in its cell walls that are partially reversed in the ser er-1 double mutants.

A role for the cell wall in Arabidopsis immunity has been proposed based on the identification of cell-wall mutants that show altered resistance to pathogens (Ellis and Turner 2001; Hernandez-Blanco et al. 2007; Vogel et al. 2002, 2004; Vorwerk et al. 2004). Moreover, some RLK, such as THE1, FEI1/FEI2, and some WAK members, have been suggested to regulate cell-wall structure or integrity (Hématy and Hofte 2008; Hématy et al. 2007; Xu et al. 2008). Based on these observations and the enhanced susceptibility to different pathogens of er mutants (Llorente et al. 2005), we decided to explore whether ER function might impact the plant cell-wall structure. The walls from er-1 and wild-type leaves were subjected to Fourier transform infra red (FTIR) spectroscopy to obtain qualitative spectrotypes (i.e., cell-wall phenotype). The comparison of averaged FTIR spectra (n = 15) between samples from er-1 and wild-type plants showed strong differences between the two genotypes (Fig. 3A). Much of the sample variation (96%) was explained by principal component 1 (PC1) (Fig. 3B). Absorptions at 948 cm\(^{-1}\), 1,066 cm\(^{-1}\), 1,186 cm\(^{-1}\), common in the fingerprint for several cell-wall polysaccharides (Kauráková et al. 2000), and 1,402 cm\(^{-1}\), 1,590 cm\(^{-1}\), and 1,708 cm\(^{-1}\) seemed to differ between er-1 and wild-type plants (Fig. 3C). In addition, FTIR spectra of a different mutant allele, er-115, in a distinct genetic background (Col-0) showed significant differences in the cell wall when compared with that of wild-type Col-0 (Supplementary Fig. 3). The pattern of the PC1 spectrum obtained (that explains 99% of the total variability) is similar to PC1 obtained from er-1/WT (Landsberg background), suggesting that er mutation produces similar changes in the cell wall in both genotypes.

To obtain a more quantitative analysis of the cell-wall changes in er-1, we compared the noncellulosic neutral monosaccharide composition as well as the cellulose and uronic acid content of cell walls (Table 1). In the ser1 er-1 mutants, the characteristic bands at 896 cm\(^{-1}\) and 948 cm\(^{-1}\) were lower (Fig. 3D), suggesting that the cell walls were partially insensitive to the different treatments. This result is consistent with the observation that ser1 er-1 plants have weak resistance to P. c c u c u m e r i n a (Berrocal-Lobo et al. 2002). The FTIR spectra also show a decrease in the absorbance at 896 cm\(^{-1}\), 948 cm\(^{-1}\), and 1,066 cm\(^{-1}\), which are characteristic of the abundant 4-O-methylglucuronic acid content of the cell wall (Kauráková et al. 2000).

Table 1. Response of wild-type (WT; La-0), er-1, and ser er-1 plants to various pathogens

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>WT</th>
<th>er-1</th>
<th>ser1 er-1</th>
<th>ser2 er-1</th>
<th>irx1-1 er-1</th>
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<tr>
<td>Golovinomyces cichoracearum USC1</td>
<td>S</td>
<td>S</td>
<td>WR</td>
<td>S</td>
<td>WR</td>
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<tr>
<td>Hyaloperonospora arabidopsis Noco2</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
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<tr>
<td>H. arabidopsis Cala2</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<tr>
<td>H. arabidopsis Emwa1</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<td>R</td>
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<tr>
<td>Botrytis cinerea</td>
<td>S</td>
<td>S</td>
<td>S</td>
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<td>S</td>
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<tr>
<td>Fusarium oxysporum f. sp. conglutinans</td>
<td>S</td>
<td>S</td>
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<tr>
<td>Rhizoctonia solanacearum GMI1000</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Pseudomonas syringae pv. tomato DC3000</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
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<tr>
<td>P. syringae pv. tomato DC3000 AvrRPM1</td>
<td>HR</td>
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\(^a\) HR = hypersensitive response, R = resistance, S = susceptible, and WR = weak resistance. The cell-wall mutant irx1-1 er-1 was included for comparison.
contents of the leaves from wild-type and er-1 plants (Blakeney et al. 1983; Reiter et al. 1993). The amounts of rhamnose, fucose, and xylose in er-1 samples were less than those found in wild-type cell walls (Fig. 4A). In the er-1 cell walls, the uronic acids and cellulose contents were much greater than in wild-type cell walls (Fig. 4B). Taken together, these analyses of cell-wall composition highlight the impact of ER function on cell-wall composition and structure.

