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An Arabidopsis Mutant with Enhanced Resistance to Powdery Mildew

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We have identified an Arabidopsis mutant that displays enhanced disease resistance to the fungus *Erysiphe cichoracearum*, causal agent of powdery mildew. The *edr1* mutant does not constitutively express the pathogenesis-related genes *PR-1*, *BGL2*, or *PR-5* and thus differs from previously described disease-resistant mutants of Arabidopsis. *E. cichoracearum* conidia (asexual spores) germinated normally and formed extensive hyphae on *edr1* plants, indicating that the initial stages of infection were not inhibited. Production of conidiophores on *edr1* plants, however, was <16% of that observed on wild-type Arabidopsis. Reduction in sporulation correlated with a more rapid induction of defense responses. Autofluorescent compounds and callose accumulated in *edr1* leaves 3 days after inoculation with *E. cichoracearum*, and dead mesophyll cells accumulated in *edr1* leaves starting 5 days after inoculation. Macroscopic patches of dead cells appeared 6 days after inoculation. This resistance phenotype is similar to that conferred by "late-acting" powdery mildew resistance genes of wheat and barley. The *edr1* mutation is recessive and maps to chromosome 1 between molecular markers *ATEAT1* and *NCC1.* We speculate that the *edr1* mutation derepresses multiple defense responses, making them more easily induced by virulent pathogens.

INTRODUCTION

Plants defend themselves against pathogens through both preformed and inducible resistance mechanisms. Among the inducible responses, the hypersensitive resistance (HR) response and systemic acquired resistance (SAR) have been the most intensively studied. The HR is a localized plant response characterized by a suite of physiological changes culminating in plant cell death and cessation of pathogen growth (Goodman and Novacky, 1994). SAR is a systemic resistance response that is induced after formation of a necrotic lesion, either as part of the HR or as a symptom of disease (Ryals et al., 1996). Although the HR and SAR have been the major forms of induced plant resistance studied, evidence for other resistance mechanisms exists (Penninckx et al., 1996; Pieterse et al., 1996). The molecular mechanisms by which HR, SAR, and other resistances are induced are poorly understood. We and other investigators have been screening for mutations that either enhance or compromise induced resistance mechanisms with the expectation that such mutations will provide insight into how resistance is regulated (Kunkel, 1996).

The HR can be induced by the interaction between a plant resistance gene and a matching pathogen avirulence gene. Such gene-for-gene interactions provide a narrow range of resistance because they differentiate between races of a pathogen based on expression of a specific avirulence gene (Flor, 1971). Resistance gene products are thought to function as receptors for ligands produced directly or indirectly by avirulence genes (Staskawicz et al., 1995; Bent, 1996). Multiple biochemical events are associated with the HR, including an oxidative burst, K/Cl ion exchange, deposition of autofluorescent compounds and callose in the cell wall, synthesis of antimicrobial phytoalexins, and cell death (Goodman and Novacky, 1994). How the putative interaction of avirulence gene products and resistance gene products leads to activation of these responses is not understood.

Induction of SAR depends on salicylic acid (SA) because transgenic plants unable to accumulate SA are also unable to induce SAR (Gaffney et al., 1993; Delaney et al., 1994). In Arabidopsis, SAR is associated with the expression of three pathogenesis-related genes: *PR-1* (unknown function), *BGL2* (β-glucanase, also known as *PR-2*), and *PR-5* (a thaumatin-like protein) (Uknes et al., 1992). Arabidopsis mutants identified based on constitutive expression of *PR* genes (*cpr1* and *cpr5*) are resistant to the fungal pathogen *Peronospora parasitica* and the bacterial pathogen *Pseudomonas syringae* pv *maculicola* (Bowling et al., 1994, 1997). Other mutants that constitutively express *PR* genes have been isolated based on the development of spontaneous leaf lesions that are similar in appearance to the lesions of an HR disease (Greenberg et al., 1994; Dietrich et al., 1995). These lesion mimic mutants also show resistance to both fungal and bacterial pathogens.

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Methyl jasmonate and ethylene may induce a defense pathway that is independent of SA. Wounding as well as pathogen attack induce the production of jasmonic acid, which in turn induces defense genes other than those associated with SAR, including genes that encode defensins (Penninckx et al., 1996) and thionins (Epple et al., 1995). Defensins and thionins are low molecular weight polypeptides that have potent antimicrobial activity in vitro (Broekaert et al., 1995; Rao, 1995). Arabidopsis plants overexpressing endogenous thionin have increased resistance to the fungal pathogen *Fusarium oxysporum* (Epple et al., 1997). Transgenic Arabidopsis plants unable to accumulate SA and thus unable to express SAR are able to respond to the jasmonic acid signal and express both defensin and thionin genes (Penninckx et al., 1996; Epple et al., 1997). Mutants that constitutively express the proposed jasmonic acid pathway, but not the SA pathway, have not been reported; however, the *cpr5* and *acd2* mutants of Arabidopsis constitutively express both *PR* genes and defensin (Greenberg et al., 1994; Penninckx et al., 1996; Bowling et al., 1997).

