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Gene-for-gene disease resistance without the hypersensitive response in *Arabidopsis dnd1* mutant

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ABSTRACT **The cell death response known as the hypersensitive response (HR) is a central feature of gene-for-gene plant disease resistance. A mutant line of *Arabidopsis thaliana* was identified in which effective gene-for-gene resistance occurs despite the virtual absence of HR cell death. Plants mutated at the *DND1* locus are defective in HR cell death but retain characteristic responses to avirulent *Pseudomonas syringae* such as induction of pathogenesis-related gene expression and strong restriction of pathogen growth. Mutant *dnd1* plants also exhibit enhanced resistance against a broad spectrum of virulent fungal, bacterial, and viral pathogens. The resistance against virulent pathogens in *dnd1* plants is quantitatively less strong and is differentiable from the gene-for-gene resistance mediated by resistance genes *RPS2* and *RPM1*. Levels of salicylic acid compounds and mRNAs for pathogenesis-related genes are elevated constitutively in *dnd1* plants. This constitutive induction of systemic acquired resistance may substitute for HR cell death in potentiating the stronger gene-for-gene defense response. Although cell death may contribute to defense signal transduction in wild-type plants, the *dnd1* mutant demonstrates that strong restriction of pathogen growth can occur in the absence of extensive HR cell death in the gene-for-gene resistance response of *Arabidopsis* against *P. syringae*.**

Gene-for-gene resistance is a form of plant disease resistance that is exploited widely by plant breeders, forming a cornerstone of disease control in crop plants (1–4). The name "gene-for-gene" denotes the dependence of this resistance on matched specificity between a plant disease resistance gene and a pathogen avirulence gene (5). In a process that is reminiscent of mammalian antibody–antigen interactions, these genes apparently control receptor–ligand interactions that activate a complex defense response (4, 6, 7). There are thousands of resistance genes that mediate the recognition of specific fungal, bacterial, viral, or nematode pathogen strains. The strong defense response that is triggered after a gene-for-gene interaction includes synthesis of antimicrobial enzymes and metabolites, generation of signaling molecules that activate defense in neighboring cells, and reinforcement of plant cell walls surrounding the site of infection (4, 7, 8). One of the most prominent features of gene-for-gene defense is the death of infected plant cells within hours after initial contact with pathogen, a process known as the hypersensitive response (HR) (9, 10). HR cell death is a programmed cell death response that bears features of the apoptotic cell death processes that occur in other metazoan organisms (8). Although HR cell death is a hallmark of gene-for-gene disease resistance, the relative importance of cell death in this form of disease resistance is not clear and may vary depending on the target pathogen species (7–10).

Proposed roles for cell death in the resistance response include mass release of antimicrobial enzymes and metabolites into the extracellular matrix, the elimination of a cell that the pathogen is exploiting for life support, and the release of signals that activate defense in neighboring and distant cells (7–10). Alternatively, HR cell death may be a side effect caused by exceptionally strong activation of signaling responses such as ion channel gating or oxidative burst or by the extensive build-up of toxic antimicrobial compounds within the cell. In forms of plant disease resistance other than gene-for-gene resistance, defense responses often are activated at a lower level and host cells typically do not undergo programmed cell death (11, 12). However, these other forms of disease resistance are less effective at blocking pathogen growth.

It has been difficult to assess experimentally the utility of cell death in gene-for-gene disease resistance because cell death is usually a central feature of this response. However, prior studies have provided some evidence that the HR is not always required for gene-for-gene resistance. In normal gene-for-gene reactions, where cell death is observed, components of gene-for-gene resistance such as an oxidative burst, salicylate production, or induction of PR gene expression are activated before HR cell death (7, 12). Components of the plant defense response also have been observed in plants in which HR cell death was delayed artificially by incubation in very low oxygen or in high humidity (13, 14). Rare examples have been reported of avirulence gene-specific resistance genes that do not provoke cell death during the restriction of pathogen growth (15, 16). As an additional example, reduced growth of an avirulent race of the obligate biotroph *Erysiphe graminis* f. sp. *hordei*, in the absence of an HR, was observed when barley tissue was treated with the transcriptional inhibitor cordycepin (17). Finally, *Arabidopsis ndr1* mutants exhibit the converse phenotype of susceptibility to avirulent *Pseudomonas syringae* despite retention of the HR phenotype in response to a subset of those pathogens (18). Although these examples suggest that cell death may not be essential for gene-for-gene resistance, there is other evidence that cell death is essential for successful restriction of pathogen growth in some gene-for-gene interactions. For example, separate experiments from the above-cited studies of Schiffer *et al.* provide evidence that *Mla*-type resistance is rendered ineffective by inhibition of HR cell death (17). The widespread association of HR cell death with gene-for-gene disease resistance in vascular plants suggests that it confers an adaptive benefit.

