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EDM2 is required for *RPP7*-dependent disease resistance in Arabidopsis and affects *RPP7* transcript levels

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Summary

Specific disease resistance of *Arabidopsis thaliana* against the *Hyaloperonospora parasitica* isolate Hiks1 (*Hp*Hiks1) is mediated by *RPP7*. Although this disease resistance gene encodes a typical nucleotide binding site leucine-rich repeat (NB-LRR) disease resistance protein, its function is independent of the defense hormone salicylic acid and most known genes required for plant immune responses. We identified *EDM2* (enhanced downy mildew 2) in a genetic screen for *RPP7* suppressors. Mutations of *EDM2* phenocopy *RPP7* mutations, but do not affect other tested disease resistance genes. We isolated *EDM2* by map-based cloning. The predicted EDM2 protein is structurally unrelated to previously identified components of the plant immune system, bears typical features of transcriptional regulators, including plant homeodomain (PHD)-finger-like domains, and defines a plant-specific protein family. In *edm2* mutants both constitutive and *Hp*Hiks1-induced *RPP7* transcript levels are reduced, suggesting that EDM2 is either a direct or an indirect regulator of *RPP7* expression. Microarray analyses defined a set of defense-associated genes, the expression of which is suppressed during successful *Hp*Hiks1 colonization of either *rpp7* or *edm2* plants. This transcriptional phenotype is counteracted by an EDM2/RPP7-dependent mechanism.

Keywords: *Arabidopsis thaliana*, *Hyaloperonospora parasitica*, disease resistance gene, EDM2 (enhanced downy mildew 2)-like proteins (ELPs), plant homeodomain (PHD) finger.

Introduction

Disease resistance of plants to pathogenic microorganisms is often triggered by specific *R* genes that mediate the recognition of distinct races of biotrophic microorganisms by genetically interacting with pathogen-derived avirulence (*avr*) genes (gene-for-gene resistance; Dangl and Jones, 2001). In this case, the plant/pathogen interaction is incompatible (resistant plant; avirulent pathogen). *R*-mediated pathogen recognition triggers a complex defense program at infection sites, which typically involves the production of reactive oxygen intermediates (ROI, oxidative burst), nitric oxide (NO) and salicylic acid (SA), cell-wall modifications, production of antimicrobial metabolites/proteins and programmed cell death (HR; Nimchuk *et al.*, 2003). Many of these physiological changes are driven by transcriptional reprogramming of defense-associated genes (Eulgem, 2005; Katagiri, 2004). The absence of *R*-mediated recognition allows pathogen growth and the development of disease symptoms in the plant (compatible interaction; susceptible plant; virulent pathogen). However plant-defense responses are activated to a low degree during compatible interactions,

thereby limiting the spread of disease. These basal defense responses can be triggered by pathogen-associated molecular patterns (PAMPs), such as peptides derived from bacterial flagellin, which are ubiquitously present in broad classes of pathogens (Chisholm *et al.*, 2006; Jones and Dangl, 2006; Zipfel *et al.*, 2004).

Numerous *R* genes have been cloned from *Arabidopsis thaliana* (Arabidopsis) and other higher plant species. They typically encode proteins with multiple C-terminal leucine-rich repeats (LRRs), a central nucleotide binding site (NB) and either an N-terminal coiled coil (CC) or a 'toll/interleukin 1 resistance' (TIR) domain (Dangl and Jones, 2001). Mutant screens and yeast-two-hybrid experiments led to the discovery of other plant genes that are required for NB-LRR-dependent resistance. Their products appear to serve one or more of the following three main functions: (1) controlling conformational changes and stability of NB-LRR proteins as molecular chaperones or co-chaperones (Hubert *et al.*, 2003; Schulze-Lefert, 2004); (2) facilitating pathogen recognition as host proteins targeted by *avr* products and guarded by NB-LRR proteins (Chisholm *et al.*, 2006; Dangl and Jones, 2001; Jones and Dangl, 2006); and (3) relaying NB-LRR-derived signals to activate defense reactions as signal transducers (Nimchuk *et al.*, 2003).

Abundant evidence supports the existence of complex signaling mechanisms shared by multiple *R* genes and the basal defense system (Katagiri, 2004; Tao *et al.*, 2003). These can variably involve coordinated production of H_2O_2, NO and SA (Delledonne *et al.*, 2002). SA appears to be a central component for basal defense and for several, but not all, *R*-mediated responses. Elevated SA levels potentiate ROI production leading to signal amplification (Shirasu *et al.*, 1997) and trigger transcriptional upregulation of defense-associated genes (Schenk *et al.*, 2000). In Arabidopsis, two separate signaling branches feed into SA signaling, one dependent on EDS1 and PAD4 (Feys *et al.*, 2001), and the other dependent on NDR1 (Aarts *et al.*, 1998; Coppinger *et al.*, 2004). Several Arabidopsis *R* genes appear to function independently of SA (Bittner-Eddy and Beynon, 2001; McDowell *et al.*, 2000; Tör *et al.*, 2002; Tör *et al.*, 2004), including *RPP7* that conditions resistance to the Hiks1 isolate of the biotrophic oomycete *Hyaloperonospora parasitica* (*Hp*; Slusarenko and Schlaich, 2003). *RPP7* encodes a CC-NB-LRR protein that is largely independent of *NDR1*, SA and most other known *R* signaling components (McDowell *et al.*, 2000; JMM, unpublished data). *SGT1b* is the only gene described to date that dramatically affects *RPP7* resistance (Slusarenko and Schlaich, 2003). Thus, *RPP7* resistance is a promising target for identifying new defense components by genetic screens.

Here we describe the Arabidopsis *EDM2* (enhanced downy mildew 2) gene. *EDM2* is required for *RPP7* function and operates upstream of defense-associated ROI production as well as HR. Conserved features of EDM2 and EDM2-like proteins (ELPs) define a new plant-specific protein family with a possible role in transcriptional regulation. Accordingly, we found that EDM2 is required for the proper control of *RPP7* transcript levels. Furthermore, microarray analyses revealed that *EDM2* and *RPP7*-dependent functions counteract the *Hp*-induced suppression of defense-associated genes.

Results

The edm2-1 *mutation defines a locus essential for* RPP7 *function*

RPP7 [in Arabidopsis accession Columbia (Col)] mediates strong resistance to the *Hp* isolate Hiks1 (*Hp*Hiks1; McDowell *et al.*, 2000; Tör *et al.*, 2002). We screened a population of Arabidopsis mutants (50 000 M2 seedlings derived from 6000 M1 Col-5 plants subjected to fast-neutron bombardment) to identify the loci required for *RPP7* function. A total of eight mutants were recovered. Seedlings of one mutant (*edm2-1)* were fully susceptible to *Hp*Hiks1, lacked HR and allowed significant development of *Hp* hyphae, oospores and sporangiophores (Figure 1a,b; Table 1). No obvious developmental or morphological phenotypes of *edm2-1* seedlings were observed. A single recessive mutation in *edm2-1* is responsible for susceptibility to *Hp*Hiks1 (not shown). Complementation tests revealed that *edm2-1* is defective in a locus distinct from *RPP7* and *SGT1b* (not shown). The functions of other Col *R* genes, mediating resistance to either *Hp* or *Pseudomonas syringae* (*RPP2*, *RPP4*, *RPM1*, *RPS2*, *RPS4* and *RPS5*), appear not to be affected by *edm2-1* (not shown). Hence *EDM2* is required specifically for *RPP7* function, at least with respect to the *R* genes tested in this study.

We tested the effect of *edm2-1* on defense-associated ROI production, an early downstream component of *R* signaling (Torres and Dangl, 2005). *Hp*Hiks1-infected seedlings were stained with 3,3′-diaminobenzidine (DAB; Figure 1c). DAB staining results in the deposition of a brownish precipitate in the presence of H_2O_2 (Torres *et al.*, 2002). Infection sites on cotyledons of wild-type (WT) seedlings exhibited DAB staining by 24 h post-inoculation (24 hpi). DAB-stained infection sites were completely absent in cotyledons from *edm2-1* and *rpp7-1* seedlings. Hence, *edm2-1* either affects ROI production directly or alters an *RPP7*-dependent signaling step upstream of this response.