Another defense pathway that is independent of SA is induced by the biocontrol bacterium *Pseudomonas fluorescens* and is termed induced systemic resistance (ISR) (Pieterse et al., 1996). ISR is observed when Arabidopsis plants grown in soil containing *P. fluorescens* are challenged with virulent bacterial and fungal pathogens. Under these conditions, the Arabidopsis plants develop less severe disease symptoms than do control plants grown in soil alone. ISR is not associated with the expression of *PR* genes and is observed in plants unable to accumulate SA, indicating that this pathway is independent of SAR (Pieterse et al., 1996). It has not been determined whether the proposed jasmonic acid pathway contributes to ISR.

We have undertaken a genetic approach to identify defense pathways that are independent of SAR. Specifically, we screened for Arabidopsis mutants that displayed enhanced disease resistance (reduced susceptibility) without constitutive *PR* gene expression (see below). Screens for plant mutants that display enhanced resistance to virulent pathogens have been performed with several crop species. From these studies, barley resistant to powdery mildew (the *mlo* mutation; Jorgensen, 1976), sugarcane resistant to smut (Jagathesan, 1982), mulberry resistant or tolerant to nematodes (Fujikata and Wada, 1982), mulberry resistant to Dogare disease (Nakajima, 1973), and peppermint resistant to Verticillium wilt (Murray, 1969) were identified. Of these, only the *mlo* resistance has been well characterized. The *mlo* mutation of barley mediates resistance to all common races of the powdery mildew fungus *Erysiphe graminis* f sp *hordei* and thus provides a broader spectrum resistance than do the gene-for-gene type of resistance genes (Jorgensen, 1992). Resistance in *mlo* mutants correlates with the formation of cell wall appositions that may prevent fungal penetration (Jorgensen, 1992; Wolter et al., 1993) and with plant cell death (Peterhänsel et al., 1997). Defense genes are not constitutively expressed in *mlo* mutant barley; however, they appear to be induced more rapidly upon infection by *E. graminis* (Peterhänsel et al., 1997). The wild-type *Mlo* gene has been cloned and is hypothesized to be a negative regulator of defense responses such that mutant *mlo* alleles mediate resistance by allowing abnormal defense responses to occur both spontaneously and during an *E. g. hordei* infection (Wolter et al., 1993; Büschges et al., 1997).

Although the resistance observed in *mlo* barley affects multiple races of *E. g. hordei*, it does not affect the virulence of rust fungal pathogens (Jorgensen, 1992). It is unclear why resistance mediated by *mlo* does not affect pathogens other than powdery mildew, but it may be related to the cell type attacked by the pathogen. *E. g. hordei* infects only living epidermal cells, whereas rust pathogens infect mesophyll cells (Jorgensen, 1992).

We screened for Arabidopsis mutants that displayed enhanced resistance (reduced susceptibility) to the bacterial pathogen *P. s.* pv *tomato* DC3000 in the absence of constitutive expression of *PR-1.* Here, we describe our screen and the initial characterization of one mutant that displays enhanced resistance to the DC3000 strain of *P. s.* pv *tomato* and to the fungal pathogen *E. cichoracearum.*

RESULTS

Isolation of Arabidopsis Mutants Resistant to Disease

Arabidopsis ecotype Columbia (Col-0) is susceptible to *P. s. tomato* DC3000. Disease symptoms develop on rosette leaves 4 to 5 days after inoculation and appear as gray lesions surrounded by chlorosis (Whalen et al., 1991). To identify Arabidopsis mutants with reduced susceptibility, we inoculated mutagenized Col-0 plants (M_2 generation) with *P. s. tomato* DC3000 and scored for disease lesion severity 4 to 5 days later. Plants were inoculated with a high dose of *P. s. tomato* DC3000 (10^9 colony-forming units per mL), because at this concentration, susceptible plants frequently died, facilitating the identification of living resistant plants. Living plants displaying a decrease in the severity of disease symptoms were selected for further analysis. Reduced disease symptoms included fewer leaves showing disease, smaller lesions, and a lack of lesions on inner rosette leaves. Approximately 25,000 mutagenized Col-0 plants were screened, and 78 putative mutants were selected. The mutant phenotype of decreased disease symptoms was found to be heritable in 36 of the 78 plants selected.

To determine whether reduced symptoms correlated with reduced bacterial growth, we quantified bacterial numbers in leaves over a 4-day period. Of the 36 mutants, 25 showed a reduction in bacterial growth in the leaves compared with wild-type Col-0 plants (data not shown).

To eliminate mutants that were constitutively expressing SAR, we analyzed expression of the *PR-1* gene in uninoculated plants. *PR-1* gene expression was assayed using RNA

gel blot analysis. Of 19 mutants analyzed (six were not tested), six displayed strong expression of *PR-1*, seven displayed weak expression, and six did not have detectable expression of *PR-1* (data not shown). The latter six mutants represent a novel class because they are less susceptible to a virulent pathogen by a mechanism independent of constitutive expression of SAR. These mutants have been termed enhanced disease resistant (*edr*).

