

Genetic Analysis of Developmentally Regulated Resistance to Downy Mildew (*Hyaloperonospora parasitica*) in *Arabidopsis thaliana*

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Although developmentally regulated disease resistance has been observed in a variety of plant-pathogen interactions, the molecular basis of this phenomenon is not well understood. *Arabidopsis thaliana* ecotype Columbia-0 (Col-0) expresses a developmentally regulated resistance to *Hyaloperonospora parasitica* isolate Emco5. Col-0 seedlings support profuse mycelial growth and asexual spore formation in the cotyledons. In contrast, Emco5 growth and reproduction is dramatically (but not completely) restricted in the first set of true leaves. Subsequent leaves exhibit progressively increased resistance. This adult resistance is strongly suppressed by expression of the salicylic acid-degrading transgene *NahG* and by loss-of-function mutations in the defense-response regulators *PAD4*, *NDRI*, *RARI*, *PBS3*, and *NPRI*. In contrast to Col-0, the Wassilewskija-0 (Ws-0) ecotype supports profuse growth of Emco5 at all stages of development. Gene-dosage experiments and segregation patterns indicate that adult susceptibility in Ws-0 is incompletely dominant to adult resistance in Col-0. Genetic mapping in a Col × Ws F2 population revealed a major locus on the bottom arm of chromosome 5, which we named *RPP31*. Analysis of T-DNA insertion lines indicated that the Columbia allele of *RPP8*, though tightly linked to *RPP31*, is not necessary for adult resistance.

Additional keywords: adult plant resistance, age-related resistance, gene-for-gene resistance, haploinsufficiency, oomycete.

Plants have evolved multiple lines of defense against pathogens and pests. These include preformed barriers as well as inducible defenses that are activated only when pathogens are perceived by the plant (Hammond-Kosack and Jones 1996; Osbourn 1996). Several distinct inducible defense mechanisms have been posited on the basis of genetic and molecular studies. The best-studied defense mechanism is “gene-for-gene” resistance, which is based on perception of pathogen aviru-

lence (*avr*) gene products by corresponding resistance (R) proteins in the plant (Dangl and Jones 2001; Flor 1955). In some cases, *avr* genes have been shown to encode virulence factors (effectors) that function inside plant cells to interdict plant regulatory pathways and create a more favorable environment for the pathogen (Chang et al. 2004; van’t Slot and Knogge 2002). R genes encode receptors that might either physically interact with the cognate effector, detect the molecular consequences of effector action, or both (Dangl and Jones 2001; Van der Biezen and Jones 1998). This molecular recognition activates a complex signaling network that controls activation of defense responses in the vicinity of infection sites (Hammond-Kosack and Parker 2003; McDowell and Dangl 2000). These localized defenses include programmed cell death (the hypersensitive response), physical reinforcements, and biosynthesis of antimicrobial compounds (e.g., phytoalexins and pathogenesis-related proteins) (Hammond-Kosack and Jones 1996). Genetic studies in *Arabidopsis* have defined a distinct layer of “basal” resistance that acts in the absence of gene-for-gene recognition to limit the growth of virulent pathogens (Glazebrook et al. 1997). Mutant analyses and microarray studies have revealed extensive regulatory overlap between basal resistance and R gene-dependent resistance, suggesting that R genes function to activate a faster and more potent elaboration of basal defenses (Katagiri 2004; Tao et al. 2003). Finally, two types of induced, systemic defense responses have been shown to provide resistance against a relatively broad spectrum of otherwise virulent pathogens: systemic acquired resistance (SAR), which can be induced throughout the plant after a localized pathogen attack and requires the phenolic signal salicylic acid (SA) (Durrant and Dong 2004) and induced systemic resistance (ISR), which is induced by rhizosphere bacteria through a mechanism that uses components of the ethylene and jasmonic acid (JA) pathways (Pieterse et al. 2003).

In some plant-pathogen interactions, defenses are effective only at specific developmental stages. This phenomenon has been referred to as developmental resistance, adult plant resistance, mature plant resistance, ontogenic resistance, or age-related resistance. Examples of developmental resistance to viruses, bacteria, fungi, oomycetes, and insects have been documented in many crops (Panter and Jones 2002). Several sources of developmental resistance have been defined genetically and deployed effectively in the field through classical breeding. For example,

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genes for adult resistance to powdery mildew (Griffey et al. 1993; Liu et al. 2001), stem rust (Sunderwirth and Roelfs 1980), and leaf rust (Kolmer 1996) have provided relatively durable disease control in wheat. It has been suggested that developmental resistances may be more stable over evolutionary time because susceptible tissue provides a refugium for the pathogen, thereby attenuating selection for resistance-breaking mutations (Parniske et al. 1997). However, developmental resistance can also provide windows of opportunity for pathogens to cause significant damage to susceptible tissue (Brophy and Laing 1992). A better understanding of why defenses are inactive at certain developmental stages might open new avenues for engineering developmentally uniform resistance. From a more fundamental perspective, genetic analysis of developmental resistance provides an opportunity to explore cross talk between molecular networks that regulate development and immunity. However, developmental resistance mechanisms have not been extensively studied at the molecular level (Panter and Jones 2002).

