Acquired Resistance in Barley

The Resistance Mechanism Induced by 2,6-Dichloroisonicotinic Acid Is a Phenocopy of a Genetically Based Mechanism Governing Race-Specific Powdery Mildew Resistance

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Treatment of susceptible barley (Hordeum vulgare) seedlings with 2,6-dichloroisonicotinic acid (DCINA) induces disease resistance against the powdery mildew fungus (Erysiphe graminis f. sp. hordei). A cytological analysis of the interaction reveals the hypersensitive cell collapse in attacked, short epidermal cells, along with the accumulation of fluorescent material in papillae, that appear at the time of fungal arrest. The cell-type-specific hypersensitive reaction occurs prior to formation of haustoria, reminiscent of the mechanism identified in genetically resistant barley plants containing the functionally active Mlg gene (R. Gorg, K. Hollricher, P. Schulze-Lefert [1993] Plant J 3: 857–866). This observation indicates that the mechanism of DCINA-induced resistance is a phenocopy of the mechanism governed by the Mlg locus. The onset of acquired resistance correlates with high-level transcript accumulation of barley defense-related genes encoding pathogenesis-related protein-1, peroxidase, and chitinase but not β-1,3-glucanase. Subcellular localization of peroxidase activity shows an increase in enzyme activity in the epidermal cell layer and in the intercellular fluids of barley leaves. Four out of more than 10 identified extracellular isozymes are induced by DCINA. The epidermal cell layer contains a major constitutively formed isozyme, together with two isozymes specifically induced by DCINA. The data support the hypothesis that host cell death and high-level accumulation of defense-related gene transcripts are not only commonly controlled in certain types of race-specific resistance (A. Freialdenhoven, B. Scherag, K. Hollricher, D.B. Collinge, H. Thordal-Christensen, P. Schulze-Lefert [1994] Plant Cell 6: 983–994) but also in acquired resistance, which confers protection to a broad spectrum of different pathogens.

In many plant species, an initial inoculation by a necrotizing microorganism can induce a defense status that protects the plant against subsequent infection. This SAR was first documented in 1901 and is thought to play an important role in the preservation of plants in nature (Chester, 1933). Particularly well characterized examples of this type of resistance is the SAR in tobacco and in cucumber (Kessmann et al., 1991). This SAR is thought to play an important role in the preservation of plants in nature (Chester, 1933). The economic importance of powdery mildew of barley throughout the temperate world along with the well-characterized genetics of the pathosystem (Pryor, 1987) motivated us to choose this interaction for a detailed analysis of acquired resistance. A prerequisite for understanding the molecular changes leading to acquired resistance is the cytological inspection of the fungal development in the host. An infection triggers a series of events in the host that can be followed at both the microscopic and the molecular level.

Abbreviations: DCINA, 2,6-dichloroisonicotinic acid; Egh, Erysiphe graminis f.sp. hordei; HR, hypersensitive reaction; ICF, intercellular fluid; INA, isonicotinic acid; JIP, jasmonate-induced protein; PL, isoelectric point; PR, pathogenesis-related; SAR, systemic acquired resistance.
The formation of a highly localized subcellular cell-wall apposition (papilla) directly beneath the appressorium and a characteristic hypersensitive cell death are prominent defense responses in genetically resistant barley (Aist and Israel, 1986; Koga et al., 1990; Görg et al., 1993). It was our intention to contrast this type of mechanism to that induced through treatment with DCINA.

Because host cell death and high-level accumulation of defense-related gene transcripts were shown to be under common control of specific genes (Freialdenhoven et al., 1994), we expanded our study to an analysis of defense-related proteins in response to DCINA treatment. Among the PR proteins induced by DCINA, we selected peroxidase for detailed analysis of the subcellular accumulation of enzyme activity and isozyme pattern. The results may help in the elucidation of the physiological role that the different members of the enzyme family exhibiting peroxidatic activities play in acquired resistance.