To determine whether any of the *edr* mutants displayed broad-spectrum disease resistance, we tested them for resistance to *E. cichoracearum*, causal agent of powdery mildew. Arabidopsis ecotype Col-0 is susceptible to the UCSC strain of *E. cichoracearum*, developing the macroscopic disease symptoms of powdery mildew (a white powder resulting from production of asexual spores; Adam and Somerville, 1996) on the leaves 7 to 10 days after inoculation. One of the six *edr* mutants displayed resistance to *E. cichoracearum*, developing almost no visible powder. Here, we provide a phenotypic and genetic analysis of this mutant, which we have designated *edr1.* The five *edr* mutants that were not resistant to *E. cichoracearum* will be described in a subsequent report.

E. cichoracearum Is Arrested at a Late Stage of the Infection Process in *edr1* Plants

The infection process of *E. cichoracearum* on Arabidopsis has been described by Adam and Somerville (1996). Spores first produce appressorial germ tubes that penetrate the underlying epidermal cells. Inside the epidermal cells, the fungus forms a haustorium, which is a baglike invagination surrounded by host cell plasma membrane. Fungal development then proceeds via formation of secondary hyphae and haustoria and terminates with formation of conidiophores (stalks of asexual spores) 5 to 7 days after infection. It is these conidiophores that produce the "powdery" appearance for which the disease is named.

Approximately 7 days after inoculation with asexual spores of *E. cichoracearum*, wild-type Arabidopsis plants displayed abundant conidiophores (visible white powder) on mature plant leaves. As shown in Figure 1A, *edr1* plants displayed strong disease resistance to *E. cichoracearum.* Starting 6 days after inoculation, the mature leaves of *edr1* first became slightly chlorotic and then developed distinct necrotic and collapsed regions. Over the next 3 days, the necrosis spread to consume large portions of the leaf (Figure 1B). During this same time period, wild-type Col-0 leaves displayed abundant conidiophores with some chlorosis but no necrosis. Visible necrosis in *edr1* plants began just before the development of visible powder on wild-type Col-0 plants. The *edr1* mutant developed only small scattered patches of powder.

To determine the stage of fungal development that was affected on *edr1* plants, we stained infected leaves at various time points with trypan blue, which detects both fungal structures and dead plant cells (Koch and Slusarenko, 1990). As shown in Table 1, on both Col-0 and *edr1* leaves, ~60% of the spores developed appressorial germ tubes 1 day after inoculation. By 3 days after inoculation, *E. cichoracearum* spores developed extensive branched hyphae with secondary germ tubes that invaded underlying epidermal cells. The average length of hyphae did not differ between germlings on *edr1* leaves and Col-0 leaves (Table 1).

As shown in Figures 1C and 1D, by 5 days after inoculation, *E. cichoracearum* developed extensive hyphal growth that nearly covered the leaf surface on both *edr1* and wild-type Col-0 plants. By day 7, *E. cichoracearum* developed abundant conidiophores on wild-type Col-0 plants (Figure 1E); however, these structures were severely reduced in number on *edr1* leaves (Table 1 and Figure 1F). The conidiophores that were present on *edr1* often were not septated and appeared to be underdeveloped compared with those on wild-type Col-0 leaves at the same time point (data not shown). These observations indicate that *E. cichoracearum* development is arrested just before formation of conidiophores, a relatively late stage in the infection process.

Defense Responses Are More Strongly Induced in *edr1* Plants

The necrotic patches observed on *edr1* plants after infection with *E. cichoracearum* indicated that cell death was occurring. Therefore, we determined whether *edr1* plants displayed microscopic patches of dead cells before pathogen exposure, as has been reported for *mlo* and lesions simulating disease (*lsd*) mutants (Dietrich et al., 1995; Peterhänsel et al., 1997). Dead cells were visualized using trypan blue staining (Koch and Slusarenko, 1990). No difference between *edr1* and wild-type plants was observed before pathogen exposure (data not shown). Five days after inoculation with *E. cichoracearum*, both *edr1* and wild-type Col-0 plants displayed small scattered groups of dead cells that did not correlate with the presence or absence of fungal hyphae. Leaves from *edr1* plants, however, also contained large clusters of dead mesophyll cells (>30 cells) that were invariably associated with areas of dense hyphal growth (Figures 1D and 1G). Large clusters of dead cells were not observed in wild-type Col-0 leaves (Figure 1C).

Plant cells undergoing an HR accumulate callose and autofluorescent compounds in the cell wall (Goodman and Novacky, 1994). To determine whether the necrosis observed in *edr1* plants shared the biochemical properties of an HR, we assayed infected leaves for deposition of autofluorescent compounds and for callose. Both wild-type Col-0 and *edr1* plants displayed punctate staining of callose in the cell walls of epidermal cells ~3 days after inoculation (Figure 1H and data not shown); however, only *edr1* showed callose staining in large clumps of mesophyll cells (Figure 1H). The bright punctate staining observed in epidermal cells of both Col-0 and *edr1* is absent in noninoculated plant leaves (data