Disease resistance mechanisms in *Arabidopsis* have been defined primarily through the use of *Pseudomonas syringae* (Quirino and Bent 2003) and the oomycete *Hyaloperonospora parasitica* (Holub and Beynon 1996; Slusarenko and Schlaich 2003) as model pathogens. *H. parasitica* causes downy mildew disease on wild populations of *Arabidopsis* and is a destructive pathogen of cultivated Brassicas (Clark and Spencer-Phillips 2000). Extensive genetic diversity has been documented in the interaction between *Arabidopsis* and *H. parasitica*, indicative of rapid and dynamic co-evolution between the host and the parasite (Holub and Beynon 1996; Slusarenko and Schlaich 2003). This intraspecific polymorphism has proven useful for molecular genetic analysis of resistance, because it has permitted genetic definition and molecular cloning of *RPP* (isolate-specific resistance to *Hyaloperonospora parasitica*) genes. To date, 30 distinct *RPP* genes have been genetically defined (Holub 1997; Holub and Beynon 1996; Holub et al. 1994; Holub 2001) and 11 have been molecularly cloned by map-based methods (Slusarenko and Schlaich 2003; Tor et al. 2004). Every *RPP* gene cloned to date (with one exception [Tor et al. 2004]) belongs to the nucleotide binding site-leucine-rich repeat (NBS-LRR) resistance gene superfamily (Jones and Jones 1996; Tameling et al. 2002).

Age-related resistance in *Arabidopsis* against two pathogens of *P. syringae* was recently defined and was shown to be genetically distinct from other forms of resistance (Kus et al. 2002). In this report, we describe a developmentally regulated resistance in *Arabidopsis* to the Emco5 isolate of *H. parasitica*. This resistance is incomplete in the first two true leaves and becomes more efficient as development proceeds. This resistance is activated specifically in the Col-0 ecotype and is not functional in Ws-0. A previous paper noted that this resistance was correlated with an oxidative burst and programmed cell death (Torres et al. 2002). In this report, we characterized the resistance in detail, to address the following questions: Is this resistance race-specific? Does the developmentally regulated disease resistance require genetic components of previously documented disease resistance mechanisms? Is developmentally regulated resistance in Col-0 genetically dominant to susceptibility in Ws-0, and how many loci relevant for expression of this resistance can be defined in a segregating Col-0 × Ws-0 population?

RESULTS

Developmentally regulated resistance to *H. parasitica* Emco5 is activated in true leaves of *Arabidopsis* Col-0 but not Ws-0.

As previously documented (Aarts et al. 1998; Holub et al. 1993; McDowell et al. 1998, 2000) and shown in Figure 1,

seedlings of *Arabidopsis* ecotype Columbia (Col-0) are highly susceptible to the Emco5 isolate of *H. parasitica*. Visual examination (Fig. 1A) and trypan blue staining (Fig. 1B) of Col-0 seedlings 7 days after inoculation (dai) revealed abundant asexual fruiting bodies (sporangiophores) and sexual spores (oospores) as well as an extensive mycelium of intracellular hyphae. Wassilewskija (Ws-0) seedlings are also susceptible to Emco5 (Figs. 1 and 2A) (McDowell et al. 2000). Landsberg *erecta* (Ler) seedlings exhibited complete resistance to Emco5, due to race-specific recognition provided by the *RPP8-Ler* gene (Fig. 1A) (Holub et al. 1993; McDowell et al. 1998).

In contrast to susceptible Col-0 cotyledons, true leaves of Col-0 exhibited significant resistance to Emco5. This was first apparent in 14-day-old seedlings, where the first two true leaves

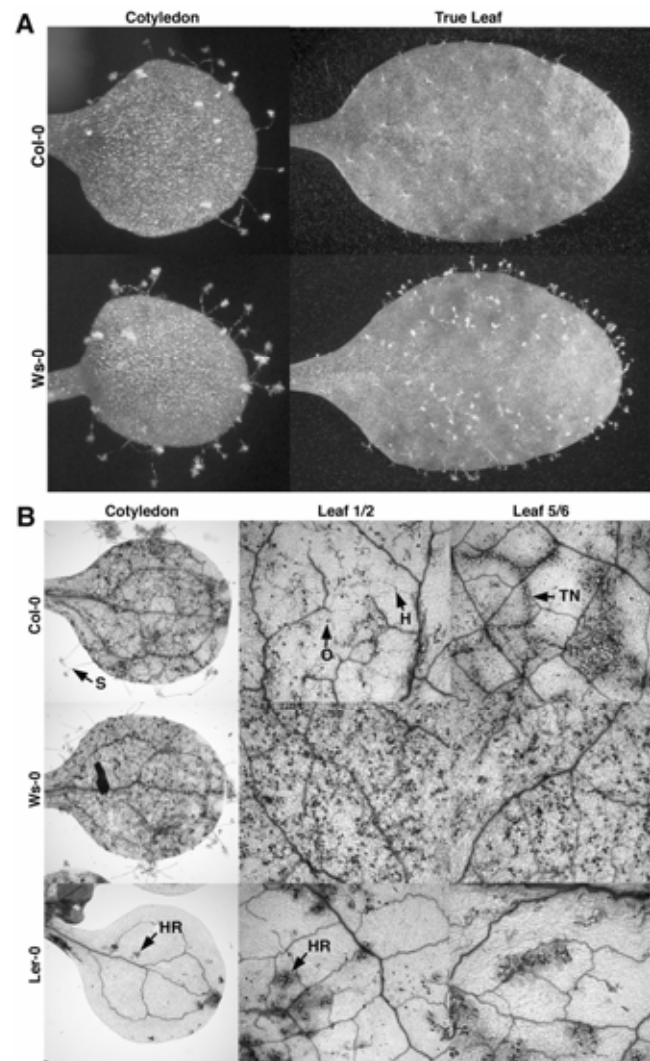


Fig. 1. Vegetative growth and asexual reproduction of *Hyaloperonospora parasitica* Emco5 during development of the Col-0 and Ws-0 ecotypes of *Arabidopsis*. All images were captured at 7 days after inoculation. The images depict phenotypes observed in three to eight independent experiments, each with at least five plants. **A**, Asexual sporulation of Emco5 on a representative cotyledon from seedlings inoculated at 7 days after germination and a representative true leaf from plants inoculated at five weeks after germination. **B**, Cotyledons or representatives from the first (leaf 1/2) or third pair (leaf 5/6) of true leaves, from Columbia (Col-0), Wassilewskija (Ws-0), or Landsberg *erecta* (Ler-0) ecotypes, stained with trypan blue. True leaves are numbered according to the order in which they emerged (Telfer et al., 1997). Trypan-blue staining highlighted asexual sporangiophores (S), vegetative hyphae (H), sexual oospores (O), hypersensitive response (HR) sites, and trailing necrosis (TN) that results from a delayed HR after limited hyphal growth.