The edm2-1 *mutation maps to the lower arm of chromosome 5*

RPP7 is also present in the Arabidopsis accession Landsberg erecta (Ler; Tör *et al.*, 2002). To map the *edm2-1* mutation, we selected 516 *Hp*Hiks1 susceptible F2s from crosses between *edm2-1* and Ler WT plants. Because of a second

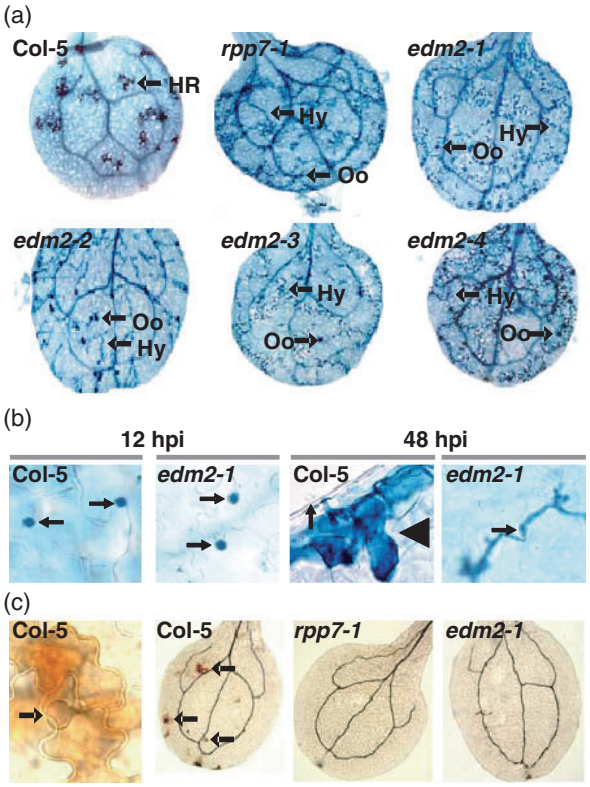

Figure 1. Mutations in *EDM2* (enhanced downy mildew 2) block *RPP7*-mediated resistance, programmed cell death (HR) and defense-associated reactive oxygen intermediates (ROI) production.
(a) Typical trypan blue-stained cotyledons of 2-week-old Col-5 (*RPP7*/*EDM2*), *rpp7-1*, *edm2-1*, *edm2-2*, *edm2-3* and *edm2-4* seedlings 7 days post infection with 5×10^4 *Hyaloperonospora parasitica* isolate Hiks1 (*Hp*Hiks1) spores ml^{-1}. Trypan blue stains *Hp* structures as well as HR sites dark blue. Hy, *Hp* hyphae; HR, HR sites; Oo, oospores. *edm2-2* (SALK_014520), *edm2-3* (SALK_144312) and *edm2-4* (SALK_142563) are T-DNA alleles.
(b) Typical examples of trypan blue-stained cotelydons of Col-5 and *edm2-1* seedlings at either 12 or 48 h post-inoculation (hpi) with *Hp*Hiks1. Arrows point to *Hp*Hiks1 spores attached to the host epidermis. The arrowhead points to an HR site.
(c) 3,3′-Diaminobenzidine (DAB)-stained cotyledons of Col-5 and mutant seedlings. Typical cotyledons of 2-week old Col-5, *edm2-1* and *rpp7-1* seedlings that were stained with DAB 24 hpi with 5×10^4 *Hp*Hiks1 spores ml^{-1}. The left-hand panel shows a germinated *Hp*Hiks1 spore (marked by arrow) surrounded by tissue showing DAB staining resulting from localized ROI production. Arrows indicate DAB stained infection sites. Three repetitions with >10 seedlings per host genotype gave identical results.

*Hp*Hiks1 recognition gene in Ler, *RPP27*, which is absent in Col-5, and is *EDM2* independent (Tör *et al.*, 2004), *Hp*Hiks1 susceptibility segregated in *edm2-1* × Ler F2s as two recessive loci on the lowest arms of chromosomes 1 and 5. As *RPP27* was mapped to the lowest arm of chromosome 1 (Tör *et al.*, 2004), we concluded that *EDM2* was localized on the lowest arm of chromosome 5.

Fine mapping localized *EDM2* to a 58.8-kb interval (covering the 3′-end of bacterial artificial chromosome (BAC) MCO15 and the 5′-end of BAC MTE17) defined by one recombinant at each border (not shown). This region harbors 22 genes (from At5g55280 to At5g55490). Seven of these encode Wax-Synthases and two encode DNA-Topoisomerases, all of which were unlikely *EDM2* candidates. We tested SALK T-DNA insertion mutant lines (Alonso *et al.*, 2003) that were available for seven of the remaining 13 genes of this interval. Three lines homozygous for independent insertions in the same gene, At5g55390, were fully *Hp*Hiks1 susceptible (Figure 1a, Table 1). The location of each *EDM2* T-DNA insertion was confirmed by PCR and sequencing. All other tested insertion lines were resistant to *Hp*Hiks1 (not shown). Sequencing of At5g55390 in the *edm2-1* mutant revealed a 2-bp deletion in the 17th exon, which creates a premature stop codon. Because At5g55390 is localized within the genetically defined *EDM2* interval, and because four independent mutations in this gene resulted in complete *Hp*Hiks1 susceptibility, we have demonstrated that At5g55390 is *EDM2*.

Table 1 Results of *Hyaloperonospora parasitica* isolate Hiks1 (*Hp*Hiks1) infections

Arabidopsis line	Sporangiophores/cotyledon
Col-5 (WT)	0.03 ± 0.03
edm2-1	12.3 ± 1.1
edm2-2 (SALK_014520)	9.10 ± 1.0
edm2-3 (SALK_114312)	14.4 ± 1.1
edm2-4 (SALK_142563)	14.3 ± 0.9
rpp7-1	10.1 ± 0.9

Two-week-old seedlings, 7 days after spray inoculation with 5×10^4 *Hp*Hiks1 spores ml^{-1}. In each case 40 randomly chosen cotyledons were scored. Similar results were obtained with the *edm2-1* mutation after two backcrosses to Col-5 (not shown).

EDM2 has structural features of transcriptional regulators and signal transduction proteins

By RT-PCR with gene-specific primers we cloned a 4080-bp full-length *EDM2* cDNA (Figure S1). Consistent with this, RNA blotting with poly(A)$^+$ RNA from Col using the full-length *EDM2* cDNA as a probe resulted in a weak single band of approximately 4000 nucleotide length (not shown). Semiquantitative RT-PCRs indicated that *EDM2* transcript levels are reduced in *edm2-1* plants and absent in *edm2-2* plants (Figure 2).

The *EDM2* gene structure based on the full-length cDNA is shown in Figure 3a. The predicted EDM2 protein (Figure 3b) consists of 1297 amino acids and has no similarity with known proteins required for *R* function. A scan for putative functional domains using Prosite (http://www.expasy.org/cgi-bin/scanprosite) revealed two putative bipartite nuclear localization signals (NLS), two zinc-finger-like motifs, a Proline-rich region and a large aspartic acid-rich region (Figure 3b). The latter two motifs as well as two smaller acidic regions may be involved in transcriptional activation (Blau *et al.*, 1996). Both zinc-finger-like stretches recognized

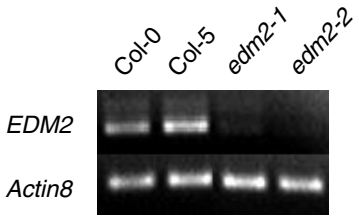

Figure 2. *EDM2* (enhanced downy mildew 2) transcript levels are reduced in *edm2* mutants.
Semi-quantitative RT-PCRs performed with total RNA from untreated 2-week-old Col-0, Col-5, *edm2-1* and *edm2-2* plants using a primer pair annealing to the 5′ region of the EDM2 cDNA. Col-0 is the background of *edm2-2*; Col-5 is the background of *edm2-1*. As a reference, transcript levels of the *Actin8* gene were measured.

by Prosite strongly resemble the PHD (plant homeodomain)-finger motif, which also has been implicated in transcriptional regulation (Aasland *et al.*, 1995; Kalkhoven *et al.*, 2002). Some structural features of EDM2 suggest functions in signal transduction. For example, certain sub-types of PHD fingers act as sensors of phospholipid messenger molecules in animals (Gozani *et al.*, 2003). In addition, a stretch with some similarity to G-protein γ subunit domains was recognized by Prosite. Collectively these motifs suggest a potential role of EDM2 as a gene regulator interacting with other signaling proteins.