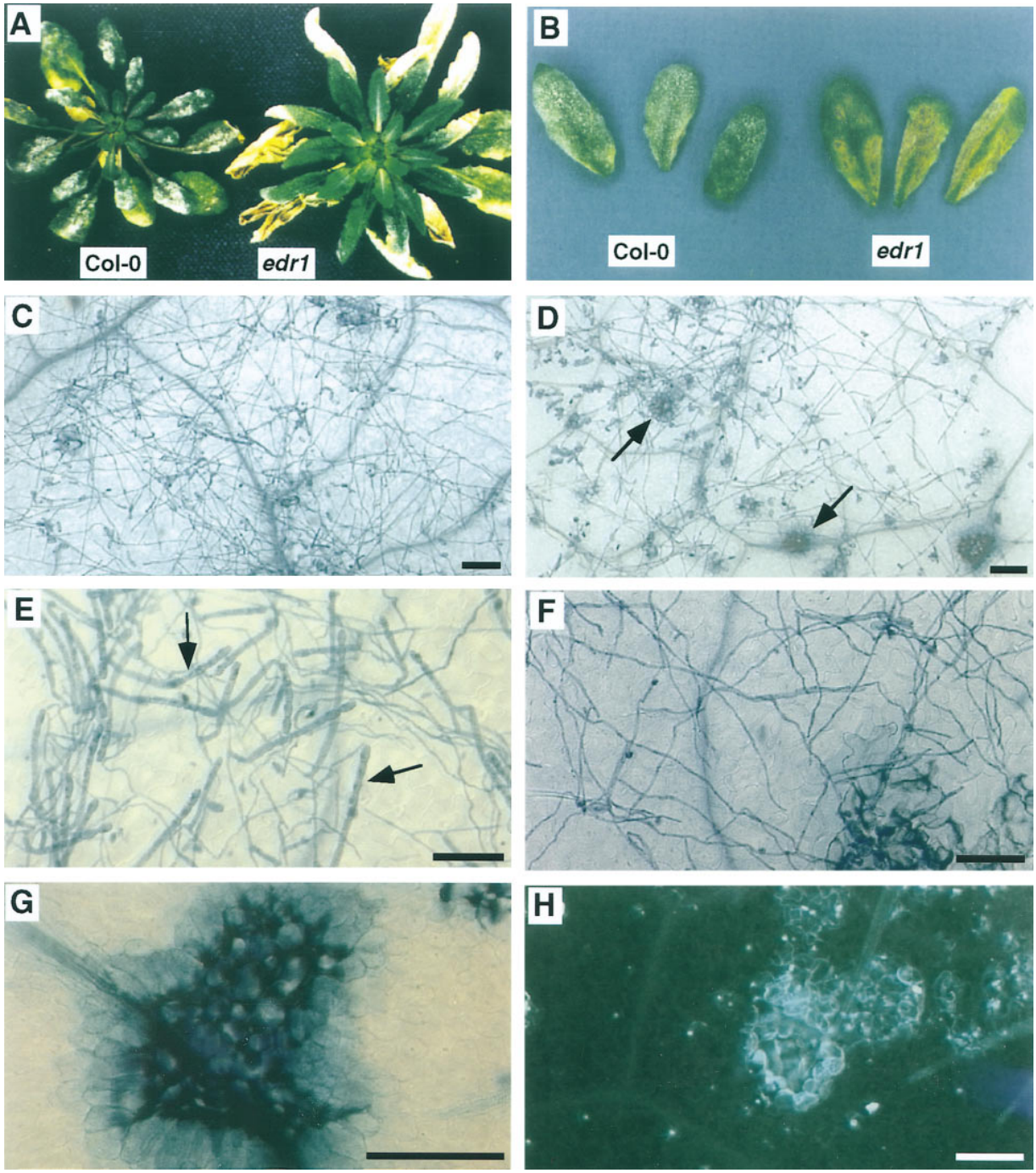

Figure 1. Response of Arabidopsis Wild-Type and *edr1* Plants to *E. cichoracearum.*

(A) Wild-type Col-0 and *edr1* plants 8 days after inoculation. Disease symptoms included white powder on Col-0 leaves. The lower leaves of *edr1* plants displayed regions of chlorosis and necrosis.
(B) Individual leaves removed from Col-0 and *edr1* plants 8 days after inoculation.
(C) Secondary hyphae on the surface of a wild-type Col-0 leaf 5 days after inoculation (stained with trypan blue). Bar = 100 μm.
(D) Secondary hyphae on the surface of an *edr1* leaf 5 days after inoculation. Arrows indicate dead mesophyll cells (stained with trypan blue). Bar = 100 μm.

Table 1. *E. cichoracearum* Development on Wild-Type Col-0 and *edr1* Leaves

Plants	Stage of Development		
	Germination[a] (%)	Hyphal Length[b] (mm)	Conidiophores/ mm Hyphae[c]
Col-0	65.3 (49)	1.99 ± 0.2 (16)	2.47 ± 0.34 (7)
edr1	66.0 (50)	1.86 ± 0.2 (18)	0.38 ± 0.12 (12)

[a] Asexual spore germination measured 1 day after inoculation. Numbers within parentheses indicate the number of spores.
[b] Total length of secondary hyphae per germling measured 3 days after inoculation. Values are expressed as the mean ±SE; numbers within parentheses indicate the number of germlings.
[c] Measured 5 days after inoculation. Values are expressed as the mean ±SE; numbers within parentheses indicate the number of microscopic fields.

not shown) and probably represents a collar of callose-containing plant material. Other investigators have demonstrated that susceptible and resistant plants respond to fungal penetration by generating a callose-containing papilla at the infection sight (Skou et al., 1984).