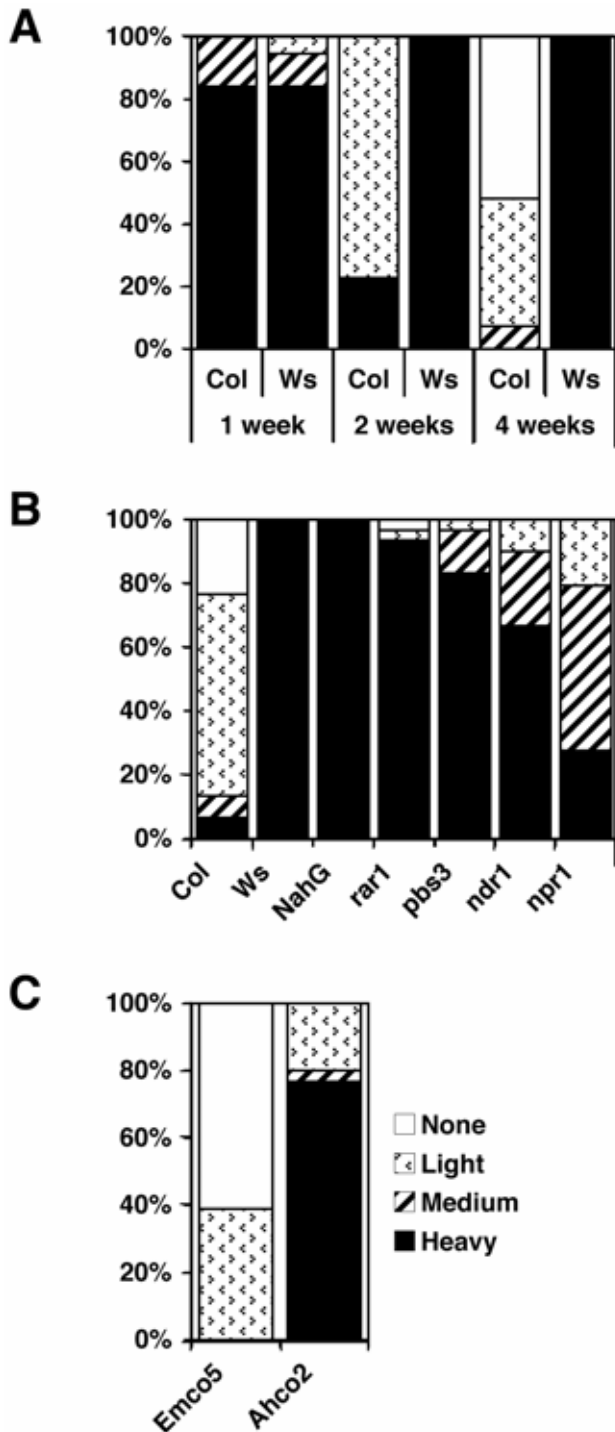


Fig. 2. Disease scores in seedlings and adult plants, based on sporangiophore production. Bar graphs depict percentages of cotyledons or true leaves that were scored as none (no sporangiophores per cotyledon or leaf), light (1 to 10 sporangiophores), medium (11 to 19), or heavy (>19), at 7 days after inoculation. Each genotype was tested in at least three independent experiments with similar results. Representative experiments are shown. **A**, Col-0 or Ws-0 plants, inoculated with Emco5 at 1, 2, or 4 weeks after germination. The 1-week percentages were calculated from 20 cotyledons scored from 10 plants. The 2- and 4-week percentages were calculated from 19 to 40 true leaves scored from 4 to 6 individual plants. **B**, 3-week-old Col-0, Ws-0, Col::*NahG*, *rar1-20*, *pbs3*, *ndr1-1*, or *npr1-1* plants inoculated with Emco5. The *pad4-1* mutant also displayed a phenotype of strong susceptibility (data not shown). Percentages were calculated from 29 to 30 leaves from five plants of each genotype. **C**, 4-week-old Col-0 or Ws-0 plants inoculated with Emco5 or Ahco2. Percentages were calculated, respectively, from 30 or 36 leaves from five or six plants.

supported markedly reduced numbers of sporangiophores, as compared with cotyledons (Fig. 2A). This phenotype is even more pronounced in true leaves of three- and four-week-old plants (Fig. 2A and B). Trailing necrosis, indicative of a delayed hypersensitive response, was observed to a limited extent in the first two true leaves and was much more extensive in leaves that subsequently emerged (Fig. 1B). Conversely, we typically observed that the first two true leaves supported significantly higher levels of hyphal growth, oospores, and sporangiophores than did subsequent leaves (Fig. 1B). Older plants (i.e., five to seven weeks after germination) often displayed complete resistance in all leaves (Fig. 1A). Control Col-0 plants expressing an *RPP8-Ler* transgene were completely resistant to Emco5 at all developmental stages (Fig. 3).

In contrast to Col-0, Ws-0 was highly susceptible to Emco5 throughout development. True leaves of Ws-0 supported very high levels of mycelial growth, oospores, and sporangiophores, regardless of developmental stage (Figs. 1 and 2A and B). Thus, the developmental resistance to Emco5 is specific to the Columbia ecotype and is not a general *Arabidopsis* response. In addition, Ws::*NahG* transgenic plants (Delaney et al. 1994) supported even higher levels of Emco5 sporulation than did wild-type Ws-0 (data not shown). This suggests that Ws-0, although susceptible, expresses a basal defense mechanism that limits the growth of virulent Emco5. Basal defense against *H. parasitica* has been documented previously (Parker et al.