EDM2-like proteins constitute a plant-specific family

The Arabidopsis genome harbors a gene closely related to *EDM2* (At5g48090), which we term *AtELP1* (*EDM2-like protein 1*; Figure 3c). Based on release 4 of the TIGR rice genome annotation (http://www.tigr.org/tdb/e2k1/osa1/), the rice genome contains eight *EDM2* orthologs (*OsELP1–8*; Figure 3c). Particularly conserved are regions containing the tandem repeat of PHD-finger-like motifs and the G-protein γ-like domain (Figure 4a,b). An additional highly conserved domain of ~260 amino acids has no obvious similarities to known protein domains (ELP domain; Figure 4c). The strongest similarity to a non-plant protein was to a region comprising the first two of four directly adjacent PHD-fingers of human NSD1, a transcriptional co-factor implicated in human malignancy (Rayasam *et al.*, 2003). We found no non-plant proteins with obvious similarity to EDM2-like proteins outside the putative zinc-finger region. EDM2, its Arabidopsis paralog, and its rice orthologs therefore define a plant-specific protein family, which we designate as ELPs. There are two characteristic features of ELPs: (1) a structural cassette consisting of a tandem repeat of PHD-like zinc-finger motifs and a G-protein γ-like domain; (2) a novel conserved domain of unknown function (ELP domain). Conservation of ELPs between distantly related dicots (Arabidopsis) and monocots (rice) suggests conserved functions across all higher plant species.

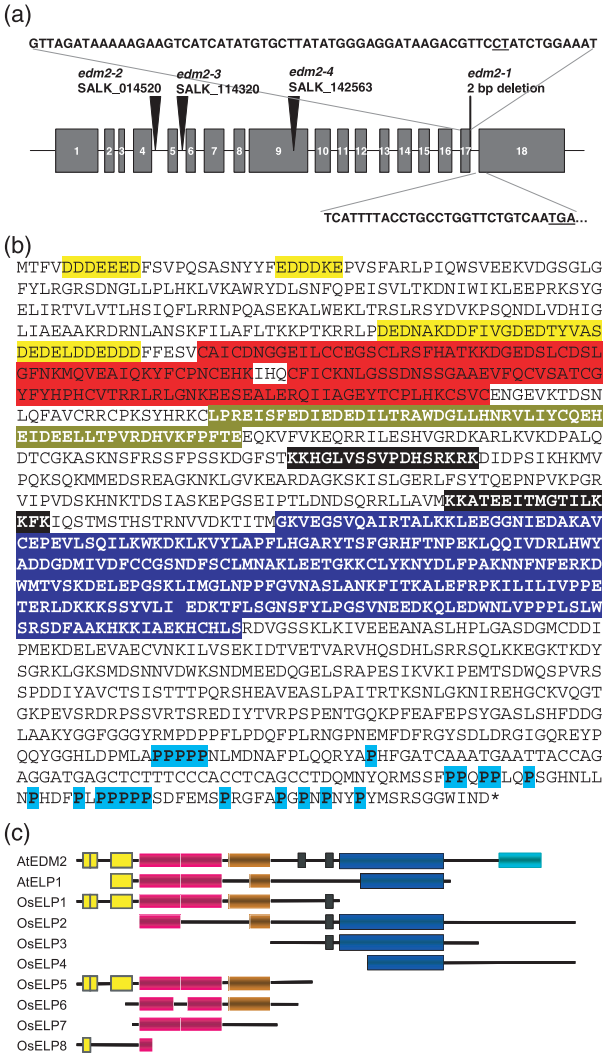

Figure 3. *EDM2* (enhanced downy mildew 2) locus and sequence.
(a) Predicted structure of *EDM2* based on a full-length cDNA. Exons are represented by grey boxes. Three SALK T-DNA insertions (*edm2-2*, *edm2-3* and *edm2-4*) and the *edm2-1* deletion are indicated. The sequence of the 17th exon is given above the schematic representation of *EDM2*. The 2-bp region that is deleted in *edm2-1* (CT) is underlined. The first 29 bp section of exon 18 is given below the schematic *EDM2* representation. The 2-bp deletion in exon 17 results in a frameshift and a premature stop codon in exon 18 (underlined in the exon-18 sequence).
(b) EDM2 protein sequence (based on the full-length cDNA).
(c) Schematic representation of conserved domains shared by ELPs. *AtELP1*, At5g48090; *OsELP1*, Os08g24946; *OsELP2*, Os01g32720; *OsELP3*, Os08g39250; *OsELP4*, Os08g24930; *OsEPL5*, Os08g24880; *OsELP6*, Os12g12300; *OsELP7*, Os03g15990; *OsELP8*, Os01g32710. Color coding for b and c: yellow, acidic regions; red, plant homeodomain (PHD) finger-like motifs; cyan, prolines in the proline-rich region; black, bipartite nuclear localization signals; brown, G-protein γ-like subunit domain; blue, EDM2-like protein (ELP) domain.

EDM2 *and* RPP7 *control transcription of a common cluster of genes*

We repeated our previous analyses of *RPP4*, *RPP7* and *RPP8* transcriptional profiling (Eulgem *et al.*, 2004) with custom

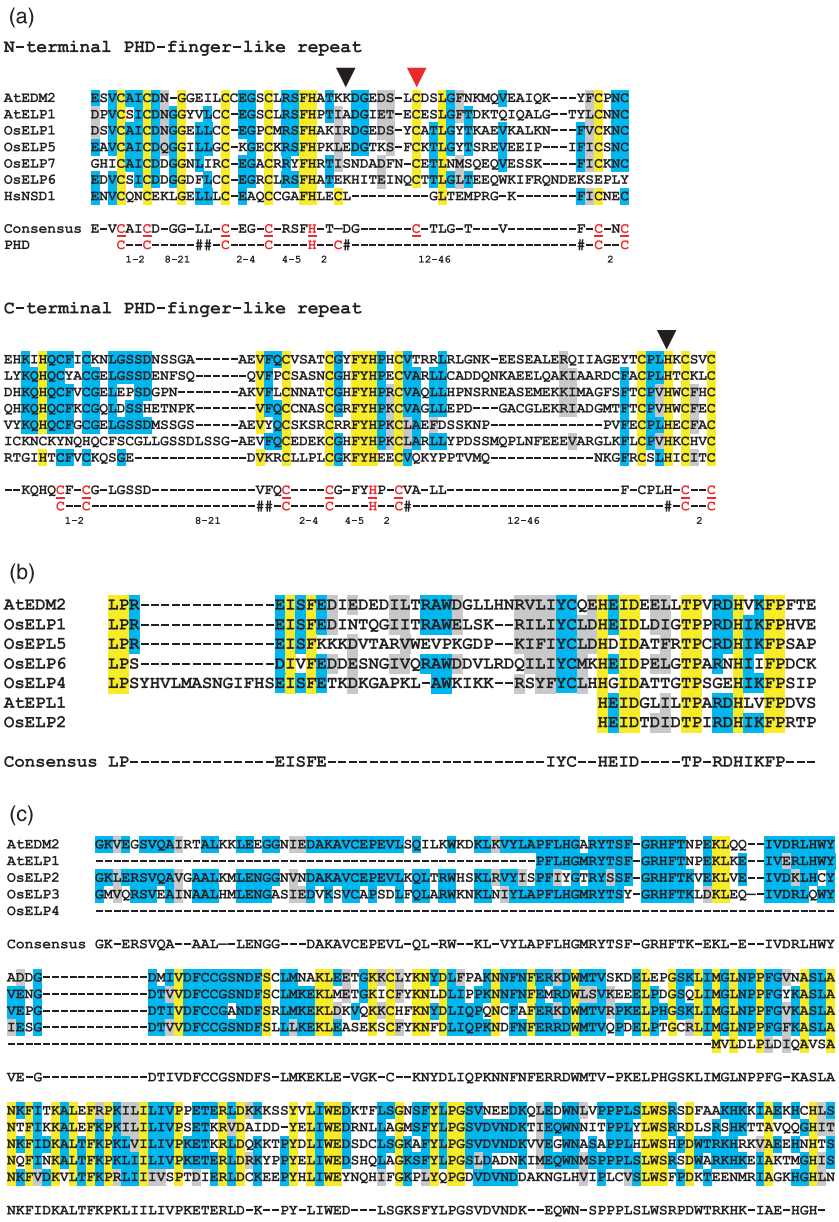

Figure 4. Conserved structural features of EDM2 (enhanced downy mildew 2)-like proteins (ELPs) define a plant-specific protein family.
(a)A tandem repeat of plant homeodomain (PHD)-finger-like motifs of EDM2 is conserved in plant and animal proteins. Alignment of the PHD-finger-like regions of EDM2, AtELP1, four rice orthologs of EDM2 (OsELPs) and HsNSD1 (AAK92049). Four additional rice ELPs that contain either partial or interrupted PHD-like repeats were not included. For each protein both displayed peptide stretches are directly adjacent to each other and constitute one continuous sequence. The consensus sequences of both PHD-finger-like regions from At and Os ELPs, as well as the consensus Cys/His pattern of PHD fingers (PHD; with spacings; Kalkhoven *et al.*, 2002; Kwan *et al.*, 2003), are given below the alignments. Conserved Cys and His residues that correspond to zinc-coordinating residues in PHD-fingers are red and underlined. The position of conserved bulky hydrophobic residues is marked by # in the PHD-finger consensus sequence. Discrepancies between EPLs and conserved hydrophobic residues of the PHD finger consensus are marked by black arrowheads. The red arrowhead points to a conserved Cys residue that is shifted in the ELP sequences by either eight or nine positions towards the C-terminus relative to the PHD-finger consensus pattern.
(b)Alignment of a region with moderate similarity to G-protein γ subunits that is conserved among ELPs. The consensus sequence of the ELP G γ-like domain is given below the alignment. This region is absent in OsELP3, OsELP7 and OsELP8.
(c)Alignment of ELP domains. This region is absent in OsELP5, OsELP6, OsELP7 and OsELP8. Conserved residues are highlighted in A, B and C as follows: Yellow, strict identity; blue, strongly conserved identity; grey, conserved similarity.