In susceptible plants (and in some resistant plants; see Discussion), the fungus penetrates through papillae that subsequently become a collar around the penetration peg (Skou et al., 1984). Callose deposition in the mesophyll cells of *edr1* is evident 3 days after inoculation with *E. cichoracearum*, which is prior to the appearance of dead cells. The pattern of autofluorescence was similar to that observed for callose (data not shown). Autofluorescing mesophyll cells accumulated in *edr1* leaves beginning 3 days after inoculation. Col-0 leaves showed only scattered epidermal cells autofluorescing at the same time point.

Analysis of *PR* Gene Expression

One of the criteria used to identify the *edr1* mutant was the lack of constitutive *PR-1* gene expression. It was possible, however, that the enhanced resistance of *edr1* was mediated by a more rapid or stronger induction of SAR or of SAR-associated genes other than *PR-1.* Therefore, we used RNA gel blot analysis to assay for expression of three SAR-associated genes during infection by *E. cichoracearum.*

As shown in Figure 2, little to no *PR-1* and *BGL2* mRNA was detectable before inoculation or 1 day after infection. By 3 days after infection, significant levels of *PR-1* and *BGL2* were observed in both wild-type and *edr1* plants. We quantified the levels of mRNA detected by using a PhosphorImager. The level of *PR-1* message in *edr1* leaves at day 3 was approximately four times higher than in Col-0 leaves. *PR-1* and *BGL2* transcript levels increased at days 5 and 7 after infection, but the relative difference between *edr1* and wild-type plants was less (Figure 2). By day 7, *PR-1* levels were higher in wild-type Col-0 than in *edr1.* Analysis of *BGL2* and *PR-5* transcript levels also revealed a small but reproducible increase in *edr1* plants relative to wild-type Col-0 on days 3 and 5 after inoculation.

Genetic Analysis of *edr1*

To determine the inheritance of the enhanced resistance phenotype, the *edr1* mutant was crossed with Arabidopsis ecotype Landsberg *erecta* (L*er*), which is susceptible to *E. cichoracearum.* The F_2 progeny were inoculated with *E. cichoracearum* conidia and scored 7 to 9 days later for development of necrotic lesions and lack of visible powdery mildew. These two traits cosegregated and behaved as a recessive mutation, producing approximately a 1:3 ratio of resistant-to-susceptible plants (85:266; $\chi^2 = 0.115$; $P > 0.1$).

To obtain a chromosomal map position for the mutation in *edr1* plants, a total of 1223 F_2 plants from the L*er* cross were scored for *E. cichoracearum* resistance, and 235 plants displaying resistance to *E. cichoracearum* were selected for mapping. DNA was isolated from the resistant F_2 plants and analyzed for linkage to simple sequence length polymorphism (SSLP) and codominant amplified polymorphic sequence (CAPS) markers (Konieczny and Ausubel, 1993; Bell and Ecker, 1994). The *edr1* mutation mapped 3.2 centimorgans centromeric from the SSLP marker *ATEAT1* (15 recombinant chromosomes) and 0.85 centimorgans telomeric from the CAPS marker *NCC1* (four recombinant chromosomes) on chromosome 1.

Because the *edr1* mutant was originally selected for its resistance to *P. s. tomato* DC3000, we wished to determine whether the bacterial resistance cosegregated with the fungal

Figure 1. (continued).
(E) Conidiophores (arrows) and secondary hyphae on the surface of a wild-type Col-0 leaf 7 days after inoculation (stained with trypan blue). Bar = 100 μm.
(F) Secondary hyphae on the surface of an *edr1* leaf 7 days after inoculation. Note the lack of conidiophores (stained with trypan blue). Bar = 100 μm.
(G) A patch of dead mesophyll cells in an *edr1* leaf 5 days after inoculation (stained with trypan blue). Bar = 100 μm.
(H) Callose deposition in mesophyll cell walls of an *edr1* leaf 3 days after inoculation (stained with aniline blue). Bar = 100 μm.

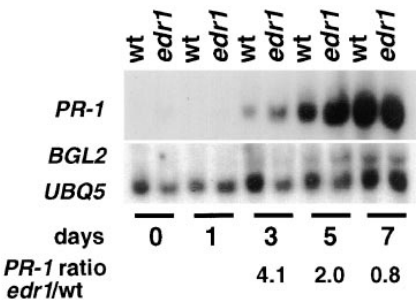

Figure 2. Time Course of *PR* Gene Expression in Arabidopsis Infected with *E. cichoracearum.*

An RNA gel blot was hybridized with *BGL2* and *UBQ5* (ubiquitin) probes and then stripped and rehybridized with a *PR-1* probe. *PR-1* expression is reported as a ratio of *edr1* to wild-type (wt) mRNA at 3, 5, and 7 days after inoculation (see Methods). This blot is representative of at least three independent experiments.

resistance. Therefore, we assayed resistance in 20 F_3 families derived from a backcross to Col-0. Although resistance to *E. cichoracearum* was robust and easily scored, resistance to *P. s. tomato* DC3000 was too weak to be scored reliably in these families. Thus, we were unable to determine whether bacterial resistance is caused by the *edr1* mutation.