We next analyzed the cell wall of the ser er-1 double mutants to determine whether ser mutations suppressed some of the er-1–associated cell-wall changes. PC analysis of the FTIR spectra (n = 15) of leaves from the different genotypes showed that ser mutations strongly reverted the er-1 cell-wall spectrum and also introduced some changes that make ser spectra different from that of wild type (Fig. 3D through F). PC1 explained almost 97% of the total variability in the data set (Fig. 3E). The absorbance features, which differed among the spectratypes of the four genotypes tested (PC1) (Fig. 3E and F), are shared by several cell-wall polysaccharides (Kauráková et al. 2000). This observation and the complexity of the PC spectra suggest that the cell-wall differences among er-1, ser1 er-1, and ser-2 er-1 cannot be assigned to a defect in a single cell-wall polymer.

We also analyzed the amounts of noncellulosic neutral sugars and the cellulose and uronic acid content of the leaves of the ser er-1 mutants. The ser2 er-1 cell walls showed higher levels of all six neutral sugars than did er-1 cell walls (Fig. 4A). Except for xylose and arabinose, the amounts of these monosaccharides in ser1 er-1 samples were similar to those found in the wild-type plants (Fig. 4A). In line with these results, we found that the total noncellulosic neutral sugar content in the cell walls of ser1 er-1 were restored to the wild-type levels, whereas in ser2 er-1, the content was increased about 30% (wt/wt) relative to wild-type cell walls (Fig. 4A). Also, ser1 er-1 and ser2 er-1 plants showed lower levels of uronic acids than er-1 and their cellulose content was slightly reduced compared with that of er-1 plants (Fig. 4B).

To further characterize ser er-1–associated cell-wall modifications, we used immunodetection of cell-wall carbohydrate epitopes present in different polysaccharides. Though this type of qualitative analysis may be affected by differential accessibility of the antibodies to specific epitopes due to structural modifications of cell-wall polysaccharides, immunodetection may allow the determination of a differential pattern distribution of carbohydrate epitopes in the cell walls of the mutants and wild-type plants (Fig. 4A and B). Immunodetection showed that the pattern of (1→3)-β-D-galactan and (1→5)-α-L-arabinan epitopes was relatively similar in ser2 er-1, ser1 er-1, and er-1 leaves (Fig. 4C). The higher levels of neutral sugars together with the immunolabeling patterns found in the ser er-1 mutants, especially in ser2 er-1, suggests that rhamnogalacturonan-I (RG-I) accumulates to higher levels in the ser2 er1 mutant and to modestly higher levels in the ser1 er-1 mutant, compared with er-1 plants. In addition, the pattern of a xylogalacturonan epitope, which is found in xylem vessels (Willats et al. 2004), was found to be slightly different in ser1 er-1 and ser2 er-1 than in wild-type and er-1 vascular bundles (Fig. 4C). Even though differences in fucose levels were observed between er-1 and the ser er-1 mutants (Fig. 4A), no differences among wild-type plants, er-1, and the ser er-1 mutants were detected for the terminal nonreducing (1→2)-linked α-L-Fucp units (data not shown) usually present on the xyloglucan backbones and in RG-I polymers (Puhllmann et al.