Affymetrix (Santa Clara, CA, USA) whole genome exon arrays representing ~26 000 Arabidopsis genes (26 K set) using RNA preparations from an independent set of *Hp*Hiks1 and *Hp*Emoy2 infection time-courses (Table 2). *Hp* spores do not germinate synchronously and only small patches of plant tissue are infected, leading to some variability in microarray experiments (Eulgem *et al.*, 2004 and data not shown). Despite this, we identified a robustly reproducible cluster of genes sharing a sustained and relatively late *Hp*-induced increase of transcript levels (coinciding with the appearance of HR) in WT plants when either *RPP4* or *RPP7* signaling is activated. We had previously defined this as cluster II using an 8 K array (Eulgem *et al.*, 2004). We renamed cluster II as *LURP* (late upregulated in response to *H. parasitica* recognition). The typical expression patterns of *LURP* genes are a continuous increase of transcript levels over the first 48 h after the triggering of *RPP7* resistance, and a substantial transcript level increase between 12 and 48 h after the triggering of disease resistance via a second *R* gene, *RPP4* (Eulgem *et al.*, 2004). The accumulation of *LURP* transcripts is either delayed or attenuated in lines deficient for the respective *RPP* functions, resulting in pronounced differences of transcript levels in comparisons between resistant and susceptible genotypes. These differences are particularly obvious at 48 hpi with *Hp*Emoy2 (triggering *RPP4*) and at 12 hpi with *Hp*Hiks1 (triggering *RPP7*) for a core set of nine *LURP* genes, which were co-clustered in the hierarchical cluster analysis we performed for all *LURP*

Table 2 Microarray experiments performed with 26 K whole-genome Affymetrix chips

Arabidopsis line	*Hp* isolate & cognate *R* gene	Interaction type	Time-points [hpi]	Comments
Col-0	Emoy2/*RPP4*	I	0, 12, 48	1 technical replicate
pad4	Emoy2/*RPP4*	C	0, 12, 48	1 technical replicate
nahG	Emoy2/*RPP4*	C	0, 12, 48	1 technical replicate
Col-5	Hiks1/*RPP7*	I	0, 12, 24, 48	2 technical replicates
rpp7-1	Hiks1/*RPP7*	C	0, 12, 24, 48	2 technical replicates
edm2-1	Hiks1/*RPP7*	C	0, 12, 24, 48	2 technical replicates
Col-0:*RPP8*	Emco5/*RPP8*	I	0, 12	Rehybridization[a]
Col-0	Emco5/*RPP8*	C	0, 12	Rehybridization[a]

C, compatible; hpi, h post-inoculation; I, incompatible.
[a]RNA samples from previous 8 K Affymetrix chip set (Eulgem *et al.*, 2004) were re-hybridized to the whole-genome array.

genes combining data from the 8 K (Eulgem *et al.*, 2004) and the 26 K data sets (Figure 5a, compare with and without asterisks; Table S1). The effects of the *rpp7-1* and *edm2-1* mutations were similar at 12 hpi, resulting in strongly reduced transcript levels of core *LURP* genes compared with Col plants.

Strikingly, transcript levels of core *LURP* genes consistently exhibited a transient decline in *rpp7* plants within 12 hpi with *Hp*Hiks1, relative to their levels at 0 hpi. In contrast, core *LURP* transcript levels increased in *Hp*Hiks1-infected Col plants during the same time interval (Eulgem *et al.*, 2004). This trend was reproduced in the 8 K and 26 K data sets. To examine if this feature applies broadly, we selected a set of 79 genes from the 26 K data set that show a minimum Pearson correlation of 0.80 to the average pattern of the core set of *LURP* genes (Figure 5b). In Col plants transcript levels of these genes tend to steadily increase after *Hp*Hiks1 infection. Within the first 12 hpi following infection of *rpp7-1* with *Hp*Hiks1, their transcripts typically exhibit a transient drop in relative accumulation (relative to their levels at 0 hpi). The *edm2-1* mutation had similar effects on transcript profiles of this extended set of 79 *LURP* genes at 12 hpi (Figure 5b). However, levels of some *LURP* transcripts were already lower at 0 hpi in *edm2-1*, resulting in a less steep decline between 0 and 12 h in *edm2-1*, compared with *rpp7-1*. Semi-quantitative RT-PCR analysis using RNA from biological replicates that were independent of those used for our microarray experiments confirmed that transcript levels of all nine core *LURP* genes exhibit a decline in *rpp7-1* and *edm2-2* (an mRNA-null allele) as early as 2–4 hpi with *Hp*Hiks1. Their levels rise in Col plants during the same time interval (Figure 5c). Thus, in the absence of functional *RPP7* or *EDM2*, the levels of *LURP* transcripts drop transiently within 12 h of infection with *Hp*Hiks1, resulting in a pronounced suppression relative to their levels in Col plants.

edm2 *mutants express reduced* RPP7 *transcript levels*

RPP7 is not represented on either the 8 K or the custom 26 K Affymetrix Arabidopsis Genome Arrays (Eulgem *et al.*, 2004). Therefore, we used semi-quantitative RT-PCR to test whether *RPP7* transcript levels are altered in *edm2* plants (Figure 5d). *RPP7* transcripts are moderately induced in Col plants at early time-points after *Hp*Hiks1 infection. All four *edm2* alleles, including *edm2-2*, exhibited reduced *RPP7* transcript levels (Figure 5d and not shown). Thus, EDM2 contributes to *RPP7* transcript regulation in either a direct or an indirect manner.

Discussion

RPP7 provides robust resistance to *Hp*Hiks1 (McDowell *et al.*, 2000). We identified *EDM2* as a defense regulator that is apparently specific to *RPP7*-mediated resistance. The deduced EDM2 protein is unrelated to any previously identified components of defense signaling. The *rpp7-1* and *edm2-1* mutations both confer strong susceptibility to *Hp*Hiks1. Moreover, both mutations eliminate *Hp*Hiks1-induced host ROI production, as well as HR, and generate similar alterations to the *RPP7*-mediated expression of a signature set of defense response genes. The similarity of their effects on downstream responses as well as the specificity of their genetic interactions suggests a close functional relationship between *EDM2* and *RPP7*. *EDM2* is, thus far, specifically required for *RPP7* function.

EDM2 has several structural features typical for transcriptional regulators, most notably two PHD-finger-like motifs. Many mammalian PHD-finger proteins have been implicated in gene regulatory mechanisms involving histone modifications and nucleosome remodeling (Kalkhoven *et al.*, 2002; Nielsen *et al.*, 2004). Most PHD-finger proteins contain DNA-binding homeodomains (Aasland *et al.*, 1995). Although these family members can bind directly to DNA, other PHD-finger proteins require interactions with separate DNA binding proteins (Rayasam *et al.*, 2003). EDM2 lacks known DNA binding domains, and may therefore interact with one or more DNA-binding proteins. The parsley homeodomain-containing PHD-finger protein, PRHP, binds to the promoter of a *PR-10*-type defense-related gene (Korfhage *et al.*, 1994). However, a function for PRHP in disease resistance has not been demonstrated. Besides the presence of PHD-finger-like