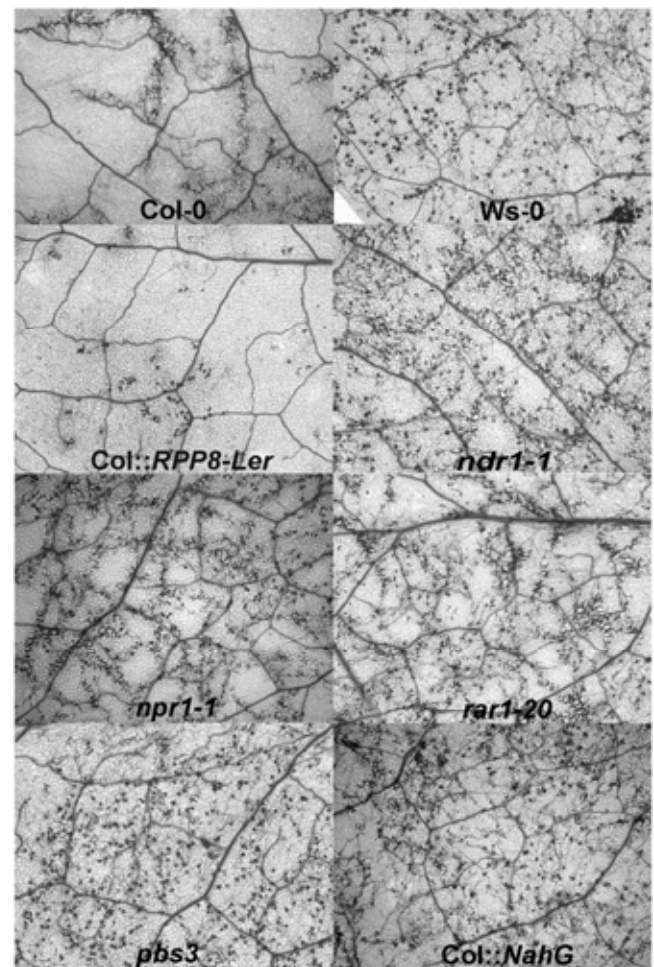


Fig. 3. Adult-resistance phenotypes of transgenic lines or mutants in the Columbia background inoculated with Emco5. True leaves were inoculated at 3 weeks after germination and were stained with trypan blue at 7 days after inoculation. The images depict phenotypes observed in two independent experiments.

1996). As in Col-0, the *RPP8-Ler* transgene in adult Ws-0 plants provided strong resistance to Emco5 (data not shown), demonstrating that Ws-0 contains all components necessary for resistance to Emco5 except for an appropriate *R* gene.

Adult resistance in Col-0 is race-specific and is suppressed by defense signal transduction mutants.

The incompatibility between Emco5 and Col-0 adult leaves could reflect a race nonspecific adult resistance against many *H. parasitica* isolates, as previously documented for some adult resistances (Panter and Jones 2002). However, we and others have previously observed that adult Col-0 plants are susceptible to *H. parasitica* isolate Noco2, suggesting that the developmental resistance in Col-0 against Emco5 is race-specific (J. M. McDowell, unpublished data) (Bowling et al. 1994; Clarke et al. 1998; Korves and Bergelson 2003). To generalize this further, we inoculated 4-week-old Col-0 plants with the Ahco2 isolate, previously shown to be virulent on Col-0 seedlings (Holub and Beynon 1996; Holub et al. 1993). We observed that true leaves of Col-0 are highly susceptible to Ahco2 (Fig. 2C). Thus, adult resistance in Col-0 against Emco5 does not reflect a general resistance against all *H. parasitica* isolates.

The trailing necrosis/hypersensitive response in true leaves (Fig. 1B) along with hydrogen peroxide production at the infection site (Torres et al. 2002) indicates that the incompatibility of adult Col-0 and Emco5 results from an active defense response in the host. To substantiate this further and to determine whether the resistance in Col-0 true leaves is genetically distinct from previously documented resistance mechanisms in *Arabidopsis*, we challenged three-week-old, isogenic Col lines containing mutations or transgenes known to suppress disease resistance responses. These included a Col::*NahG* transgenic line (Delaney et al. 1994) and loss-of-function mutants *npr1-1*

and *pad4-1* (Cao et al. 1997; Jirage et al. 1999; McDowell et al. 2000). Each of these lines is deficient in SA-mediated signaling, thereby impairing basal resistance, SAR, and certain *R* genes. We also challenged the *ndr1-1* mutant, in which certain *R* genes as well as SA-mediated responses are suppressed (Century et al. 1997; Coppinger et al. 2004; McDowell et al. 2000; Shapiro and Zhang 2001), the *rar1-20* mutant that exhibits reduced R protein stability (Bieri et al. 2004; Shirasu and Schulze-Lefert 2003; Tornero et al. 2002), and the *pbs3* mutant, in which certain *R* genes are suppressed through an unknown mechanism (Warren et al. 1999). Adult resistance to Emco5 was significantly suppressed in each of these backgrounds. The strongest suppression was observed in *NahG* transgenic plants, in which Emco5 mycelial growth and sporulation matched or slightly exceeded Ws controls. In contrast, the *npr1-1* mutation only partially suppressed resistance and displayed limited trailing necrosis indicative of a delayed resistance response. The *pad4-1*, *rar1-20*, *pbs3*, and *ndr1-1* phenotypes were intermediate to those of *NahG* and *npr1-1*. The jasmonate-response mutant *jar1* (Staswick et al. 2005) displayed no alteration of adult resistance (data not shown). Taken together, these results demonstrate that the adult resistance in Col-0 requires a variety of regulatory components previously associated with inducible defenses.

Genetic analysis of adult resistance.