Here we report the identification and characterization of an *Arabidopsis* mutant, *dnd1*, that does not develop the HR in response to avirulent *P. syringae* pathogens. The *dnd1* mutant

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Abbreviations: HR, hypersensitive response; PR, pathogenesis-related; cfu, colony-forming units.
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exhibits gene-for-gene restriction of pathogen growth in the absence of extensive HR cell death and also exhibits a constitutive systemic acquired resistance phenotype.

MATERIALS AND METHODS

Inoculations with *P. syringae*. Original mutants and their progeny were tested for the HR by pipet inoculation of individual leaves with *P. syringae* pv. *glycinea* Race 4 pV288 (*avrRpt2*$^+$) or Race 4 pVSP61 (no *avr* gene) at $\approx 2 \times 10^8$ colony forming units (cfu)/ml (19, 20). Additional *P. syringae* strains used to test for gene-for-gene HR included *P. syringae* pv. *glycinea* Race 4 pAvrRpm1 (*avrRpm1*$^+$) and Race 4 pVB01 (*avrB*$^+$) (19, 21). Positive and negative *Arabidopsis* controls included the use of wild-type Col-0, Col-0 *rps2–201/rps2–201*, and Col-0 *rpm1/rpm1* ("*rps3–1*") mutants (19, 21). For bacterial growth experiments and for gene expression studies, *P. syringae* pv. *tomato* strain DC3000 and *P. syringae* pv. *maculicola* strain 4326 were used with the above plasmids or with pKec218 (*avrRps4*$^+$) (22). Quantitative determinations of bacterial growth in leaves were performed by dilution plating of homogenized leaf tissue on selective media, as described in ref. 23.

Mutant Screen and Crossing. *Arabidopsis thaliana* ecotype Col-0 seeds were mutagenized with ethyl methane sulfonate; M2 populations were obtained from Lehle Seeds (Round Rock, TX). To test for activation of the HR, *P. syringae* pv. *glycinea* Race 4 pV288 (*avrRpt2*$^+$), at a concentration of $\approx 2 \times 10^8$ cfu/ml in 10 mM $MgCl_2$, was introduced by vacuum infiltration into leaf mesophyll tissue of ≈11,000 M2 seedlings. Leaves were observed 24 and 40 h after infiltration, and plants with reduced, delayed, or no leaf collapse were saved for further analysis. Lines of potential interest were crossed with the wild-type Col-0 parent to initiate backcrossing and with ecotype No-0 to initiate genetic mapping. For complementation tests, *Arabidopsis* Col-0 *dnd1/dnd1* plants were crossed to homozygous *cpr1* and *cpr5* mutants, which also display a reduced rosette size (24, 25). Dominance/recessiveness and genetic complementation were deduced by observation that all F1 plants were wild-type in appearance and displayed the HR after inoculation with *P. syringae* pv. *glycinea* Race 4 pV288.

Microscopy. To monitor HR cell death at the cellular level, pipet infiltration was used to introduce *P. syringae* pv. *glycinea* Race 4 pV288 (*avrRpt2*$^+$) or Race 4 pVSP61 (no *avr* gene) into 40–70% of the mesophyll space of individual leaves, at the bacterial concentrations indicated. Leaves were removed from plants after 24 h, fixed in 2% formaldehyde, 5% acetic acid, and 40% ethanol for 30 min, and then cleared sequentially in 50% ethanol and 95% ethanol (20). Leaf parenchyma cells then were examined for HR-associated autofluorescence by using fluorescence microscopy with a fluorescein filter set (Ex 495 $\pm$ 20 nm, Em $>$ 505 nm) (26). Alternatively, Evan's Blue (Sigma) was infiltrated into leaves as a 1% aqueous solution 22–26 h after pathogen inoculation (26). After at least 10 min of staining, leaves were removed from plants, a portion of the epidermis was peeled back, and leaves were rinsed in H_2O, mounted in H_2O, and observed by light microscopy. Leaf areas damaged by physical handling were not considered when evaluating the proportion of dead and living cells.