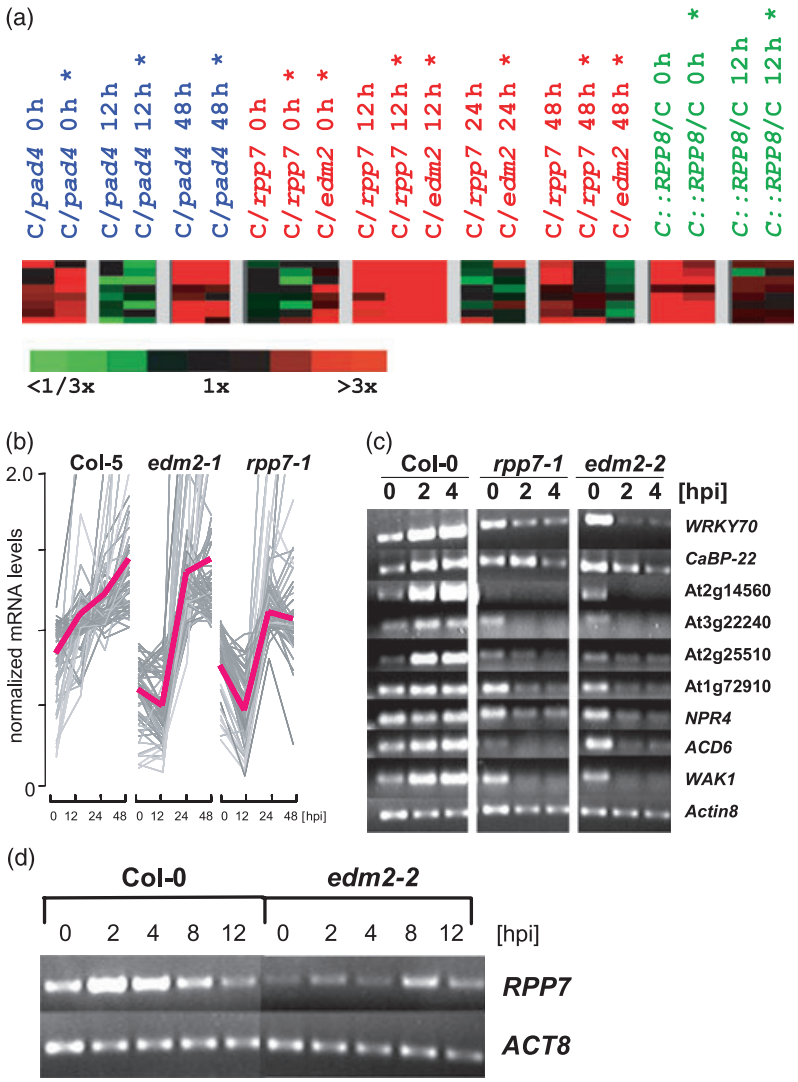

Figure 5. *EDM2* (enhanced downy mildew 2) affects transcript levels of a set of late/sustained upregulated genes and *RPP7*.

(a)Transcript profiles of a sub-cluster of nine genes that exhibit robust late/sustained upregulation following *Hyaloperonospora parasitica* (*Hp*) recognition [core late upregulated in response to *Hp* recognition (*LURP*) genes]. The displayed sub-cluster resulted from hierarchical cluster analysis performed with all 38 members of the *LURP* cluster (formerly called cluster II; Eulgem *et al.*, 2004) combining data from the 8 K (Eulgem *et al.*, 2004) and 26 K microarray analyses (labeled by asterisks; see Table 2). Transcript level ratios from the indicated comparisons between resistant and susceptible plant genotypes at several time points post infection with *Hp* are represented. Red signals indicate higher transcript levels in plants with intact *R* signaling relative to susceptible lines, whereas green signals indicate the opposite. Maximal color intensity represents an either 3-fold or higher expression difference. Treatments diagnostic for dependency on the tested *R* genes are highlighted as follows: *RPP4*, blue (infected with *Hp*Emoy2); *RPP7*, red (infected with *Hp*Hiks1); *RPP8*, green (infected with *Hp*Emco5). Col plants (C) contain *RPP4* and *RPP7*, but lack *RPP8*. C::*RPP8*, transgenic Col-0 line containing the *RPP8* from Ler (McDowell *et al.*, 1998); *pad4* is a mutant compromised in *RPP4*-mediated resistance.

(b)Normalized transcript levels of 79 genes co-expressed with core *LURP* genes in Col-5, *edm2-1* and *rpp7-1* plants at the indicated time points post *Hp*Hiks1 infection. These data were generated with custom Affymetrix whole-genome exon arrays. The transcript patterns shown exhibit a minimum Pearson correlation coefficient of 0.80 to the weighted average pattern of core *LURP* genes. Pearson correlation coefficients consider general patterns and not amplitudes of data (Knudsen, 2002). The weighted average expression pattern of all 79 genes is shown in red. Signal intensities for treatments and probe sets represented in this figure are listed in Table S2.

(c, d)Semi-quantitative RT-PCR assays monitoring transcripts from nine core *LURP* genes (c) and *RPP7* (d) in 2-week-old Col-0 and *edm2-2* plants at various time points after infection with *Hp*Hiks1. As a reference, transcript levels of the *Actin8* (*ACT8*) gene were measured.

motifs there are no other structural similarities between PRHP and EDM2.

RPP7 is constitutively expressed in unchallenged Col seedlings. *Hp*Hiks1 recognition triggers a moderate early and transient increase of *RPP7* transcript levels. Both the basal and transiently increased *RPP7* transcript levels are partially dependent on *EDM2*. Thus, EDM2 positively contributes to *RPP7* expression. The effect of EDM2 on *RPP7* expression may be either direct or indirect and might require interactions with DNA-binding proteins. Multiple transcrip-

tion factors have been implicated in the regulation of the plant defense transcriptome and disease resistance to biotrophs (reviewed in Eulgem, 2005). Those described in detail to date operate downstream in defense signaling and require the accumulation of SA. In contrast, EDM2 appears to act much earlier and seems to function, at least in part, by affecting the expression of the *R* gene with which it specifically functions.

Defined steady-state levels of NB-LRR proteins like RPP7 are a key factor for their function (Bieri *et al.*, 2004; Holt *et al.*, 2005; Hubert *et al.*, 2003). Post-translational control mechanisms involving HSP90 chaperones as well as the putative co-chaperones RAR1 and SGT1 appear to control NB-LRR protein levels and activity. Our results indicate that transcriptional regulation might also contribute to *R* gene activity. A moderate reduction of *RPP7* transcript levels in *edm2* mutants is correlated with a loss of *RPP7* function. It is currently unclear if loss of disease resistance in *edm2* mutants is caused by the reduction of constitutive *RPP7* transcript levels, by the delay and attenuation of *RPP7* upregulation, by the deregulation of *LURP* genes or by some combination of these.

Although constitutive *RPP7* expression is likely to occur at defined levels throughout most plant tissues, its *Hp*Hiks1-induced upregulation is probably limited to infection sites. Thus, the moderate increase of *RPP7* transcript levels observed at early time points in our RT-PCR experiments may reflect a substantial upregulation in a small number of cells. This may lead to an increased *Hp*Hiks1 recognition capacity in cells at (and surrounding) infection sites, resulting in a stronger protection against *Hp*Hiks1 hyphae that escape the first line of defense and a more efficient recognition of additional infection attempts. Therefore, both constitutive and upregulated *RPP7* expression may be important for *Hp*Hiks1 resistance.

Upregulation of known and putative *R* genes in response to defense stimuli has been reported. For example, transcripts of multiple NB-LRR genes were found to be upregulated in response to flagellin (Navarro *et al.*, 2004). Moreover, the *RPP8* gene, which is distantly related to *RPP7*, is upregulated in response to *Hp* (JMM, unpublished data). Additionally, transcriptional upregulation of the atypical *R* genes, *RPW8.1*, *RPW8.2* and *Xa27*, as well as the *RPP8* allele *HRT* occurs, and is correlated with their various disease-resistance functions (Chandra-Shekara *et al.*, 2004; Gu *et al.*, 2005; Xiao *et al.*, 2003). Transcriptional upregulation of *R* and other defense-signaling genes during basal defense responses has been suggested as a mechanism that could sensitize plant cells for subsequent *R*-mediated pathogen recognition (Navarro *et al.*, 2004; de Torres *et al.*, 2003). Future experiments will have to address whether defense-associated accumulation of *R* transcripts is required for full disease resistance, or is an attendant response involved in 'boosting' basal defense responses to prepare for specific *R*-mediated responses.

Our microarray analyses suggest that, besides *RPP7*, a large number of additional genes (including *LURPs*) exhibit reduced transcript levels in *edm2-1*. Thus, mutations in *EDM2* might disrupt the defense network at multiple levels. Therefore, we cannot rule out that full *Hp*Hiks1 susceptibility in *edm2* mutants is caused by combined effects resulting from the reduced activity of *RPP7* and additional *EDM2*-dependent genes. However, such additional effects of *edm2* mutations might be dependent on *RPP7*, as they do not affect function of the limited number of additional *RPP* genes accessible for us to test in Col. Future experiments will address the question of whether the effects of *edm2* mutations on *Hp*Hiks1 resistance and *LURP* expression can be complemented by driving *RPP7* expression independently from *EDM2*.