DISCUSSION

The *edr1* mutant displays enhanced resistance to powdery mildew, but it does not constitutively express pathogenesis-related genes, such as *PR-1* and *BGL2.* The latter observation indicates resistance is being conferred by a mechanism that differs from previously described Arabidopsis disease-resistant mutants (Bowling et al., 1994, 1997; Greenberg et al., 1994; Dietrich et al., 1995). Our data show that multiple defense responses are induced more rapidly in *edr1* plants than in wild-type plants when infected with a virulent strain of powdery mildew. These observations suggest that the *edr1* mutation leads to a "hair trigger" inducibility of these responses.

We obtained the *edr1* mutant by screening for plants that displayed enhanced resistance to the bacterial pathogen *P. s. tomato* DC3000, suggesting that the *edr1* mutation also enables a more rapid defense response against bacteria. However, the resistance to *P. s. tomato* DC3000 was variable, and we were unable to show that bacterial resistance cosegregated with the *edr1* mutation in either a backcross to wild-type Col-0 or a mapping cross with L*er.* The *edr1* mutant also displayed variable resistance to a second strain of *P. syringae*, *P. s. maculicola* M2 (data not shown). These observations suggest that *edr1*-mediated resistance to *P. syringae* is influenced by unknown environmental factors.

The weak effect of the *edr1* mutation on *P. syringae* infection compared with its effect on *E. cichoracearum* might be related to the different modes of infection of these pathogens. *P. syringae* colonizes the intercellular spaces of the leaf mesophyll, reaching maximum population levels within 2 to 3 days after infection. In contrast, *E. cichoracearum* remains on the leaf surface and does not produce spores until 6 to 7 days after infection. The defense responses induced in *edr1* plants by these pathogens may be too little too late to affect *P. syringae* significantly, but they are sufficient to prevent *E. cichoracearum* sporulation, which occurs late in fungal development. Alternatively, *E. cichoracearum* may be a stronger inducer of defense responses in *edr1* plants than is *P. syringae.*

To further evaluate the spectrum of resistance mediated by the *edr1* mutation, we have sent this mutant to colleagues in other laboratories for assay with additional pathogens. No enhanced resistance to several strains of *P. parasitica* (an oomycete fungus) was observed (J. McDowell, personal communication). As with the weak resistance to *P. syringae*, this observation may reflect *P. parasitica*'s site of infection; haustoria are produced in mesophyll cells rather than epidermal cells (Dangl et al., 1992). Perhaps the *edr1* mutation sensitizes these two cell types differently.

In contrast to the *P. parasitica* result, the *edr1* mutant was found to be resistant to a second species of *Erysiphe*, *E. cruciferarum* UEA1. This pathogen induced a resistance phenotype essentially the same as that induced by *E. cichoracearum*, including mesophyll cell death associated with a dramatic decrease in conidiophore production (J. Turner and X. Shunyuan, personal communication). This result suggests that the *edr1* mutation confers broad-spectrum rather than race-specific resistance against powdery mildew.

Resistance to *E. cichoracearum* in *edr1* plants is manifested at a relatively late stage in the infection. *E. cichoracearum* spores germinated on the leaf surface and developed extensive networks of secondary hyphae on both *edr1* and wild-type Col-0 plants. Asexual reproduction was dramatically reduced on *edr1* plants; both the number of conidiophores formed as well as the number of conidia that make up each conidiophore were decreased. These observations suggest that the *edr1* resistance response affects the fungus primarily after onset of conidiophore formation at day 4.

Consistent with the observed effect on fungus development, we did not detect enhanced defense responses in *edr1* plants until 3 days after infection with *E. cichoracearum.* The earliest response detected was deposition of callose and autofluorescent compounds in epidermal cells and underlying mesophyll cells. We also observed a slightly enhanced expression of *PR* genes at day 3; however, cell death was not observed until 5 days after inoculation.

The defense responses observed in *edr1* plants are slow compared with that conferred by most classical disease resistance genes. For example, barley plants containing the *Mla1* gene induce a single cell HR within 14 hr of infection by an avirulent strain of *E. g. hordei*, preventing the fungus

from forming secondary hyphae (Boyd et al., 1995). Not all powdery mildew resistance genes confer a rapid HR, however. The resistance phenotype of *edr1* plants to *E. cichoracearum* is similar to the phenotypes conferred by the *Pm2* and *pm5* genes of wheat and the *Mla3* and *Mla7* genes of barley. These resistance genes affect powdery mildew growth after the development of secondary hyphae but before conidiophore production, and they are associated with the accumulation of large masses of dead mesophyll cells (Hyde and Colhoun, 1975; Boyd et al., 1995). In addition, plants with rapidly acting resistance genes occasionally allow fungal germlings to form secondary hyphae. Growth of such escapees is usually halted before conidiophore formation and is associated with mesophyll cell death (Hyde and Colhoun, 1975).