Genetic Mapping. F2 populations from a No-0 × Col-0 *dnd1/dnd1* cross were used for mapping. The HR phenotype was assessed visually 24 and 48 h after pipet inoculation of leaves with *P. syringae* pv. *glycinea* Race 4 pV288 (*avrRpt2*$^+$) resuspended to $\approx 1 \times 10^8$ cfu/ml in 10 mM $MgCl_2$. Informative F2 lines were retested for HR in selfed F3 families. PCR-based cleaved amplified polymorphic sequence and microsatellite markers were used as described in refs. 27 and 28; a set of 17 markers spanning all five *Arabidopsis* chromosomes was used for initial linkage analysis.

Inoculations with Other Pathogens. Tobacco ringspot virus grape strain was applied to plants, and virus multiplication was monitored by using ELISA as described in ref. 29. *Xanthomonas campestris* pv. *campestris* strain 2669 (30) and *X. c.* pv. *raphani* strains 1946, 2345, and 2586 (31) were applied at a concentration of $\approx 1 \times 10^7$ cfu/ml and monitored as described in ref. 31. *Peronospora parasitica* isolate Noco2 was applied and monitored as described in ref. 32. For all experiments, *Arabidopsis* ecotype Col-0 served as a susceptible control for pathogen multiplication and virulence.

Gene Expression Studies. *P. syringae* pv. *tomato* strains DC3000 (pV288) or DC3000 (pVSP61) were introduced into leaf mesophyll of intact plants by vacuum infiltration (as above), typically at a dose of 5×10^4 cfu/ml. Total RNA was extracted from leaf material and equal quantities of RNA from each sample were separated in agarose–formaldehyde gels, blotted, and hybridized with ^{32}P-radiolabeled probe essentially as described in ref. 33. DNA probes were from Cao *et al.* (25). Hybridization was quantified by using a storage phosphor imaging system according to the manufacturer's instructions (Molecular Dynamics). Signal for PR-1 or β-glucanase in each lane was normalized to the control β-ATPase signal for that lane to correct for slight differences in gel loading, and normalized signals then were divided by the signal for the Col-0/no-pathogen sample to establish a relative scale.

Salicylic Acid Determinations. Salicylic acid determinations were performed as described in ref. 34 on leaf material from uninoculated 6-week-old plants.

RESULTS

Mutant Screen and Initial Analysis. To address the relationship between HR cell death, resistance gene-mediated defense signal transduction, and the actual restriction of pathogen growth, we sought to isolate and characterize mutants of *Arabidopsis thaliana* that are deficient in the HR. A mutagenized M_2 population of *Arabidopsis* line Col-0, which expresses the *RPS2* resistance gene, was screened by inoculating plants with a strain of the bacterial plant pathogen *P. syringae* pv. *glycinea* expressing the *RPS2*-complementary avirulence gene *avrRpt2* (19). An extremely high titer of pathogen, 2×10^8 cfu/ml, was used so that plants undergoing a wild-type HR would exhibit visible collapse of leaf tissue. The *dnd1* mutant was recovered from this screen as a line display-

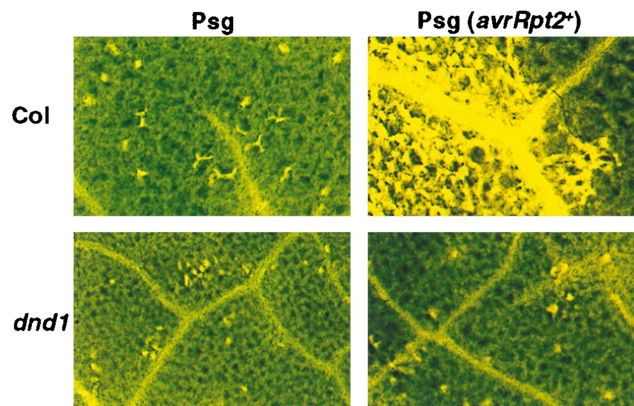

FIG. 1. HR cell death defect in *dnd1* mutant. Leaves of wild-type parent (Col) and *dnd1* mutant (*dnd1*) plants were inoculated with a high dose (2×10^8 cfu/ml) of avirulent, HR-stimulating *P. syringae* pv. *glycinea* Race 4 pV288 (Psg *avrRpt2*$^+$) or the isogenic, nonavirulent control strain *P. syringae* pv. *glycinea* Race 4 pVSP61 (Psg). At 24 h postinoculation, leaves were harvested, fixed, and examined for autofluorescent dead cells by using a fluorescence microscope. (*Upper Right)* The edge of an inoculated zone, revealing confluent cell death in response to bacteria only on the left (inoculated) side.