Relative transcript levels of core *LURP* genes (and possibly additional genes showing a *LURP*-type expression pattern) show a striking drop between 0 and 12 hpi with *Hp*Hiks1 in both *rpp7* and *edm2* mutants. In *Hp*Hiks1-infected Col plants this relative diminution of expression was either absent or much less pronounced. Thus, we can rule out circadian rhythms and other periodic mechanisms as causes of this effect. *Hp*Hiks1-suppressed core *LURP* genes include *NPR4*, *WRKY70* and *ACD6*, which are known to contribute to disease resistance (Tables S1 and S2; Knoth et al., in press; Li *et al.*, 2004; Liu *et al.*, 2005; Lu *et al.*, 2003; Veronese *et al.*, 2003). Additional members of this gene set are required for full basal resistance to *Hp* (Knoth *et al.*, in press). Therefore, it is unlikely that core *LURP* transcript levels are actively downregulated by the Arabidopsis immune system. A more likely explanation is that this transient drop results from a virulence-promoting function of *Hp*.

Despite the absence of *R*-mediated recognition, basal defense responses (including the expression of *LURP* and other defense-associated genes) are moderately activated during compatible *Hp* interactions, and probably contribute to limiting disease (Eulgem *et al.*, 2004; Glazebrook, 2001; Jones and Dangl, 2006). To compromise basal defense reactions, *Hp* may secrete effector proteins into host cells that transiently suppress core *LURP* genes, by analogy with the well-studied type-III effector proteins from phytopathogenic bacteria (Chang *et al.*, 2004; Mudgett, 2005). Recent findings indicate that oomycetes including *Hp* express effectors that function inside host plant cells (Allen *et al.*, 2004; Armstrong *et al.*, 2005; Rehmany *et al.*, 2005). Conserved RXLR motifs in these proteins may mediate trafficking into host cells, where they promote virulence (Birch *et al.*, 2006). Interestingly, a RLXR protein from the oomycete *Phytophthora infestans* carries a functional nuclear localization signal that may target it to host nuclei during infection (Birch *et al.*, 2006). Interference of this protein with host transcription and defense, however, has not yet been

reported. Hence, transcriptional activation of the core *LURP* genes is potentially a target for (an) *Hp*-derived virulence function(s).

*Hp*Hiks1-induced suppression of core *LURP* genes is absent in WT Col plants with functional *RPP7* and *EDM2*. Formally, the lack of their suppression in WT plants may simply result from *RPP7*-mediated *EDM2*-dependent interference with *Hp* growth. However, the relative drop of core *LURP* transcript levels takes place between 0 and 12 hpi, and is detected by RT-PCR as early as 2 hpi (Figure 5c). We observed neither *Hp*Hiks1 spore germination nor hyphal growth in either resistant or susceptible plants during the first 12 hpi (Figure 1b; Eulgem *et al.*, 2004). Therefore, a reasonable scenario explaining our observations involves the antagonism of signals from multiple sources: (1) the recognition of *Hp*Hiks1 spores activates basal defense responses including the expression of core *LURP* genes; (2) the concurrent expression of other *Hp*Hiks1-derived signals transiently suppresses core *LURP* expression, potentially by targeting EDM2, in order to promote disease; (3) the *RPP7*-mediated *EDM2*-dependent recognition of an *Hp*Hiks1-derived molecule triggers rapid and high-amplitude defense responses that counteract the *Hp*-derived disease-promoting signals. This scenario could include direct targeting of EDM2 by an *Hp*Hiks1-derived virulence factor and the subsequent indirect activation of RPP7, in accordance with the Guard Hypothesis for NB-LRR activation (Chisholm *et al.*, 2006; Dangl and Jones, 2001; Jones and Dangl, 2006). Such intricate communication between host and pathogen reflects the high degree of co-evolution between obligate biotrophs and their hosts, and bears further scrutiny.

Experimental procedures

Infection of Arabidopsis seedlings and staining of cotelydon tissue

Hp was grown, propagated and applied to Arabidopsis as described previously (McDowell *et al.*, 2000). Arabiodpsis seedlings were grown on soil in a clean growth chamber (10-h day, 14-h night, 21°C; 100 μeinstein m^{-2} sec^{-1}) and spray inoculated with 50 000 spores ml^{-1} of *Hp*Hiks1. Trypan blue and DAB staining of infected Arabidopsis cotelydons was performed as described previously (McDowell *et al.*, 2000; Torres *et al.*, 2002).

Mutant screening and map-based cloning of EDM2

The *edm2-1* mutant was identified in screens for *Hp*Hiks1-susceptible fast neutron Col-5 mutants as described previously (Tör *et al.*, 2002). Crude mapping was performed with 50 Hiks1-susceptible F2s from crosses of *edm2-1* to Ler-0 plants (homozygous for Col-5 alleles at *edm2*). Using a set of simple sequence length polymorphism (SSLP) markers distributed over the genome and polymorphic between Col-5 and Ler-0 (Lukowitz *et al.*, 2000) we mapped *EDM2* to the lower arm of chromosome 5. Fine mapping was performed with a total of 516 Hiks1-susceptible F2s from *edm2*/Ler crosses using additional SSLP markers on the lowest arm of chromosome 5. These new SSLP markers were designed using known Col-0/Ler-0 polymorphisms (http://www.arabidopsis.org/Cereon/index.jsp) (Lukowitz *et al.*, 2000). Two markers were identified that localized *EDM2* to a 58.8-kb interval: MCO15-D (based on indel CER455334 at position 22439798 of chromosome 5) and MTE17-B (based on indel CER457157 at position 22498662 of chromosome 5). One recombinant was identified with each marker. No recombinants were identified with marker MTE17-A (based on indel CER457146 at position 22447025 of chromosome 5), which is located between MCO15-D and MTE17-B. Sequences of primers used for MCO15-D, MTE17-A and MTE17-B are listed in Table S3a. SALK T-DNA lines (Alonso *et al.*, 2003) with insertions in the *EDM2* interval were ordered from the ABRC Stock Center at Ohio State University (http://www.biosci.ohio-state.edu/~plantbio/Facilities/abrc/abrchome.htm). T-DNA lines homozygous for the respective insertions were identified as described previously (Alonso *et al.*, 2003).

Microarray analyses

Microarray experiments were performed as described previously (Eulgem *et al.*, 2004) with custom Affymetrix Arabidopsis whole-genome exon arrays representing 26367 Arabidopsis genes (http://syngentabiotech.com/EN/partnership/array_information.aspx). For each *Hp*Hiks1 experiment of the 26 K set, two technical replicates were performed. Reproducibility between these replicates was excellent. For each pair of technical replicates the correlation coefficient was at least 0.990. Averages of the signal intensities from the respective replicates were used for further analysis. Raw data for all chips, as well as information about the microarray design, are deposited at ArrayExpress (http://www.ebi.ac.uk/aerep/) according to the MIAME guidelines under the accession numbers E-TABM-96 and A-MEXP-366, respectively. Scanned images were acquired and processed by Affymetrix MAS5.0. Normalized expression levels were computed by a custom algorithm (Zhou and Abagyan, 2002). Cluster analysis was performed using CLUSTER and TREEVIEW (Eisen *et al.*, 1998) as described previously (Eulgem *et al.*, 2004). Genes co-expressed with core *LURP* genes were defined using GeneSpring (Agilent Technologies, Santa Clara, CA, USA) by selecting genes with normalized mRNA profiles showing a Pearson correlation of ≥0.80 to the weighted average profile of the *LURP* core set (http://www.silicongenetics.com/cgi/TNgen.cgi/GeneSpring/GSnotes/Notes/want_average).

RT PCRs

Total RNA was extracted from 2-week-old Arabidopsis seedlings using RNAwiz (Ambion, Austin, TX, USA). After the treatment of RNA with Dnase I (NEB, Beverly, MA, USA), 2 μg of the RNA was used for synthesis of 1st-strand cDNA using SuperScript III Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA). The resulting cDNA (1 μl) was used for RT-PCR in a total reaction volume of 20 μl with 5 U of *Taq* polymerase (NEB). PCR product (8 μl) was loaded for gel electrophoresis. The cycle numbers for PCRs were determined by comparing the intensities of PCR products after gel electrophoresis and are as follows: 30 for *EDM2*, 28 for *RPP7*, 25 for *WRKY70* (At3g56400), 26 for *CaBP-22* (At2g41090), 35 for At2g14560, 26 for At3g22240, 23 for At2g25510, 32 for At1g72910, 29 for *NPR4* (AT4G19660), 29 for *ACD6* (AT4G14400), 27 for *WAK1* (AT1G21250) and 21 for *Actin8*. The used PCR primers are listed in Table S3b,c.