Mesophyll cell death thus appears to correlate with late activation of defense responses. Because *Erysiphe* spp do not infect mesophyll cells, activation of cell death in this layer must be triggered by a signal that is transmitted from the epidermal cells. We speculate that production of this signal requires the presence of a functional haustorium in the overlying epidermal cell. Accordingly, when the HR arrests fungal development before or shortly after formation of the haustorium, only the infected epidermal cell dies. If the fungus is not arrested at this stage, however, the signal reaches the mesophyll cell layer, triggering cell death in more cells.

The similarity between the *edr1* resistance phenotype and that conferred by late-acting resistance genes suggests that the *edr1* phenotype is mediated by normal defense pathways. How the *edr1* mutation enables such activation is not clear. A possibly related phenomenon has been reported in maize. Three novel alleles of the *Rp1* disease resistance gene, *Rp1-D21*, *Rp1-MD19*, and *Rp1-NC3*, have been shown to confer a non-race-specific resistance response to rust (*Puccinia sorghi*), and the resistance is correlated with extensive host cell necrosis (Hu et al., 1996; Hulbert, 1997). The lesions do not appear on axenically grown plants, indicating that the response is triggered by a biotic stimulus (Hulbert, 1997). Because these three alleles arose from recombination within the *Rp1* complex, it is tempting to speculate that a recombination event has altered one or more disease resistance genes, and the resulting gene(s) has lost specificity and gained a hair trigger for inducing defense responses (Hulbert, 1997). It is conceivable that the *edr1* mutation has altered an existing disease resistance gene that now responds to *E. cichoracearum.*

The *edr1* resistance phenotype shares some attributes with resistance mediated by the *mlo* mutation of barley. The *mlo* mutation is recessive and confers resistance to multiple races of *E. g. hordei*, but it has no apparent effect on other pathogen species tested, such as *Puccinia hordei*, the causative agent of rust on barley (Jorgensen, 1992; Wolter et al., 1993). Similarly, *edr1* plants showed clear resistance to two species of powdery mildew but only weak resistance to bacterial pathogens and no detectable resistance to *P. parasitica*, the causative agent of downy mildew (J. McDowell, personal communication). Unlike *edr1*, however, *mlo* resistance is associated with the formation of callose-rich papillae that are thought to block penetration of the fungus into the initially infected epidermal cell (Aist et al., 1988; Wolter et al., 1993); secondary hyphae very rarely form on *mlo* plants. The *edr1* mutation does not map to the same location as the *Mlo*-like Arabidopsis genes identified previously (Büschges et al., 1997; C.A. Frye and R.W. Innes, unpublished data).

Elucidating how the *edr1* mutation leads to enhanced disease resistance should provide new insights into how defense responses are regulated and could lead to development of new strategies for engineering resistance. We are currently pursuing isolation of the *EDR1* gene by positional cloning.

METHODS

Bacterial and Fungal Strains and Media

Strain DC3000 of *Pseudomonas syringae* pv *tomato* was obtained from D. Cuppels (Agricultural Canada–Research Center, London, Ontario, Canada), and strain M4 of *P. s.* pv *maculicola* was provided by J. Dangl (University of North Carolina, Chapel Hill). Both *P. syringae* strains were cultured at 30°C on either King's medium B (King et al., 1954) or trypticase soy agar (Becton Dickinson, Cockeysville, MD) supplemented with 100 mg/L rifamycin (Sigma).

Strain UCSC of *Erysiphe cichoracearum* was kindly provided by S. Somerville (Carnegie Institute of Washington, Stanford, CA) and was maintained on *Arabidopsis thaliana* ecotype Columbia (Col-0) by brushing diseased plants onto new plants. Inoculated plants were maintained under a 14-hr day length at 22°C.

Seed Sources

Wild-type Arabidopsis ecotype Col-0 seed was obtained from B.J. Staskawicz (University of California, Berkeley). Mutagenized seed (M_2 generation) was obtained from Lehle Seeds (Round Rock, TX; fast-neutron mutagenized) or M. Estelle (Indiana University, Bloomington; ethyl methanesulfonate mutagenized and γ-irradiated). In all cases, M_1 generation seeds were mutagenized, planted, and allowed to self-pollinate to generate the M_2 population. M_2 populations were bulked from $\sim$500 M_1 plants. The *edr1* mutant was isolated from the γ-irradiated population. Third and fourth generations (M_3 and M_4) of the *edr1* mutant were used interchangeably for phenotypic analyses and crosses.

Arabidopsis Growth and Bacterial Inoculation

Arabidopsis seeds were sown in 4-inch-diameter pots filled with Perlite Plug Mix (Grace Sierra, Milpitas, CA). Pots were covered with 1.3-mm nylon mesh (window or door screen), and plants were allowed to grow through the screen. Seeded pots were covered and held at 4°C for 3 days before being placed in growth rooms under a 9-hr day length (100 to 150 μE m^{-2} sec^{-1} of light) at 24°C. Covers were

removed after the seeds sprouted and the first true leaves were emerging.

Adult plants (4 to 6 weeks after sowing) were inoculated by dipping whole rosettes in a suspension of 10^9 colony-forming units of *P. s. tomato* DC3000 per mL suspended in 10 mM $MgCl_2$ supplemented with 0.025% [v/v] L77 Silwet (OSI Specialties, Danbury, CT). Inoculated plants were maintained under growth conditions described above with humidity domes for ~24 hr. Disease symptoms were scored 4 to 5 days after inoculation.