ing reduced rosette size and a clear HR^- phenotype. Progeny lines derived from the *dnd1* mutant failed to produce an HR not only when inoculated with pathogens expressing *avrRpt2* but also in response to *P. syringae* that express avirulence genes *avrRpm1* or *avrB* (19, 21). Two separate resistance genes (*RPS2* and *RPM1*) control responsiveness to these three separate avirulence genes. Accordingly, we hypothesize that the *dnd1* line is disrupted in a common component of the plant defense response that is shared by initially distinct gene-for-gene signal transduction pathways.

Microscopic Analysis of HR Cell Death. To confirm the absence of hypersensitive cell death in response to avirulent pathogens in the *dnd1* mutant, fluorescence microscopy was used to monitor cells within inoculated leaf tissue (26). Plant cells that undergo the HR display a marked increase in fluorescence due primarily to the production and release of phenolic compounds upon cell death. In "low titer" experiments, *P. syringae* pv. *glycinea* expressing *avrRpt2* were introduced into leaf mesophyll tissue at a concentration of $\approx 5 \times 10^5$ cfu/ml, a dose at which a majority of the plant cells are not initially in contact with pathogen. As expected, leaves from the wild-type parental line infected at this dose with *P. syringae* expressing *avrRpt2* contained numerous isolated autofluorescent cells. In contrast, very few autofluorescent foci were present in *dnd1* leaves inoculated with the same avirulent strain. The *dnd1* leaves instead resembled uninoculated leaves or leaves inoculated with the nonavirulent *P. syringae* control.

When leaves of the parental Col-0 line were inoculated with an extremely high titer of avirulent *P. syringae* (2×10^8 cfu/ml), the expected confluent collapse of host cells was observed (Fig. 1) (19, 20). However, even at this high pathogen dose, very little cell death above that seen in negative controls was detected in *dnd1* plants (Fig. 1). Separate experiments that used Evans Blue to stain dead or dying cells gave similar results. The autofluorescence assay method was preferred because of greater clarity and less laborious tissue preparation. With the autofluorescence assay, absence of HR cell death in *dnd1* plants was observed in multiple experiments, including experiments that used initial bacterial titers as high as 2×10^9 cfu/ml. A slight increase in cell death was observed in $\approx$5–8% of the *dnd1* leaves inoculated with 2×10^8 cfu/ml of avirulent *P. syringae* but only in isolated areas that represented a fraction of the inoculated tissue. Cell death in these small areas was patchy rather than confluent, and similar small patches of cell death could be observed at a lower frequency in control Col-0 plants inoculated with the nonavirulent *P. syringae* strain. Because of this isolated cell death in a small minority of inoculated leaves, we cannot absolutely conclude that the HR is abolished. However, no stimulation of cell death by avirulent *P. syringae* could be detected in the vast majority of the inoculated *dnd1* leaves.

Restriction of Avirulent Pathogen Growth. To determine whether the absence of the HR in the *Arabidopsis dnd1* mutant is associated with compromised disease resistance, growth of *P. syringae* pv. *tomato* within plants was monitored quantitatively over time (23). Pathogenic strains that express an avirulence gene are virulent on plants that do not express the corresponding resistance gene, but their growth is reduced severely on plants with the appropriate resistance gene. Fig. 2*A* shows the growth of *P. syringae* pv. *tomato* expressing *avrRpt2* in wild-type *Arabidopsis* Col-0 (*RPS2/RPS2*), in a Col-0 line lacking functional *RPS2* (*rps2–201/rps2–201*), and in the Col-0 *dnd1* mutant (*dnd1/dnd1*). Despite the absence of the HR, *dnd1* was very similar to wild type in successfully restricting the growth of *P. syringae* expressing *avrRpt2*. Strong avirulence and resistance gene-dependent restriction of pathogen growth also was observed in quantitative experiments with *P. syringae* expressing *avrRpm1*, *avrRps4*, or *avrB* (Fig. 2*B*; data not shown). These results demonstrate that extensive HR cell