Resistance to pathogens is often genetically dominant to susceptibility, particularly when conferred by an *R* gene (Crute and Pink 1996). To determine whether adult resistance in Col-0 is genetically dominant to susceptibility in Ws-0, we examined resistance to Emco5 in F1 and F2 progeny from crosses between Col and Ws-0 (Table 1, Fig. 4). None of the Ws-0 × Col-0 F1 progeny displayed a Col-like resistance phenotype. The majority of F1s were just as susceptible as parental Ws to

Table 1. Segregation of adult resistance to *Hyaloperonospora parasitica* Emco5

Cross ^a	F1			F2		χ^2 ^e
	Res ^b	Sus	Int	Res ^c	Sus ^d	
<i>rpm1-3</i> (Col-5) × Ws-0	0	8	2	89	30	1.09 (<i>P</i> > 0.3)
Col-0 × Ws-0	0	6	3			
Ws-0 × Col-0	0	2	4			
Ws-0 × CS3432	1	0	5			
CS3432 × Ws-0	0	0	6			
CS3151 × Ws-0	0	0	4			

^a Data were pooled from four experiments. The pollen recipient is listed first.

^b Res = resistant phenotype, resembling Col-0 parent; Sus = susceptible phenotype, resembling Ws-0 parent; Int = intermediate phenotype.

^c Each resistant F2 plant was inoculated twice with Emco5 to confirm that the resistant phenotype was not due to a low dose of inoculum.

^d Plants displaying an intermediate phenotype were scored as susceptible.

^e χ^2 and probability (*P*) values are given for the expected 1:3 resistant/susceptible ratio.

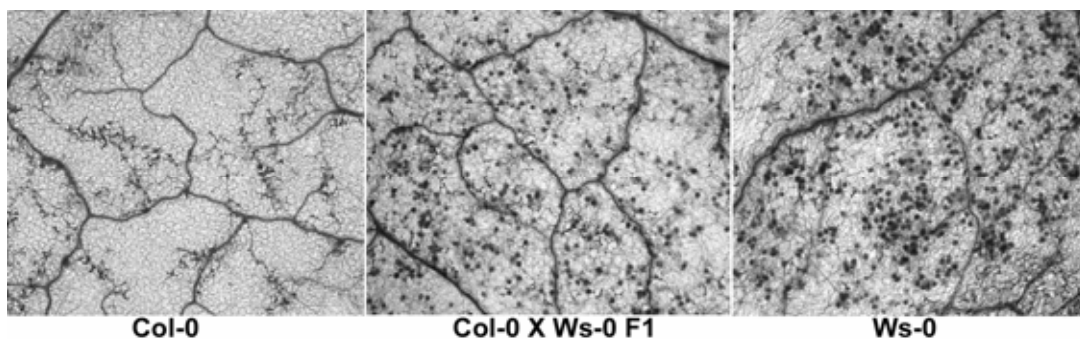


Fig. 4. Adult-resistance phenotype in F1 progeny from a cross of Ws-0 to Col-0. Representative leaves from Col-0, Ws-0, and a Ws-0 × Col-0 F1 progeny, inoculated at 3 weeks after germination and stained with trypan blue at 7 days after inoculation. The images depict phenotypes observed in four independent experiments.

Emco5, while a minority displayed intermediate levels of susceptibility. These phenotypes indicate that susceptibility is incompletely dominant to resistance. F2 segregation ratios were consistent with this interpretation. Only 24% of F2 progeny displayed Col-like adult resistance, while the remaining 76% displayed full (Ws-like) or intermediate susceptibility to Emco5. This segregation ratio of 1:3 resistant/susceptible indicates that the phenotypic difference between Col and Ws-0 is due to a single, recessive gene.

The recessivity of resistance in the Col-0 × Ws-0 hybrid could be a gene-dosage effect, in which one copy of a Col-0 allele is insufficient to activate adult resistance (termed haploinsufficiency). Alternatively, Ws-0 might express a susceptibility allele that is truly dominant, perhaps through a negative effect on a Col-0 resistance allele. To better understand the genetic basis of susceptibility in the Col-0 × Ws-0 hybrid, we conducted a gene-dosage experiment in which we examined the adult resistance phenotype of triploid plants containing two copies of each Col chromosome and one copy of each Ws-0 chromosome (genotype Col/Col/Ws-0). Triploid plants were constructed by crossing diploid Ws-0 with two tetraploid derivatives of Col (CS3151, CS3432) (Greenberg 2000). If resistance is recessive because of Col-0 haploinsufficiency in diploid Col-0 × Ws-0 F1s, then triploid plants should display Col-like adult resistance. We did not observe this phenotype; almost every F1 from crosses to CS3151 or CS3432 displayed clearly enhanced sporulation compared to Col-0, CS3151, and CS3432 parents (Table 1). However, the triploid F1s consistently supported less sporulation than either Ws-0 parents or diploid F1 progeny from wild-type Col-0 × Ws-0 crosses. These phenotypes indicate that the Ws-0 susceptibility phenotype is dominant (albeit incompletely) to resistance in Col-0.

We genetically mapped the adult resistance phenotype in Col-0 × Ws-0 F2s, using polymerase chain reaction (PCR)-based markers (cleaved amplified polymorphic sequences [CAPS] or simple sequence length polymorphisms [SSLP]) on each of the 10 *Arabidopsis* chromosome arms (Table 2). Because adult resistance is recessive, we performed the mapping with a set of 89 F2s that displayed a clear adult resistance phenotype and were presumably homozygous for one or more recessive Col-0 resistance alleles. We observed strong linkage of the resistance phenotype to the bottom arm of chromosome 5, in a region between the *spl2* and *nga129* molecular markers (Table 2). We hypothesize that a major gene for adult resistance in Col-0 resides in this interval. We named this gene *RPP31*, following the previously established convention for naming *Arabidopsis* genes that provide resistance to *Hyaloperonospora parasitica*. We also observed weak linkage to the bottom of chromosome 2, suggesting the existence of a weak modifier of the resistance phenotype (Table 2).