**Fig. 3.** Fourier transform infra red (FTIR) analysis of wild-type and mutant plant cell walls. A, FTIR averaged spectra (n = 15) from 3-week-old rosettes of wild-type plants (WT, red line) and er-1 (blue line). B, Biplot showing the separation of WT (white open circles) and er-1 (closed circles) samples. C, First principal component (PC1) from the covariance-matrix separation of the full infrared data sets WT and er-1. D, FTIR averaged spectra (n = 15) from 3-week-old rosettes of wild-type (red), er-1 (dark blue), ser1 er-1 (green), and ser2 er-1 (light blue) cell walls. E, Biplot showing the separation of wild-type (white, open circles), er-1 (black, closed circles), ser1 er-1 (gray-black circles), and ser2 er-1 (black-gray circles) samples. F, PC1 of the full infrared data sets obtained for the four genotypes shows clear differences between the er-1, wild-type, ser1 er-1, and ser2 er-1 genotypes. In B, C, E, and F, mid-infrared spectra were analyzed by the covariance-matrix approach for PC analysis (Kemsley 1996).
To determine if the differences in the uronic acid levels occurred in the homogalacturonan (HG) cell-wall fraction, we used the antibody 2F4, which labels Ca\textsuperscript{2+} crosslinked HG (Guillemin et al. 2005), but no differences were detected (data not shown).

All together, these analyses revealed that ser mutations restored some features of the er-1 cell wall to that of wild-type plants but also introduces additional cell-wall changes, further suggesting a direct correlation between cell-wall composition and structure and plant resistance to the necrotroph *P. cucumerina*.

**Arabidopsis cell-wall composition is a resistance determinant.**

Recent genetic studies have shown that variation in cell-wall composition can result in altered disease resistance outcomes with various pathogens (Ellis and Turner 2001; Hernández-Blanco et al. 2007; Osorio et al. 2008; Vogel et al. 2002, 2004). However, a direct correlation between cell-wall composition and structure and pathogen resistance has not been demonstrated. As shown in Figure 5, a positive correlation ($r = 0.9; P = 0.026$), explained by a logarithmic-X nonlinear model, was found between the *P. cucumerina* biomass and the uronic acids.

**Fig. 4.** Composition analysis of wild-type, er-1, and ser er-1 cell walls. A, Quantification of total and individual neutral sugars (milligrams per gram of dry weight) from the noncellulosic carbohydrate fraction and B, of the cellulose and total uronic acids content (milligram per gram of dry weight) of the cell walls from wild-type (white bar), er-1 (gray bar), ser1 er-1 (green bar), and ser2 er-1 (blue bar). Data represent average values ($\pm$ standard error) of three (A) or two (B) replicates. Statistical analysis of the data was performed using analysis of variance ($P \leq 0.05$) and the Bonferroni post hoc test. C, Confocal images of leaf cross-sections showing the in situ localization of different pectin epitopes in the indicated genotypes. Specific monoclonal antibodies were used: LM-5, which detects (1->4)-β-D-galactan (Jones et al. 1997); LM-6, which detects (1->5)-α-L-arabinan (Willat et al. 1998); and LM-8, which detects xylogalacturonan (Willats et al. 2004). Images are representative pictures from 15 different plants. In some cases, autofluorescence derived from waxes in the abaxial epidermis was detected. Scale bar represents 100 μm.
content in the \textit{er-1}, \textit{ser er-1}, and wild-type cell walls. Based on this analysis, the modified cell-wall uronic acids content accounted for 94\% of the variation in resistance to \textit{P. cucumerina} of the genotypes tested. Similar analyses were performed with additional cell-wall parameters, such as cellulose or noncellulosic neutral sugars contents. No correlation was found between resistance to the fungus and cellulose content in the genotype tested \((r = 0.4; P = 0.6)\), whereas a weak, negative correlation \((r = 0.83\) and \(P = 0.17\)) was identified for noncellulosic neutral sugars content (Supplementary Fig. 4).

We next asked whether the susceptibility to \textit{P. cucumerina} of \textit{er} alleles would be suppressed by additional cell-wall mutations, such as the \textit{irx1} mutant (Hernández-Blanco et al. 2007). Susceptibility of the \textit{er-1} mutant was restored to almost wild-type (La-0) levels in the \textit{er-1 irx1} double mutant (Fig. 6A). Similar results were obtained in a different genetic background (Col-0) by comparing the susceptibility to \textit{P. cucumerina} of wild-type plants, the \textit{er-115} mutant, and the \textit{irx1-6 er-115} double mutant (Fig. 6A). In contrast to the \textit{irx1-1 er-1} double mutant, the \textit{ser er-1} mutants were not more resistant to the soilborne bacteria \textit{R. solanacearum} (Table 1) (Hernández-Blanco et al. 2007). These results suggest that the enhanced susceptibility to necrotrophic pathogens of \textit{er} mutants may be related with specific alterations of their cell wall.