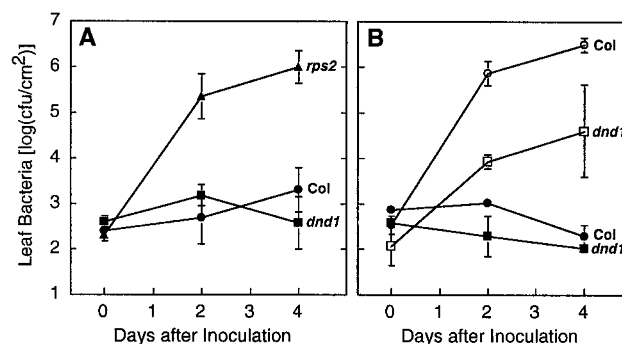

FIG. 2. Growth of bacteria within plant leaves. (*A*) *Arabidopsis* lines Col (Col-0 wild-type, *RPS2/RPS2*; *DND1/DND1*), *rps2* (Col-0 *rps2–201/rps2–201*; *DND1/DND1*), and *dnd1* (Col-0 *RPS2/RPS2*; *dnd1/dnd1*) inoculated with *P. syringae* pv. *tomato* DC3000 pV288 (*avrRpt2*$^+$). (*B*) *Arabidopsis* lines Col-0 and *dnd1* inoculated with isogenic *P. syringae* pv. *tomato* DC3000 differing by the presence (pAvrRpm1, filled symbols) or absence (pVSP61, open symbols) of avirulence gene *avrRpm1* carried on plasmid pVSP61. Both plant lines are *RPM1/RPM1* genotype. All data points are mean ± SD.

death is not always required for resistance gene/avirulence gene-dependent plant disease resistance.

Genetic Mapping. To determine the genetic basis of the *dnd1* phenotype, segregation analysis and gene mapping studies were carried out. Crosses of *dnd1* to wild-type Col-0 and No-0 ecotypes yielded F1 individuals that display the wild-type HR^+ phenotype, demonstrating the recessive nature of the mutant phenotype. F2 of a Col-0 × *dnd1* cross segregated 24:7 for HR^+:HR^-, F2 of a No-0 × *dnd1* cross segregated 154:55, and F2 of a reciprocal *dnd1* × No-0 cross segregated 132:45. These data are consistent with a 3:1 ratio (for χ^2 test, $P = 0.59$, 0.66, and 0.90, respectively), indicating that a single mutant locus controls the observed phenotypes. The reduced rosette size phenotype was also recessive, and absolutely co-segregated with the HR^- phenotype in these and all other F2 plants analyzed. The gene symbol *DND1* was chosen for this locus, reflecting the mutant phenotype of *D*efense with *N*o HR cell *D*eath. PCR-based microsatellite and cleared amplified polymorphic sequence genetic markers were used to map the mutated locus. No linkage was detected except to markers for the top arm of chromosome 5. Fine-structure mapping with 536 F2 individuals from No-0 × *dnd1* crosses yielded only six recombinant chromosomes between *dnd1* and nga106 and a different 11 recombinant chromosomes between *dnd1* and CHS1. These experiments placed *DND1* within the $\approx$1.6-cM interval between CHS1 and nga106 on the upper arm of *Arabidopsis* Chromosome 5. This location defines a map position that has not been associated previously with defense-related genes.

Response to Virulent Pathogens. Having established that *dnd1* plants are resistant to avirulent *P. syringae* despite the absence of the HR, the response of the *dnd1* mutant to virulent *P. syringae* was examined. Fig. 2*B* shows the growth of the virulent *P. syringae* pv. *tomato* strain DC3000 (pVSP61) in wild-type Col-0 and in Col-0 *dnd1/dnd1* plants (open symbols). This strain does not trigger gene-for-gene resistance in plants of the Col-0 genotype (19, 23), yet leaf populations of this strain were reduced 10- to 100-fold in experiments with the *dnd1* mutant. Similar results were obtained in multiple experiments and in studies with the virulent *P. syringae* pv. *maculicola* strain 4326 (data not shown). The *dnd1* plants express a level of resistance to virulent *P. syringae* that is typical of plants exhibiting systemic acquired resistance, induced systemic resistance, or other forms of resistance gene-independent disease resistance (11, 35). This broad spectrum resistance phenotype co-segregated with the other *dnd1* mutant phenotypes in all cases tested.