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Supplementary Material

The following supplementary material is available for this article online:

Figure S1. EDM2 full length cDNA sequence.

Table S1 Transcript ratios of all genes shown in Figure 5a

Table S2 Signal intensities for all genes shown in Figure 5b. Listed are raw signal intensities (average difference values). Values <25 were raised to 25 to eliminate noise (Eulgem *et al*., 2004). In addition to AGI locus identifiers, common names for some genes with known or putative roles in defense are given

Table S3 (a) Primers of key markers used for EDM2 fine mapping
(b) Primers used for RT-PCR analyses of *EDM2* transcripts
(c) Primers used for RT-PCR assays shown in Figure 5

This material is available as part of the online article from http://www.blackwell-synergy.com

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EDM2 full length cDNA sequence

ATGACGTTCGTTGACGATGATGAAGAGGAAGACTTCTCTGTTCCTCAATCAGCTTCCAATTATTATTTCGAAGATGATGATAAAGAGCC
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ACTCACATGTGTAGCTAGTGGGCCTTTTAGCTGTTTAATAATATAAAAGAAAAAGAAGCCAGCTTCTATTGTCTT

transcript ratios of core *LURP* genes represented in figure 3A

Locus Identifier	common name/putative function	pad4 0 8k	pad4 0 26k	pad 4 12 8k	pad4 12 26k	pad 4 48 8k	pad4 48 26k	rpp7 0 8k	rpp7 0 26k	edm2 0 26k	rpp7 12 8k	rpp7 12 26k	edm2 12 26k	rpp7 24 26k	edm2 24 26k	rpp7 48 8k	rpp7 48 26k	edm2 48 26k	rpp8 0 8k	rpp8 0 26k	rpp8 12 8k	rpp8 12 26k
At1g21250	NPR4	3.7428571	1.1456	0.983553	0.425678933	6.40963855	8.28520752	0.75659824	1	1	5.967948718	3.1908	3.1908	0.769967	2.493	2.429844098	1.017433	0.584857	5.236364	1.786	1.5528647	1.615049187
At1g72910	TIR-NB-LRR disease resistance gene	1.96875	3.41816737	0.712963	0.996910445	1.47154472	1.583991086	0.992462312	0.937171	3.2368	3.426229508	3.6256	3.6256	0.927162	0.589211	1.332575758	1.069799	0.720023	1.475593	1.335420467	1.6189376	1.459906718
At2g14560	unknown	1.12	2.7152	0.109195	0.114889762	12.7981651	5.890687916	1	0.291969	1.040975	13	3.843739	6.4152	0.487613	0.841238	3.629186603	1.01717	0.630641	6.76	7.553728489	1.6361768	1.651190389
At2g25510	unknown	1.8877551	1.95079404	0.598364	0.822110842	1.61165919	1.07517651	0.836975994	0.873297	1.604721	1.585638298	5.245704	4.157732	0.992224	0.880549	1.201694915	1.398423	1.152639	3.156008	2.164836731	1.4206224	1.080272814
At2g41090	CaBPP22	1.52	2.41000257	0.754237	1.066695603	2.86740332	2.617277665	0.866855524	0.911875	2.607145	2.573033708	12.427577	5.451776	1.069193	0.891477	1.724615385	1.131416	0.538086	3.3	5.145684366	1.3817382	1.315696616
At3g22240	unknown	3.1333333	4.2672246	0.187313	0.587806125	3.98245614	1.305590361	0.903092784	1.718835	2.789859	13.75471698	7.187721	10.01404	2.425686	0.892931	2.619097587	4.585583	0.998073	4.900498	3.627591457	1.3041412	1.457364297
At3g56400	WRKY70	1	3.5812	0.971831	0.538986963	4.75	5.690888352	1	0.563111	1.3134	4.24	6.188	4.50889	0.721388	0.992569	3.434782609	0.937965	0.645011	3.6	2.32929068	0.5401929	1.120631206
At4g14400	ACD6	1.7054264	2.38915471	0.787037	0.374481558	2.98777506	3.883551779	0.819875776	0.45641	1.402	4.064327485	13.547438	7.627671	0.707028	1.591007	1.897689769	1.418757	0.650717	9.696429	6.184806753	1.1895425	1.129769058
At4g19660	unknown	1.96875	1.4256	1.577778	1.033193571	2.42105263	1.763583355	0.524590164	0.992768	1.133956	1.888888889	1.310971	1.174128	0.867717	1.446886	2.288888889	0.923906	0.774191	2.622222	1.113982199	1.1969697	0.833697732

signal intensities of genes co-expressed with core *LURP* genes (Pearson correlation coefficiens =/> 0.8) represented in figure 3B