The \textit{irx}-mediated resistance has been demonstrated partially or distinct. Here, we show that at least some genetic components of one or more ER-associated signaling pathways regulating immunity and development were identical with the oomycete \textit{Hyaloperonospora arabidopsis} or after treatment with \textit{fig22} (Gomez-Gomez et al. 1999; Llorente et al. 2005). This selective effect on \textit{P. cucumerina}–induced callose accumulation in \textit{er-1} mutants suggests that ER potentially sensitizes the plant callose response to this necrotrophic fungus. We decided to explore whether \textit{ser er-1} double mutants deposited callose at sites of \textit{P. cucumerina} infection. As shown in Figure 7, callose deposition following inoculation with \textit{P. cucumerina} was substantially higher in \textit{ser2 er-1} than in \textit{er-1} and wild-type plants and was more similar to wild-type in \textit{ser1 er-1} plants. In contrast, the deposition of callose upon wounding was found to be similar in all genotypes (Fig. 7), showing that the lack of callose accumulation in \textit{er-1} upon \textit{P. cucumerina} infections was not due to a defect in callose synthesis or deposition per se. The expression of the \textit{PMR4} gene, encoding a component of the pathogen- and wound-inducible callose synthase (Nishimura et al. 2003), was analyzed in the different genotypes under study; however, no difference in \textit{PMR4} expression upon infection was found (data not shown). Thus, an inverse relationship exists between the disease ratings for \textit{P. cucumerina} infections and callose deposition in the genotypes under study, suggesting that either ER has multiple independent pleiotropic effects on plants or that these features may be mechanistically linked through ER.

**DISCUSSION**

ER was initially identified as a LRR RLK that regulates development in several ways, including organ shape and floral architecture determination and stomatal patterning (Lease et al. 2001; Masle et al. 2005; Torti et al. 1996). More recently, ER has also emerged as a key regulator of basal resistance, as \textit{Arabidopsis} \textit{er} mutants (e.g., \textit{er-1}) display an enhanced susceptibility to different types of pathogens, including the bacterium \textit{R. solanacearum}, the fungus \textit{P. cucumerina} and the oomycete \textit{Phytophthora irregulare} (Adie et al. 2007; Godiard et al. 2003; Llorente et al. 2005). It was unknown whether the molecular components of one or more ER-associated signaling pathways regulating immunity and development were identical or distinct. Here, we show that at least some genetic components (e.g., \textit{SER1} and \textit{SER2}) of the ER signaling pathway regulate these biological processes in a opposite ways, as \textit{ser er-1} double mutants suppressed the disease susceptibility of \textit{er-1} plants (Fig. 1), while they enhanced the \textit{er}-associated developmental alterations (Fig. 2). Interestingly, the enhanced susceptibility of \textit{er-1} to \textit{R. solanacearum} was not modified by the \textit{ser} muta-
tions (Table 1), suggesting that specific defensive mechanisms effective against only a subset of pathogens might operate downstream of ER. The genetic mapping of the SER1 and SER2 genes indicate that ser phenotypes were not due to either

an intragenic mutation in the ER gene, a mutation in any of the already described ER genetic interactors (e.g., ERL1, ERL2, and TMM [Shpak et al. 2003, 2004, 2005]), nor a constitutive activation of the YUCCA5 gene, which restored the er-associated developmental alterations to wild-type morphology (Woodward et al. 2005). Thus, SER1 and SER2 are novel genetic components that deserve additional investigation.

Resistance to necrotrophic fungi such as P. cucumerina depends on the coordinate regulation of multiple signaling pathways, including the SA, ET, JA, and ABA pathways (Berrocal-Lobo et al. 2002; Hernández-Blanco et al. 2007; Llorente et al. 2005). Expression analysis of defense marker genes suggested that none of these pathways are constitutively activated in the ser1 er-1 and ser2 er-1 mutants (Figs. 1B and C and 6B). However, as it has been reported in the irx1-1 er-1 mutant (Hernández-Blanco et al. 2007), the CYP79B2 and CYP79B3 genes were constitutively expressed in ser1 er-1 (Fig. 6C), which may lead to enhanced accumulation of indole glucosinolate–derived metabolites and enhanced resistance to some pathogens, including G. cichoracearum (Table 1). In contrast, the PAD3 gene, required for plant resistance to several pathogens including the necrotroph B. cinerea (Ferrari et al. 2007), was not constitutively upregulated in ser mutants, indicating that ser-mediated resistance may not be dependent on an enhanced accumulation of camalexin in the ser er-1 mutants.