No *RPP* gene has been previously mapped in the Columbia ecotype to the interval between *spl2* and *nga129*. However, the *RPP8* resistance gene from the Landsberg *erecta* ecotype (*RPP8-Ler*) is located approximately 1 cM south of *spl2*, based on mapping in the Dean-Lister Col × Ler recombinant inbred population (McDowell et al. 1998). *RPP8-Ler* was previously defined by its ability to provide resistance to Emco5 when expressed as a transgene in Emco5-susceptible Col-0 or Ws-0 seedlings (McDowell et al. 1998, 2000). The Col-0 allele of *RPP8* is transcribed, and encodes an NBS-LRR protein with 92% amino acid identity to *RPP8-Ler*. Although *RPP8-Col* does not provide resistance to Emco5 in seedlings, it could potentially function in adult resistance. To test this possibility, we challenged two lines from the Salk T-DNA mutant collection that contained independent insertions in *RPP8-Col* (SALK_046059, SALK_001957) (Alonso et al. 2003). The SALK_46059 insertion maps to codon 13 in exon 1, just downstream of the translation initiation site. The SALK_001957 insertion maps to codon 647 in exon 3, within the fifth of 14 LRR. These insertions presumably result in null alleles. We used PCR to confirm that each line contains T-DNA at the documented location. We inoculated 38 and 22 individuals, respectively, from T3 populations segregating for the SALK_046059 and SALK_001957 insertions. Each of these individuals displayed a clear adult resistance phenotype, indistinguishable from Col-0 controls (data not shown). Subsequent PCR tests of 12 Emco5-challenged plants from each population confirmed that the T-DNA insertions in *RPP8-Col* were segregating at approximately the expected genotypic ratio of 1:2:1 wild-type/hemizygote/homozygous knockout, in both populations. The absence of Emco5-susceptible adult plants in these populations indicated that *RPP8-Col* is unnecessary for adult resistance to Emco5.

DISCUSSION

Although developmentally regulated disease resistance has not been well-studied at the molecular level, several experimentally tractable pathosystems have been recently developed. Initial studies with these systems indicate that many distinct mechanisms underlie the phenomenon of developmental resistance (Panter and Jones 2002). In several cases, resistance has been correlated with the transition to reproductive growth. For example, developmental resistance in *Arabidopsis* to *P. syringae* (referred to by the authors as age-related resistance [ARR]) has been correlated with the transition to flowering and is also activated by abiotic stress (Cameron and Zaton 2005; Kus et al. 2002). This resistance requires SA accumulation in the intercellular space but is not compromised by the *npr1-1*, *pad3-1*, or *eds7-1* mutations. These results indicate

Table 2. Genetic mapping of the adult-resistance phenotype

Marker ^a	Chromosome	cM ^b	Recombinant chromosomes	Total chromosomes	Recombination frequency (%)	χ^2 ^c
mi310	II	18.6	29	44	54	0.30 ($P > 0.59$)
m429	II	73.2	12	34	35	2.94 ($P > 0.09$)
nga168	II	73.8	75	172	44	2.81 ($P > 0.09$)
athBIO2	II	76.1	77	178	43	3.24 ($P > 0.07$)
nga249	V	23.7	23	55	41	1.79 ($P > 0.18$)
nga76	V	68.4	22	80	28	16.20 ($P < 0.001$)
SO191	V	79.9	29	172	17	75.6 ($P < 0.001$)
spl2	V	91.6	13	168	8	120.0 ($P < 0.001$)
nga129	V	105.4	21	168	13	94.5 ($P < 0.001$)

^a Only markers on chromosomes 2 and 5 are shown. No linkage was observed with the following markers: nga63 and nga128 on chromosome 1; nga162, glabrous 1, and PUR5 on chromosome 3; nga8 and nga11 on chromosome 4.

^b cM = centimorgans; map positions are from the Dean-Lister Columbia × Landsberg recombinant inbred population.

^c χ^2 and probability (P) values are given for a 1:2 ratio of recombinant versus total chromosomes (as expected for loci unlinked to developmentally regulated resistance)

that ARR is mechanistically distinct from SAR, ISR, and basal resistance (Kus et al. 2002). Flowering tobacco plants also display enhanced resistance to several pathogens that are highly virulent at earlier developmental stages, including *Tobacco mosaic virus* and the oomycete pathogens *Phytophthora parasitica* (black shank) and *Peronospora tabacina* (blue mold) (Hugot et al. 1999; Reuveni et al. 1986; Wyatt et al. 1991; Yalpani et al. 1993). Gene expression studies in tobacco have revealed that defense-associated genes are globally upregulated after the transition to flowering (Fraser 1981; Hugot et al. 1999, 2004; Lotan et al. 1989; Uknes et al. 1993; Yalpani et al. 1993). This upregulation occurs independently of pathogen attack and is triggered by endogenous developmental signals that include SA. Interestingly, tobacco resistance to blue mold is activated before the transition to reproductive growth in late-flowering tobacco lines, and the authors of this study speculate that resistance could be triggered by the onset of senescence (Wyatt and Kuc 1992). This idea is supported by reports that defense-associated genes can be activated during senescence (Morris et al. 2000; Quirino et al. 1999). Panter and Jones (2002) have postulated distinct “flowering-induced” and “senescence-induced” pathways for developmentally regulated resistance.