Important to note, Fig. 2*B* also shows that growth of populations of *P. syringae* that do express *avrRpm1* (closed symbols) was restricted to a much greater extent than was growth of the virulent pathogen strain. A 1,000- to 10,000-fold reduction of pathogen growth was observed if the otherwise-virulent *P. syringae* strains DC3000 or 4326 expressed avirulence genes *avrRpm1* or *avrRpt2* (Fig. 2*B*; data not shown). These experiments demonstrated that gene-for-gene resistance can be induced over and above the weaker resistance gene-independent resistance in *dnd1* plants.

To examine the extent of the lower level resistance to virulent pathogens in the *dnd1* mutant, plants were inoculated with virulent strains of other pathogen species (29, 30, 36, 37). Tobacco ringspot virus spread systemically in only 9% of *dnd1* plants as opposed to 71% for wild-type Col-0. *Xanthomonas campestris* pv. *campestris* and *X. c.* pv. *raphani* (bacteria) only produced mild yellowing on *dnd1* rather than the necrotic lesions produced on Col-0. *Peronospora parasitica* (oomycete) produced three-fold fewer spores on *dnd1* as opposed to Col-0 [3.0 ± 2.2 vs. 10.7 ± 3.1; mean $\pm$ SE of (spores $\times 10^3$) per leaf]. Microscopy of leaves infected with virulent *P. parasitica* confirmed that restriction of mycelial growth was not associated with HR-like host cell necrosis or autofluorescence. At 3 days postinoculation, mycelia of virulent *P. parasitica* strain Noco2 typically had formed haustoria on 2–10 host cells in *dnd1* plants, whereas in wild-type Col-0 plants a typical mycelium ramified extensively and formed haustoria on 15–30 host cells. Significantly reduced growth of *Erysiphe orontii* (fungus) in *dnd1* plants also has been observed (T. L. Reuber and F. M. Ausubel, personal communication).

PR Gene Expression. Constitutively elevated broad spectrum resistance has been observed previously in a number of contexts, such as in *Arabidopsis cpr, cim, lsd*, and *acd* mutants (8), in hybrid tobacco lines derived from crosses between disparate *Nicotiana* species (38), and in plants expressing systemic acquired resistance in response to prior pathogen infection or treatment with salicylic acid or synthetic salicylic acid mimics (11). Elevated resistance often is associated with increased expression of pathogenesis-related (PR) genes (11), and examination of uninoculated *dnd1* plants revealed constitutively increased expression of the PR genes β-glucanase and PR-1 (Fig. 3 *A* and *B*) (25, 33). Although plants infected by virulent *P. syringae* pv. *tomato* displayed elevated levels of β-glucanase or PR-1 mRNA, inoculation of *dnd1* or wild-type Col-0 with avirulent *P. syringae* expressing *avrRpt2* caused an even greater elevation in PR-1 mRNA (Fig. 3*C*) (25, 33). Similar or more pronounced results were obtained with four distinct RNA sets prepared, blotted, and probed in entirely separate experiments. These results demonstrate, at the level of gene expression, that gene-for-gene signal transduction and defense response activation are functional in *dnd1* plants and are inducible over and above constitutive broad spectrum resistance.

Salicylic Acid Levels. Enhanced PR gene expression and broad spectrum resistance can be induced by elevated levels of endogenous or applied salicylic acid compounds (11). We observed constitutively elevated levels of both free salicylic acid and glucoside-conjugated salicylates in *dnd1* plants (Fig. 4). Although salicylates are likely to be a primary mediator of heightened resistance in *dnd1* plants, the mechanism by which the *dnd1* mutation causes salicylate elevation remains to be discovered.