The cell wall has emerged as a dynamic and responsive structure that regulates plant responses to external stimuli or stresses (Humphrey et al. 2007). Recent genetic data show that specific changes in cell-wall composition can result in altered immune responses to different type of pathogens, including the necrotroph P. cucumerina and the biotroph G. cichoracearum (Ellis and Turner 2001; Hernández-Blanco et al. 2007; Vogel et al. 2002, 2004). Here, we show that impairment of ER in Arabidopsis resulted in alterations of cell-wall composition that may lead to changes in structure (Figs. 3 and 4). The content of noncellulosic neutral sugars, in particular those of the pectin fraction, possibly associated with RG-I structures, were significantly lower in the cell wall of er-1 than in that of wild-type plants (Fig. 4). However, the uronic acid content was clearly higher in er-1, possibly deriving from the partially methyl-esterified HG structures, since no differences in the nonesterified HG crosslinked by Ca2+ bridges (recognized by the 2F4 antibody) were detected. However, additional analyses will be required to corroborate this conclusion, as immunodetection results are qualitative. In addition, cellulose levels were also significantly increased in er-1 compared with those in wild-type plants (Fig. 4). Notably, cellulose levels have been shown to be reduced in the irx CESA mutants, which showed an enhanced resistance to several pathogens (Hernández-
Blanco et al. 2007). It is unclear whether all these er-1–associated cell-wall changes are a direct consequence of the inactivation of ER function or an indirect effect.

Interestingly, the ser1 and ser2 mutations partially restored to wild-type levels some of these changes in er-1 cell-wall composition and introduced some additional cell-wall changes, suggesting a direct linkage between cell-wall composition and resistance to P. cucumerina. The positive correlation found between uronic acids content of the cell walls from er-1, ser1 er-1, ser2 er-1, and wild-type plants and their levels of resistance to P. cucumerina highlights the importance in disease resistance against this pathogen of this metric of cell-wall composition over other cell-wall changes and suggests that cell-wall composition and, consequently, cell-wall structure is a determinant of the success of fungal colonization (Fig. 5).

In yeast, there is a mechanism controlling cell-wall integrity that is regulated by the WSC genes (for cell-wall integrity and stress response), which encode integral membrane proteins acting as surface sensors responding to environmental stress (Philip and Levin 2001). In plants, a similar sensing system to that of yeasts has been proposed, and several members of the CrRLK1L and WAK families have been suggested to function as cell-wall integrity sensors (Hématy and Höfte 2008). For example, the thel1 mutant attenuates the development-associated phenotypes of the primary cell-wall mutants pcr and elil (Hématy et al. 2007). Interestingly, some WAK RLK have been found to bind covalently cell wall–derived pectins (Humphrey et al. 2007), and THE1 has been suggested to detect cell-wall components that accumulate upon perturbation of cellulose synthesis (Hématy and Höfte 2008). It has been demonstrated that some Arabidopsis wak mutants show disease resistance alterations (Diener and Ausubel 2005; Wagner and Kohorn 2002). The soilborne bacterium Pseudomonas syringae and conidiospores from the oomycete Hyaloperonospora conglutinans were normalized and analyzed, using the PC analysis covariance matrix method (Kemsley 1996). The data presented here and published previously (Hématy and Höfte 2008; Xu et al. 2008) suggest that some RLK may be involved in the detection of pathogen- or development-induced perturbations of cell-wall integrity. The identification of the corresponding ligands and downstream effectors of these RLK cell-wall sensors is a future challenge in the plant field.