The developmentally regulated, *RPP31*-dependent resistance characterized in this study differs significantly from the examples cited above. Most obviously, *RPP31*-dependent resistance is activated before the transition to flowering or the onset of senescence. The striking contrast in the responses of true leaves compared with those of cotyledons suggests that a component of *RPP31*-dependent resistance is activated upon the developmental phase change from postembryonic to juvenile growth (Kerstetter and Poethig 1998). Additionally, *RPP31* resistance is race-specific. This contrasts with the broad-spectrum resistance against viruses and multiple oomycete species in flowering tobacco. *Arabidopsis* ARR to *P. syringae* is effective against two strains and may be similarly broad spectrum (Kus et al. 2002). *RPP31* resistance is further distinguished from *Arabidopsis* ARR against *P. syringae* in terms of its genetic requirements. Both types of resistance are suppressed by *NahG* and, therefore, may be SA-dependent. In contrast, ARR against *P. syringae* is functional in the *npr1-1* mutant background, while *RPP31* resistance is partially suppressed by *npr1-1* (Kus et al. 2002). Finally, ARR against *P. syringae* is functional in the *Ws-0* background, while *RPP31* resistance is not.

Considering the race specificity of *RPP31* resistance as well as its correlation with an oxidative burst and cell death and its genetic dependence upon known components of *R* protein signal transduction, it seems likely that *RPP31*-dependent resistance is underpinned by a gene-for-gene interaction. In principle, the naturally variable *RPP31* locus could encode either an *R* protein, another type of resistance signaling component, or a “guardee.” Although we cannot rule out the latter two possibilities until *RPP31* is cloned, it seems likely that *RPP31* encodes an *R* protein, because every naturally variable *RPP* gene cloned to date encodes a NBS-LRR or receptor-like protein (RLP) (Slusarenko and Schlaich 2003; Tor et al. 2004). To our knowledge, no examples of intraspecific polymorphism among resistance signaling components or guardees has been described in *Arabidopsis*, with the possible exception of the *ISR1* locus that has been postulated as a regulator of ISR (Pieterse et al. 2003). Like *RPP31*, *ISR1* is functional in *Col* and nonfunctional in *Ws*, but *ISR1* has been mapped to chromosome 3 and, therefore, is not allelic with *RPP31* (Ton et al. 2001). The *RPP31* mapping interval on chromosome 5 encompasses four genetically defined resistance genes: *RPP8*, *RPS4*, *TTR1*, and *RRS1* (Deslandes et al. 2002; Gassmann et al. 1999; Lee et al. 1996; McDowell et al. 1998). Our analysis of knockouts from

the Salk collection demonstrated that *RPP8-Col* is unnecessary for adult resistance. The allelic relationships of *RPP31*, *RPS4*, *TTR1*, and *RRS1* remain to be clarified. The *RPP31* mapping interval encompasses a large number of uncharacterized NBS-LRR genes that represent potential candidates for *RPP31* (Meyers et al. 2003; Tor et al. 2004).

Several cases of developmentally regulated, gene-for-gene resistance have been documented previously. For example, the tomato gene *Cf-9B* provides resistance to the leaf mold pathogen *Cladosporium fulvum* and tomato *Mi-1.2* provides resistance to aphids and whiteflies (Goggin et al. 2004; Panter et al. 2002; Parniske et al. 1997). Both of these resistances are activated after the transition to flowering. The rice gene *Xa-21*, for resistance against sheath blight (*Xanthomonas oryzae*), is non-functional in seedlings but becomes progressively more efficient from 21 days onward (Century et al. 1999). Finally, *Arabidopsis* resistance genes in the *RPP1* cluster are functional in leaves but not roots (Hermanns et al. 2003).

One obvious hypothesis to explain developmentally regulated, gene-for-gene resistance is that expression of the *R* gene itself is developmentally regulated. Interestingly, expression studies of the genes cited above do not support this hypothesis. Transcripts from *Xa-21*, *Cf-9B*, *Mi-1.2*, and *RPP1* were detected at approximately equivalent levels in resistant and susceptible tissues (Century et al. 1999; Goggin et al. 2004; Hermanns et al. 2003; Panter et al. 2002). The *Cf-9B* promoter was shown to be functionally equivalent to the promoter of the *Cf-9* gene, which confers resistance in seedlings as well as mature plants (Panter et al. 2002). Finally, a transgene fusion of the constitutive cauliflower mosaic virus 35S promoter to the *Mi-1.2* coding sequence was highly expressed in seedlings but did not confer resistance (Goggin et al. 2004). These results indicate that differential regulation of *R* gene transcription is not a major mechanism underlying developmentally regulated resistance. However, none of the studies described above examined *R* protein abundance. Thus, it is possible that the *R* gene products are regulated by translational or posttranslational mechanisms.

The studies described in the previous paragraph speak to the question of why *RPP31*-dependent resistance is active in true leaves but not in seedlings. The simplest explanation is that *RPP31* encodes a resistance gene that is expressed in true leaves but not seedlings. Although the precedents discussed above argue against this hypothesis, it cannot be ruled out at present. It is also possible that a regulator of resistance, acting upstream or downstream of an *R* protein, is nonfunctional or is not expressed in seedlings. Finally, the developmental component of *RPP31* resistance could be provided by a differentially expressed guardee, a plant protein that is targeted by an Emco5 virulence factor and is guarded by *RPP31* (Panter and Jones 2002; Panter et al. 2002).