To monitor bacterial growth inside plant leaves, adult plants (4 to 6 weeks after sowing) were vacuum infiltrated with either 10^5 colony-forming units per mL of *P. s. tomato* DC3000 or 5×10^5 colony-forming units per mL of *P. s. maculicola* M4. Bacterial suspensions contained 0.01% L77 Silwet and 10 mM $MgCl_2$. At specific time points, samples were removed from rosette leaves using a number 2 cork borer (three discs per sample) and macerated in 200 μL of 10 mM $MgCl_2$. Dilutions were made in 10 mM $MgCl_2$, plated on trypticase soy agar containing 100 mg/L rifamycin, and incubated at 30°C. Colonies were counted 48 hr later.

E. cichoracearum Inoculation and Histology

E. cichoracearum actively growing on Col-0 plants (7 to 10 days after inoculation) was used as an inoculum. To inoculate plants, diseased plants were used to brush healthy plants, thus passing spores onto the new plants.

Fungal structures and dead plant cells were stained by collecting leaves and boiling for 2 min in alcoholic lactophenol trypan blue (20 mL of ethanol, 10 mL of phenol, 10 mL of water, 10 mL of lactic acid [83%], and 10 mg of trypan blue). Stained leaves were cleared in chloral hydrate (2.5 g dissolved in 1 mL of water) overnight at room temperature (Koch and Slusarenko, 1990). Cleared leaves were mounted under coverslips in 50% glycerol.

Autofluorescence and callose were detected as described by Adam and Somerville (1996). To observe all tissues, leaves were mounted under coverslips with 50% glycerol and observed with an Axiophot microscope (Carl Zeiss, Oberkochen, Germany). Autofluorescence and callose fluorescence were analyzed using a 4′,6-diamidino-2-phenylindole filter setting.

Quantitation of *E. cichoracearum* Growth

The percentage of germinating spores was determined 1 day after inoculation. Germination was defined as the presence of a germ tube. Hyphal length (3 and 7 days after inoculation) and conidiophore number (7 days after inoculation) were obtained from a minimum of six trypan blue–stained leaves collected from separate experiments. Microscopic images (described above) were captured and digitized using a ZVS-3C75DE 3 CCD video camera (Carl Zeiss) and PowerTower Pro 180 computer (PowerComputing, Round Rock, TX). Digitized images were viewed and printed using Adobe Photoshop software (Adobe Systems, San Jose, CA). Hyphal length at 3 days after inoculation was measured on the printed images and converted to actual measurements by comparing it with an image of a slide micrometer. To calculate conidiophores per millimeter of hyphae at 7 days after inoculation, hyphal length was estimated using a 50-μm grid on the printed image, as described by Olson (1950). Conidiophores on the printed image were counted directly. Fields with approximately equal hyphal density were chosen to ensure equal sampling.

Analysis of Pathogenesis-Related Gene Expression

RNA was purified from frozen leaf tissue using a phenol–chloroform–guanidine hydrochloride extraction procedure (Logemann et al., 1987). RNA concentration was determined spectrophotometrically by absorbance at 260 nm. Twenty-five-microgram samples of total RNA were separated by electrophoresis through a formaldehyde–agarose (1.5%) gel (Sambrook et al., 1989). RNA was transferred from the gel to a nylon membrane and hybridized to ^{32}P-dATP–labeled DNA probes, according to the manufacturer's instructions (Hybond N; Amersham). Probes were generated using a random primed DNA labeling kit (Boehringer Mannheim). DNA templates for probes were generated by polymerase chain reaction amplification of Arabidopsis genomic DNA (*BGL2*, *PR-5*, and ubiquitin [*UBQ5*]) or amplification from a cDNA clone (*PR-1*; Uknes et al., 1992) by using published primers (Glazebrook et al., 1996). Hybridization was quantitated using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). Values for *PR-1* and *BGL2* hybridization were normalized for unequal loading, using values obtained from the *UBQ5* hybridization. Images for Figure 2 were obtained by exposing the membrane to x-ray film (Fuji film RX; Fisher Scientific).

Genetic Analysis

Arabidopsis mutant *edr1* was crossed to ecotype Landsberg *erecta* (L*er*). The F_1, F_2, and F_3 plants were scored for the mutant phenotype after dusting with *E. cichoracearum* spores. Resistant F_2 plants were selected for generation of F_3 families, which were used to confirm F_2 mutant phenotypes. DNA for analysis of molecular markers was collected from one or two inner rosette leaves of resistant F_2 plants by using a hexadecyltrimethylammonium bromide extraction procedure (Bisgrove et al., 1994). Simple sequence length polymorphism (SSLP) and codominant amplified polymorphic sequence (CAPS) markers were amplified by polymerase chain reaction (Konieczny and Ausubel, 1993; Bell and Ecker, 1994). All primers for SSLP and CAPS markers were purchased from Research Genetics (Hunstville, AL). Amplified products were resolved on a 4% NuSieve gel (3:1 NuSieve:Seakem LE; FMC, Rockland, ME).

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