Comparison to Other *Arabidopsis* Mutants. We are not aware of previous reports of plant mutants that display gene-for-gene disease resistance with no HR cell death. However, other *Arabidopsis* mutants that exhibit constitutively elevated resistance have been isolated, such as the *cpr*, *cim*, *lsd*, and *acd* mutants (8, 24, 39, 40). Accordingly, *dnd1* plants were compared with a number of these lines. In contrast to the *acd* and *lsd* mutants, no lesion-mimic phenotype was observed in *dnd1* mutants when leaf tissue from uninoculated plants was inspected by naked eye, by autofluorescence microscopy as described in ref. 20, or after trypan blue staining as described in ref. 41. Genetic complementation tests demonstrated that *DND1* is a separate locus from the two published *cpr* loci, *CPR1* and *CPR5* (see *Materials and Methods*). In addition, the *dnd1* mutant apparently does not resemble many of the other unpublished *cpr* or *cim* mutants because the *dnd1* mutant does not exhibit traits observed in preliminary analysis of those mutants such as dominant or semi-dominant behavior, very low fertility, glabrousness, or distorted leaf shape (data not shown; S. Bowling and X. Dong, personal communication; K. Maleck and J. Ryals, personal communication). In particular, previously described *cpr* and *cim* mutants do not display the *dnd* phenotype of gene-for-gene defense with no HR cell death. The *dnd1* mutant does exhibit a dwarf phenotype, as is observed in *Arabidopsis cpr*, *cim*, and other constitutive PR-expression mutants, but *dnd1* plants otherwise appear normal in their growth and development.

DISCUSSION

A strong association exists between HR cell death and gene-for-gene resistance. However, there is evidence in the litera-

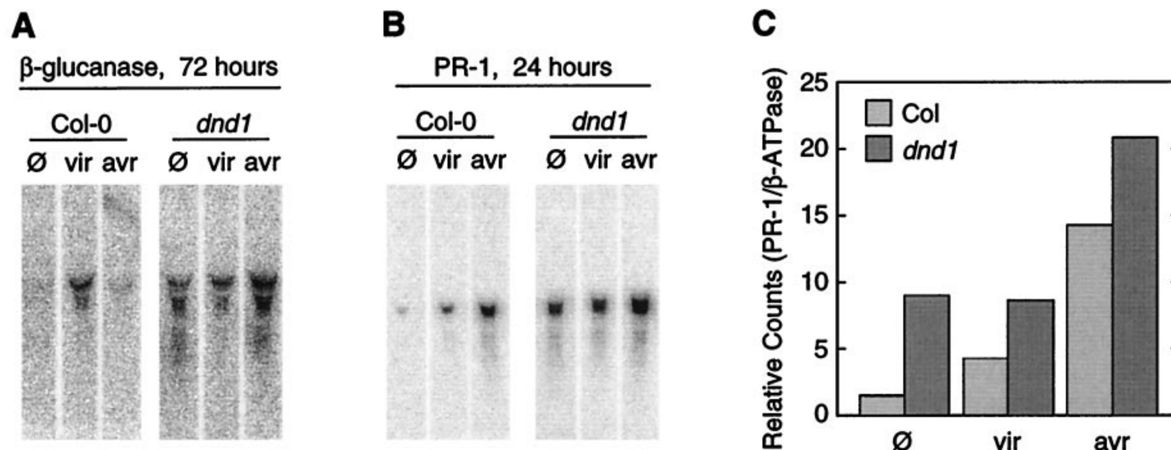

FIG. 3. Pathogenesis-related gene expression monitored by RNA blot analysis of Col-0 wild-type (Col) and Col-0 *dnd1*/*dnd1* mutant (*dnd1*) plants. (*A*) β-glucanase expression 72 h after treatment of leaves with 10 mM $MgCl_2$ containing no pathogen (ϕ), the nonavirulent control strain *P. syringae* pv. *tomato* DC3000 pVSP61 (vir), or the isogenic *avrRpt2*-expressing strain *P. syringae* pv. *tomato* DC3000 pV288 (avr). (*B*) PR-1 expression 24 h after treatment as in *A*. (*C*) Phosphorimager quantification of PR-1 expression from blot shown in *B*, normalized to level of constitutive β-ATPase mRNA. Similar results were obtained in multiple experiments.

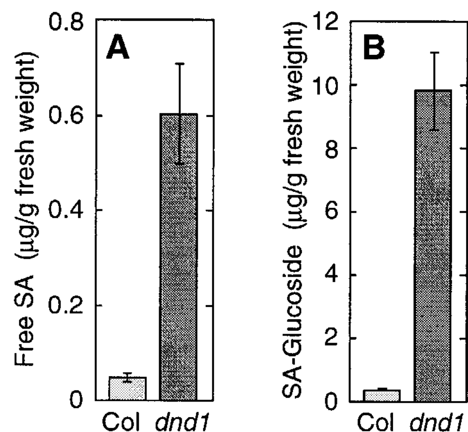

FIG. 4. Levels of salicylic acid and glucoside-conjugated salicylic acid compounds in Col-0 and mutant Col-0 *dnd1–1/dnd1–1* plants.

ture that it might be possible for gene-for-gene disease resistance to occur without HR cell death (see examples in Introduction). In the present study, a plant mutant was isolated that directly demonstrates gene-for-gene-mediated restriction of pathogen growth despite virtual elimination of HR cell death.