Another intriguing aspect of *RPP31* is its genetic recessivity. The majority of *R* genes that have been cloned are genetically dominant, and little is known about molecular differences distinguishing dominant and recessive resistance genes (Chu et al. 2004; Li et al. 2001). A small number of recessive resistance genes have been cloned, and most do not encode NBS-LRR or RLP proteins. The best-studied recessive resistance gene is *mlo* from barley, which encodes a seven-transmembrane domain protein (Büschges et al. 1997). Loss-of-function *mlo* alleles confer broad-spectrum, durable resistance to powdery mildew. The exact function of *Mlo* is unclear, but it is currently thought to function as a component of the defense machinery and as a compatibility target that the pathogen actively manipulates to facilitate colonization (Schulze-Lefert 2004). Other recessive resistance genes that have been recently cloned include *pvr1/pvr2* from pepper, *sbm1* from pea, and *moll1* and *moll2* from lettuce, all of which function in resistance

to different potyviruses (Gao et al. 2004; Kang et al. 2005; Nicaise et al. 2003; Ruffel et al. 2002). These genes encode isoforms of eukaryotic translation initiation factor 4e, which likely functions as a host compatibility factor to facilitate viral replication and movement. The rice *Xa5* and the *Arabidopsis RRS1* genes provide recessive resistance against *Xanthomonas oryzae* and *Ralstonia solanacearum*, respectively. *Xa5* encodes the gamma subunit of transcription factor IIA (Iyer and McCouch 2004). The role of this protein in resistance is currently unclear; however, the cognate Avr protein is localized to the nucleus, suggesting that *Xa5* may also function in compatibility. *RRS1* is the only cloned recessive resistance gene with molecular similarity to dominant *R* genes. *RRS1* encodes a Toll and interleukin 1 receptor (TIR)-NBS-LRR protein with an atypical WRKY DNA-binding domain at the C-terminus (Deslandes et al. 2002). *RRS1* appears to associate directly with the cognate avirulence protein in the nucleus. The molecular basis of *RRS1* recessivity is currently unknown and somewhat enigmatic, because *RRS1* transgenes are genetically dominant when expressed in an *rrs1* background (Deslandes et al. 2002, 2003).

One explanation for *RPP31* recessivity, from the precedents discussed above, is that *RPP31* encodes a naturally variable compatibility factor that is nonfunctional in the Ws-0 background. However, as discussed above, we favor the hypothesis that *RPP31* encodes an NBS-LRR resistance protein. Perhaps *RPP31* encodes a “weak” R protein with low affinity for an interacting protein, such that two doses are necessary to activate resistance. Our gene-dosage experiments indicate that resistance is dose-dependent because triploid Col/Col/Ws-0 hybrids were noticeably more resistant than diploid Col-0/Ws-0 hybrids. However, the triploid hybrids consistently permitted more pathogen growth and reproduction than did diploid or tetraploid Col. This phenotype is inconsistent with a hypothesis of simple haploinsufficiency, suggesting that a Ws-0 allele exerts a negative, incompletely dominant effect on Col-0 resistance. This and other aspects of *RPP31*-dependent resistance would likely be clarified by map-based cloning of *RPP31*.

In closing, it is relevant to note that genes for developmentally regulated resistance to *H. parasitica* have been identified in broccoli and cabbage cultivars (*Brassica oleracea*) (Coelho and Monteiro 2003; Dickson and Petzoldt 1993; Wang et al. 2000). Like *RPP31*, these genes provide resistance in true leaves but not in cotyledons. Cultivated Brassicas like broccoli and cauliflower are particularly susceptible to downy mildew disease at the seedling stage, because they are grown in propagation tray systems under high humidity that promotes disease development (Brophy and Laing 1992). Thus, it would be of potential agronomic utility as well as of fundamental interest to compare the mechanisms underlying developmental resistance to downy mildew disease in the *Brassica* and *Arabidopsis* genera.

MATERIALS AND METHODS

Plant and pathogen lines, growth conditions, and pathogen inoculations.

rpm1-3 contains a loss-of-function allele of the bacterial resistance gene *RPM1* in the Col-5 (glabrous 1) background (Boyes et al. 1998; Grant et al. 1995). CS3432 and CS3151 are tetraploid lines in the Columbia background (Greenberg 2000). Adult-resistance phenotypes in *rpm1-3*, CS3432, and CS3151 lines are indistinguishable from wild-type Col-0 (data not shown). *H. parasitica* isolates Emco5 and Ahco2 were previously described (Holub and Beynon 1996; McDowell et al. 1998) and were propagated on Ws-0 or Col-0 plants, respectively, at 7-day intervals.

Plants for pathogen-challenge experiments were grown at 23°C under 8 or 10-h day length, with light intensities of approximately 200 μE in Sunshine mix number one (Sun Gro Horticulture, Bellevue, WA, U.S.A.). Plants for adult-resistance assays were germinated in 4-in pots and were grown for 2 weeks, after which they were transplanted to 10 \times 17-in flats in matrices of 10 columns by 5 rows. Inoculations were performed 7, 14, or 21 days later, by spraying plants with a suspension of 5 \times 10⁴ spores/ml in distilled water to the point of imminent run-off. Flats were covered with a plastic dome for 12 to 18 h, were uncovered for 3 days, and then, were covered again until they were scored at 7 dai. Comparisons of age-matched transplanted and nontransplanted plants were performed to confirm that resistance and susceptibility phenotypes were not affected by transplantation. Senescence was never observed in 3- and 4-week old plants and occurred only rarely in 5-week old plants. Staining with trypan blue was performed as described (McDowell et al. 1998), and images were captured on a Zeiss Axioscop2 compound microscope with a Spot digital camera. Images of sporulating plants were captured on a Zeiss SV11 stereomicroscope using a JVC digital camera and Automontage imaging software (Syncroscopy, Inc., Frederick, MD, U.S.A.).

Genetic mapping.

Crosses were performed by manual emasculation and pollination and were verified by visual or molecular markers. F2 plants were inoculated and scored as described above. Plants that displayed a Col-like adult-resistance phenotype were immediately inoculated a second time with Emco5 and were scored one week later to rule out the possibility that the resistance phenotype was due to a low dose of inoculum during the initial screen. DNA was isolated from one leaf of each plant that passed both screenings, and genetic mapping was conducted with CAPS and SSLP markers. Molecular markers were amplified by PCR and were assayed as described by The Arabidopsis Information Resource, except for SPL2. Primers 5'-GAAATTGATGGATTGATTGC-3' and 5'-ATGCTATAACTGAACTATAGGC-3' amplified a 1.5-kb band that is polymorphic after digestion with the DdeI restriction enzyme.

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