Systematic Name	common name (or GO ter	Col-5 , Time 0 raw	Col-5 , Time 12 raw	Col-5 , Time 24 raw	Col-5 , Time 48 raw	edm2 , Time 0 raw	edm2 , Time 12 raw	edm2 , Time 24 raw	edm2 , Time 48 raw	rpp7 , Time 0 raw	rpp7 , Time 12 raw	rpp7 , Time 24 raw	rpp7 , Time 48 raw
At2g25510		1,558	3,741	3,824	5,294	970.6	899.8	4,343	4,593	1,784	713.2	3,854	3,786
At2g41090		241.2	936.9	1,052	1,091	92.51	171.9	1,180	2,027	264.5	75.39	983.7	964.2
At5g45470		37.46	48.9	50.01	69.17	25	25	64.44	81.61	35.74	25	55.31	36.81
At3g22240		341.4	1,619	1,812	2,628	122.4	161.7	2,029	2,633	198.6	225.3	747	573.1
At5g24210		93.32	206.2	158.1	233	55.28	76.44	167.4	359.9	54.13	25	156.9	138.8
At3g04210	disease resistance protein (TII	70.86	90.93	89.65	143.7	25	26.59	77.7	140.1	61.51	25	107	87.76
At1g19960		81.6	137.1	284.4	554.7	67.11	60.04	482.9	600	108.3	50.93	228.3	432.4
At3g56400	WRKY70	32.83	154.7	142.9	243.9	25	34.31	144	378.1	58.31	25	198.1	260
At2g40750	WRKY54	32.62	49.67	54.12	74.11	35.72	25	55.83	110.3	40.42	25	59.33	62.99
At4g14400	ACD6	35.05	357	254.8	412.6	25	46.8	160.1	634.1	76.79	26.35	360.3	290.8
At3g62600		49.47	46.35	59.31	74.77	32.17	25	68.28	88.32	53.47	26.74	54.94	63.72
At5g23420		32.12	47.93	44.94	50.79	25	25	47.74	43.58	36.56	25	39.44	36.06
At5g47210		283.7	334.8	354.2	524.2	189.5	134.8	404.4	450.1	342.3	153.5	452.7	345.4
At4g15150		314.8	317.8	389.6	388.7	275.6	247.5	375.1	393.1	314.4	264.6	378.6	311.8
At5g19250		319.4	395.6	424.8	512	185.5	125.4	510.7	662.5	385.1	122.2	473.1	617.3
At5g55070		146.5	148	188.5	200.7	100.8	130.4	170.1	197.1	152.6	93.99	149.2	166.5
At1g72910	disease resistance protein (TII	80.92	90.64	204	213.4	25	25	346.2	296.3	86.35	25	220	199.4
At3g04870		114.3	144.6	187.7	167.1	107.7	104	147.4	178.1	122.6	97.74	183.6	145.8
At5g03350		193.2	394.7	1,252	1,966	84.29	48.33	1,015	1,847	304.9	50.78	1,119	2,044
At5g66470		25	51.54	46.72	61.23	26.39	25	45.93	45.88	41.7	25	41.62	41.63
At4g17390		1,736	2,054	2,125	2,338	1,546	967.7	2,453	2,139	1,555	900.1	2,021	1,982
At1g07370		60.99	58.73	71.42	90.71	33.53	25	80.33	73.89	51.51	25	76.35	55.51
At5g40770		124.7	170.1	157.3	146.8	99.16	100.2	171.1	169.3	122.7	81.35	168.3	147.9
At2g46430	CNGC3, CYCLIC NUCLEOTI	25	58.69	40.26	54.39	25	25	49.97	102.6	25	25	58.74	38.88
At3g23600		341.5	433.7	412	444.8	304.9	300.1	504	550.3	339.9	386.5	396.2	508
At4g22870		35.37	37.27	52.56	59.96	25	29.64	47.32	72.38	33.12	25	25	48.74
At3g51140		140.1	167.5	157	182.8	126.5	104.5	167.6	166	135	105.5	143.9	127.6
At5g61030		94.61	102.2	112.6	113.7	39.67	25	138.9	136.1	83.46	25	119.3	86.46
At4g26780		47.62	42.12	62.31	54.81	25	25	59.81	64.98	38.44	25	61.42	50.03
At1g43920		30.19	42.19	48.7	53.67	25	25	34.88	61.36	38.19	25	42.81	28.61
At5g42530		827.1	2,306	2,021	2,351	484	1,200	2,227	1,950	1,354	1,015	2,309	1,867
At2g38810		29.12	31.95	34.15	42.06	25	25	35.47	35.76	25	25	25	31.06
At3g09630		2,587	2,547	2,817	3,020	2,271	2,217	3,050	2,838	2,071	2,214	2,779	2,931
At1g14320		2,694	2,916	3,419	3,749	1,999	1,163	3,007	3,303	2,551	1,453	3,309	2,882
At5g10920		32.47	49.22	44.86	58.83	27.36	31.82	46.94	42.67	35.67	25	44.3	36.12
At3g44940		33.76	39.85	50.91	50.5	33.74	31.32	64.14	56.44	42.87	28.05	48.66	43.97
At1g56070		2,357	2,646	3,133	3,395	2,005	1,757	3,411	2,949	2,355	1,738	3,156	2,248
At5g59870		335.6	338.1	399	509.4	192.2	79.79	387.1	406.6	288	74.82	302.9	371.9
At5g61170		1,329	1,365	1,567	1,586	845.8	530	1,794	1,592	1,154	611	1,434	1,512
At2g31060		29.9	41.38	31.35	35.92	25	27.78	37.42	46.78	31.42	29.57	33.33	37.95
At1g76960		79.5	58.08	195.2	462.6	62.53	45.22	543.9	598.5	80.94	39.5	107.2	321.4
At5g61020		111.7	157	147.4	156.7	99.62	123	132.2	172.3	138.7	105.6	162.8	130.4
At5g02870		424.6	495.3	524.6	605.9	328.9	240.1	630.3	488.7	422.7	233.1	534.2	521.6
At5g03420		44.94	59.31	54.8	54.72	37.72	36.74	53.72	59.19	46.4	33.76	52.88	39.59
At5g49910		306	330.1	594.6	664.1	137.3	83.35	519.5	478.8	333.5	117.1	582	605.8
At1g75040	PR5	78.01	127.5	155.7	204	56.52	77.4	133.9	382.1	100.8	70.46	166.3	102.8
At3g20050		44.56	58.06	53.67	61.07	39.24	45.06	58.31	58.1	54.54	34.06	61.02	57.47
At3g05060		45.8	47.34	74.18	87.65	30.4	25	52.13	76.51	48.06	25	54.8	33.08
At3g47480		26.23	69.32	51.53	85.88	25	25	116	170.3	46.44	34.15	128.2	122.2
At2g47110		4,051	4,244	3,998	4,566	3,205	1,938	5,233	4,714	3,210	2,210	3,991	3,885
At5g58310		25	31.62	25	31.89	25	25	31.82	36.56	25	25	25	29.67
At1g31580		2,078	4,003	4,864	5,877	1,348	1,619	4,958	6,075	950.1	89.68	1,473	455.2
At1g55210	similar to pathogenesis-relatec	49.3	70.82	81.99	77.68	25	25	148.7	127.2	64.02	31.35	83.07	107.3
At5g50340		36.67	35.99	53.51	60.04	28.17	25	48.24	60.22	49.69	30.93	56.54	42.49
At3g15680		73.92	61.71	92.01	96.62	46.19	31.28	90.81	91.96	71.47	27.64	78.92	66.47
At4g31700		1,196	1,106	1,273	1,390	676.8	421.9	1,696	1,396	962.5	411.2	1,131	1,158
At2g19730		765.4	886.4	838.2	973.8	463.4	330.4	897.9	846.9	598	342	751.1	546.1
At3g28540		30.82	40.2	69.74	148.3	25	25	151.3	336.3	48.28	25	184.4	150.9
At5g62840		26.85	28.22	35.09	45.97	25	25	38.16	34.9	31.84	25	33.22	30.27
At2g14560		30.74	160.4	172.3	682.5	29.53	25	204.8	1,082	105.3	41.72	353.4	670.9
At2g21280		116.7	141.9	169.6	192.7	82.88	46.49	201.7	150.4	139.1	45.4	140.8	127.8
At3g12100		49.97	47.65	48.44	52.33	31.2	40.88	61.96	61.45	43.28	40.28	49.71	47.04
At5g10910		29.61	34.9	36.11	37.59	25	25	42.44	33.9	26.89	25	31.77	28.33
At5g61790		506.5	342.3	548.6	661.4	235.4	151.3	739.8	829.8	526.4	272.2	560.9	692.7
At5g16710		94.94	102.8	103.8	126.2	72.57	61.01	92.32	102.7	84.43	60.94	94.67	82.53
At1g18080		1,525	1,474	1,695	1,697	1,338	1,035	1,770	1,702	1,353	1,164	1,570	1,819
At5g52650		934.5	982.9	1,044	1,063	611.4	544.8	1,309	1,032	783.6	571.5	1,118	1,030
At2g28000		200.4	313.3	335.1	398.5	194.3	81.33	312.4	343.6	284.7	67.07	333.5	432.2
At2g40510		362	393.9	449	480.4	256.5	166.2	600.1	470.2	366.4	169.5	471.6	341.5
At2g46440		32.74	66.81	70.6	74.29	25	25	31.74	138.5	28.08	25	72.19	39.06
At1g53120		47.53	53.65	61.78	56.97	33.22	33.01	43.38	62.59	42.6	34.1	61.12	45.4
At5g12080		47.85	39.43	59.35	49.22	27.06	25	64.11	64.07	40.89	25	54.6	44.09
At1g73350		25	34.69	34.32	31.89	25	25	32.03	35.19	25	25	35.45	25
At1g23290		1,772	1,720	1,875	2,052	1,089	834.8	2,469	1,887	1,422	852.7	1,762	1,797
At3g27060		119.4	174.6	217	177.7	91.38	48.94	257.3	178.8	123.7	43.8	191.7	144.2
At4g02930		59.01	77.13	57.87	61.94	37.28	28.39	70.62	95.37	64.53	29.02	62.62	66.93
At5g02620		31.85	32.79	39.38	38.33	25	25	41.63	47.02	40.02	25	32.24	38.58
At1g18540		823.5	748.9	851.2	1,144	491.6	280.4	788.2	829.3	593.1	261.8	737	831.2
At1g22780		805	826.6	912.6	914.5	597.6	561.6	1,042	858.8	694.1	564	938.7	751.6

Supplementary Table 3:

A: Primers of key markers used for EDM2 fine mapping

MCO15-D-F:	5'-GCAGCTACTATGCAACGAATGAG-3'
MCO15-D-R:	5'-GAGGTTTAGAGGGTCCACTCG-3'
MTE17-A-F:	5'-GGTTAATTTACTAAATTGTGAAGC-3'
MTE17-A-R:	5'-CCCATAAGAATGTGGATATCC-3'
MTE17-B-F:	5'-ATTGGCAAATCAAGTTGCAG-3'
MTE17-B-R:	5'-CGATAAAATTTGGATTGTAC-3'

B: Primers used for RT-PCRs shown in Figure 3

PR1-F:	5'-TTCCCTCGAAAGCTCAAGATAGC-3',
PR1-R:	5'-GGCACATCCGAGTCTCAC TGA C-3',
WRKY70-F:	5'- AACGACGGCAAGTTTGAAGATTC-3',
WRKY70-R:	5'-TTCTGGCCACACCAATGACAAGT-3',
CaBP-22-F:	5'-CGGAACCATCAATTT CACTGAGT-3',
CaBP-22-R:	5'-CAAAGTGCCACCAGTTGTGTCAT-3',
At2g14560-F:	5'-CTCGACGACTCTTGTGTTGTCTAC-3',
At2g14560-R:	5'-GCT AAGGGCATGTGTTTGTATTTA-3',
RPP7-F:	5'-GTCGATGACTATATGCATCCT C-3',
RPP7-R:	5'-CAGATGCATCATTTATAGGAAATGC-3',
ACT8-F:	5'-ATGAAGATTAAGGTCGTGGCAC-3'
ACT8 –R:	5'-GTTTTTATCCGAGTTTGAAGAGGC-3'.