Models for the induction of plant defense appropriately place cell death downstream of resistance gene product/avirulence gene product interaction (4, 7, 8). Cell death can be placed upstream of defense induction, given that cell death is a known inducer of responses such as PR gene expression or local and systemic acquired resistance (8, 11). However, the role of cell death in defense induction is apparently supplementary or reinforcing rather than essential. Many forms of plant disease resistance other than gene-for-gene resistance do not involve cell death (3). Even in gene-for-gene resistance, where cell death is so prevalently observed, components of gene-for-gene resistance such as an oxidative burst, salicylate production, or induction of PR gene expression are activated before HR cell death (7, 12). The fact that cell death also can activate these responses suggests that a function of HR cell death may be to reinforce or strengthen the induction of defenses (8, 11, 42). Enhanced stimulation of defense responses by host cell death may account in part for the observation that gene-for-gene resistance often provides more complete restriction of pathogen growth than systemic acquired resistance [(Fig. 2 and ref. 43; compare also refs. 19, 21, 44, and 45)]. In particular, HR cell death causes elevation of salicylic acid levels, and salicylic acid is known to potentiate enhanced responsiveness of the host to subsequent pathogen infections (8, 11, 46). The constitutive elevation of salicylate observed in *dnd1* plants may substitute for extensive HR cell death in potentiating the strong, gene-for-gene-mediated defense response. Alternatively, the *dnd1* mutation may alter production of or sensitivity to other potentiators of gene-for-gene resistance. It is also possible that the HR cell death response is unnecessary for resistance signaling. Even if cell death does contribute to defense signal transduction in wild-type plants, our data with the *dnd1* mutant demonstrate that cell death itself is not directly essential for the strong restriction of pathogen growth observed in gene-for-gene resistance of *Arabidopsis* against *P. syringae*.

In building models that account for the effect of the *dnd1* mutation, a number of defense-related plant phenotypes must be considered. Because recessive mutation of *DND1* caused constitutive activation of defense responses, it can be stated formally that the product of the wild-type *DND1* locus suppresses constitutive elevation of defenses. This effect may be direct or quite indirect, however, because absence or misfunction of *DND1* may cause any of a variety of perturbations that trigger defenses. It is likely that this perturbation acts upstream of salicylic acid production because salicylate levels are elevated in *dnd1* mutants. The elevated salicylate would seem to account for the observed elevation of PR gene expression and the enhancement of resistance against virulent pathogens. The *dnd1* defect does not significantly inhibit initial resistance gene-dependent recognition of avirulent pathogens nor does it block the defense signaling that leads to strong PR gene expression and strong restriction of the growth of avirulent *P. syringae*.

The defective HR phenotype of *dnd1* mutants may result from elevation of cell death-suppressing functions as one component of the constitutively elevated broad spectrum resistance. As early as 1970, Lozano and Sequeira reported suppression of the HR by resistance-inducing pretreatment of tobacco with heat-killed cells of *Ralstonia* (*Pseudomonas*) *solanacearum* (47). Research with many biological systems has provided evidence of programmed cell death pathways that will function unless they are suppressed (48, 49). Study of lesion-mimic plant mutants indicates that such cell death suppression pathways operate in wild-type plants to prevent excessive spread of HR cell death (8, 50, 51). However, if the *dnd1* mutants do actively suppress HR cell death, this suppression is not explained simply by constitutive elevation of salicylic acid or PR gene expression. Many other *Arabidopsis* mutants display these traits and yet do not suppress HR cell death. In addition to elevation of salicylic acid, disruption of *DND1* function apparently causes the activation of other plant responses, including processes that contribute to the suppression of HR cell death.

The availability of *dnd1* plants should facilitate future study of the mechanisms that control programmed cell death, gene-for-gene disease resistance, and broad spectrum disease resistance in plants. Study of *dnd1* mutants may also suggest novel approaches to the engineering of disease resistance in cultivated plant species.

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