**MATERIALS AND METHODS**

**Plant growth and pathogenicity assays.** Arabidopsis plants were grown either in soil or in vitro (Hernández-Blanco et al. 2007). Spores from the fungi Plectosphaerella cucumerina, Botrytis cinerea, Fusarium oxysporum f. sp. conglutinana, Golovinomices cichoracearum UCSC1, and conidiospores from the oomycete Hyaloperonospora arabidopis (isolates Noco2, Cala2, and Emwa2) were collected as reported (Hernández-Blanco et al. 2007). The bacteria Pseudomonas syringae pv. tomato DC3000 were grown on nutrient broth at 28°C as described previously (Berrocal-Lobo et al. 2002). The soilborne bacterium R. solanacearum GM11000 was grown at 28°C in B broth medium (Boucher et al. 1985).

Plant infections with fungi, bacteria, and oomycete were performed as described (Hernández-Blanco et al. 2007). After inoculation, disease progression was estimated, depending on the pathogen, as average disease rating, bacterial growth, or conidiospore formation (Hernández-Blanco et al. 2007). All the pathogenicity assays were repeated at least three times and a minimum of 20 plants per genotype were inoculated in each experiment. Since the data of all the disease-resistance experiments were homocedastic, differences in these parameters according to Arabidopsis genotype were analyzed by one-way analysis of variance (ANOVA) using plant genotype as factor. To determine whether values of analyzed traits were significantly different among classes within each factor the Bonferroni post hoc test was employed.

For callose detection, 3-week-old plants were wounded, mock-inoculated or challenged with a spore suspension (4 × 106 spores/ml) of P. cucumerina, and callose staining was performed (Vogel and Somerville 2000).

**Isolation, mapping, and characterization of ser1 and ser2 mutants.** For the identification of ser mutants, about 10,000 10-day-old plants of an ethyl methanesulfonate M2 mutagenized population of Arabidopsis er-1 were sprayed with a concentration of 2 × 106 spores/ml of P. cucumerina. SER1 and SER2 genes were mapped using 300 and 20 selected mutant individuals from ser1 er-1 × Col-0 and ser2 er-1 × Col-0 F2 populations, respectively. New markers within the SER1 mapping interval on chromosome II were generated using the CEREON database of Col-0 and er-1 (Ler) polymorphisms. The ser2 plants were obtained by crossing ser2 er-1 × Lax-0, and selecting ser2 F2 individuals lacking the er-1 mutation. The derived oligonucleotide sequences used for mapping are showed in the Supplementary Table 1. For ER sequencing, RNA was extracted from 3-week-old plants and cDNA was synthesized using oligo(dT) as reported (Llorente et al. 2005). Oligonucleotides used for cDNA amplification and sequencing were 5′-TCTTC TTCATGGAACATTGAAAGC-3′, 5′-GAGGAGATTATGC GGTCCG-3′, 5′-GGCCCAATTCAGAAGACCT-3′, and 5′-TATAGGAACTAATGTCAGCT-3′. Determination of the pedicel and silique length of the genotypes analyzed was done as described by Torii and associates (1996). The first five fruits from ten 7-week-old (wild-type, er-1, and ser1 er-1) or 10-week-old plants (ser2 er-1) were measured.

**Cell-wall analyses.** For FTIR, precleared (by solvent extractions) dried leaves from at least 30 3-week-old individual plants per genotype were pooled and homogenized by ball milling. The powder was dried and mixed with potassium bromide and, then, pressed into 13-mm pellets. For each line, 15 FTIR spectra were collected on a Thermo Nicolet Nexus 470 spectrometer (ThermoElectric Corporation, Chicago) over a range of 4,000 to 400 cm-1. For each spectrum, 32 scans were co-added at a resolution of 4 cm-1 for Fourier transform processing and absorbance spectrum calculation, using OMNIC software (Thermo Nicolet, Madison, WI, U.S.A.). Using win-das software (Wiley, New York), spectra were baseline-corrected and normalized and analyzed, using the PC analysis covariance matrix method (Kemsley 1996).

Cell-wall monosaccharides were assayed as alditol acetate derivatives (Stevenson and Furneaux 1991) by gas chromatography performed on an Agilent 6890N gas chromatograph (Wilkinson, DE, U.S.A.), and the results obtained were validated with the method previously described by Gibert and
For carbohydrate epitope detection, intact leaves were sectioned by hand and were then fixed in 4% formaldehyde for 1 to 2 h. Cross-sections were washed in phosphate-buffered saline (PBS) and were then extracted in EtOH and rehydrated in series of EtOH-PBS until 1× PBS. Sections were blocked in PBS containing 5% (wt/vol) fat-free milk powder (5% milk phosphated buffer solution [MPBS]) for 30 min. Rat monoclonal antibodies specific for various carbohydrate epitopes were used: antibody LM-5, (1→4)-β-D-galactan (Jones et al. 1997); LM-6, (1→3)-α-L-rhamnose (Willats et al. 1994), and 2F4, Ca+2 crosslinked homogalacturonans (Liners et al. 1989). Sections were incubated with the primary antibody (dilution 1:50 to 1:1,000) in 5% MPBS 1 to 2 h at room temperature and were washed twice with PBS. To visualize the primary antibody binding, anti-rat immunoglobulin coupled to alkaline phosphatase, (1:1,000) in 5% MPBS was added for 1 h at room temperature. After washing with PBS for 5 min (two times), the samples (n = 20) were observed in a laser scanning confocal microscope. As a control, primary antibodies were omitted during the labeling procedure. Confocal imaging was performed using an MRC 1024 laser scanning confocal head (Bio-Rad, Hercules, CA, U.S.A.) mounted on a Diaphot 200 inverted microscope (Nikon, Tokyo), a Zeiss 510 laser scanning confocal microscope. The samples were excited with two lasers (Ar/Kr and He/Cd) at the following wavelengths: 568 nm for checking chlorophyll fluorescence and at 488 nm for detecting the secondary antibody coupled to Alexa fluor 488 dye. Three-dimensional reconstructions of image stacks (n = 5) were carried out using Image J version 1.34 software. All images were processed with Adobe Photoshop 7.0 (Adobe Systems, Mountain View, CA, U.S.A.) and were assembled with Illustrator (Adobe Systems, San Jose, CA, U.S.A.) software.

Expression profiling.
RNA extractions from *P. cedrusina*–infected or mock-inoculated plants were done as described by Llorente and associates (2005). Real-time qRT-PCR analyses were done as previously reported (Hernández-Blanco et al. 2007). Ubiquitin (AT5G25760) expression was used to normalize the transcript level in each sample. Oligonucleotides used for cDNA amplification were used: antibody LM-5, (1→4)-β-D-galactan, 5'-GGCGAGGCTTTTGCA-3' and 5'-TCTTTTCCCGGTA-3'; ATC TTC-3' and 5'-CGCGGTTTATGGTCAACGGAT-3'; ABI2 (AT5G57050), 5'-TCAGTTGCGCGAGTAAAAGA-3' and 5'-TCCG-TTCCCAGCGCAAATC-3'; LTP3 (AT5G59320), 5'-GAAG-GACATTTTCGTTGTCACAC-3' and 5'-GTGGCATGTT ACGTCTCATGGA-3'; NCE3 (AT3G14440), 5'-AACKCTCTTTG CCAACCAAGA-3' and 5'-CACGACCTGTGTTGTCGAT-3'; PMR4 (AT4G03550), 5'-TCCTGCGGGCTATGTGAC-3' and 5'-AACGACCATGTCACGCCTGTG-3'; PMR6 (AT4G02180), 5'-CCGGTTTATGGTCAACGGAT-3'; PR1 (AT2G14610), 5'-GGCGCAGCGCCGAGGAGCTTCCCACG-3' and 5'-CTGGACCAAGCATCGCTGTTTGAG-3'; PR1; (AT2G14610), 5'-CGCAGAAGCTCAAGATAGCCACA-3' and 5'-TTTCTGCGATGCTCGAGCAGTAC-3'; PDF1.2a (AT5G44420), 5'-TCTCTTGTGTGTCGTCTGGACG-3' and 5'-GACATGCAATCTGTGTCGCCA-3'; CYP79B2 (AT4G39950), 5'-CCGCGCCCCACCCCTAATA-3' and 5'-GCGCCACCCCTGTCATA-3'; CYP79B3 (AT2G22330), 5'-AGTCTA CCGAGGCTTTTGGCA-3' and 5'-CCACACCCAAAGGCT CGAA-3'; ABI1, (AT4G26080); 5'-TGTGTTCGCCGTTCTCAC

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**LITERATURE CITED**