MATERIALS AND METHODS

Plants, Pathogens, and Inoculation

The barley (Hordeum vulgare) cv Pallas as well as Mλl 12 and Mλg backcross lines of cv Pallas were obtained from P. Schulze-Lefert (Aachen, Germany). Their generation has been described previously (Köstler et al., 1986). Plants were grown in a growth chamber at 18°C, 80% RH, and a photoperiod of 16 h (100 μE m⁻² s⁻¹). Inoculation experiments were carried out using conidia from Erysiphe graminis DC.: Fr. f.sp. hordei Em Marchal (Egh) expressing the Mλl 12 and Mλg avirulence function (Wiberg, 1974). For all inoculations, a spore density of 20 conidia mm⁻² was utilized. Inoculation was always performed at the beginning of the light period.

Application of INAs

DCINA (CGA 41396, Ciba-Geigy AG, Basel, Switzerland) or the respective dehalogenated INA (CGA 227519, Ciba-Geigy), formulated as 25% active ingredient with a wettable powder carrier (Métraux et al., 1991), was applied to 7-d-old barley seedlings as a soil drench. The compounds were used with final concentrations of 2 and 20 mg/L soil volume, respectively. The suspensions were prepared with sterile tap water.

Cytological Investigations

At the indicated times, 6-cm leaf segments were harvested, placed in a fixing solution (95% ethanol: lactophenol, 2:1 [v/v]), and boiled for 2 min to remove all Chl. The boiling step was repeated and the segments were finally transferred to fresh alcoholic lactophenol and stored in stoppered glass tubes. Whole-cell autofluorescence was observed by fluorescence microscopy (wavelength = 310 nm). For bright-field microscopy, fixed segments were stained in Coomassie blue (0.6% Coomassie brilliant blue R-250, 15% TCA, 1:1 [v/v]) for 5 s, washed in water, and mounted in 50% glycerol (v/v).

Extraction of Peroxidase, Enzyme Assay, and IEF Electrophoresis

ICFs were extracted from barley leaves according to Kerby and Somerville (1989). Only traces of GII-6-P dehydrogenase were found in ICF, indicating that contamination with intracellular material was very low. Cell-wall-bound peroxidases were prepared from leaves previously used for ICF extraction. Leaves were homogenized using a mortar and pestle in 10 mm sodium phosphate buffer, pH 6.0 (buffer A; 3 mL/g fresh weight). The homogenate was filtered through cheesecloth and centrifuged for 30 min at 10,000 g. The pellet (crude cell wall) was washed extensively (six times) with buffer A to remove all water-soluble enzyme. Iomobally bound cell-wall peroxidase was subsequently extracted from the pellet with buffer A containing 1 M LiCl. For determination of peroxidase in epidermis, abaxial epidermal strips were removed from detached primary leaves and homogenized as described above. Leaves with abaxial epidermis removed were subsequently used for the preparation of mesophyll protoplasts (Kogel et al., 1984).

Extraction of RNA and Blotting and Hybridization Techniques

Total RNA was extracted from 5-cm-long primary leaf segments at the indicated time points after inoculation with A6 conidia as described by Prescott and Martin (1987). Ten micrograms of total RNA per lane was loaded on 1% agarose gels and separated by formaldehyde electrophoreses at 120 V for 2.5 h. RNAs were blotted by a capillary transfer to Hybond-N+ membranes (Amersham, Braunschweig, Germany) in 10× SSC buffer, pH 7.0. RNAs were fixed onto the nylon membrane by baking for 1 h at 80°C. cdNA probes used for hybridization were cloned into the EcoRI restriction site of pBluescript SK+. Linearization at the 5' end of the DNA vector was done with the restriction enzyme PsI or SmaI. A digoxigenin-labeled RNA probe was prepared by in vitro transcription using a T7 RNA polymerase and digoxigenin-labeled UTP as substrate (DIG-Luminescence Detection Kit; Boehringer, Mannheim, Germany). Prehybridization of northern blots was performed for 6 h at 68°C followed by hybridization for 12 h at 68°C. Blots were finally washed with 0.5× SSC, 0.1% SDS for two times for 15 min each at 68°C. Digoxigenin-labeled nucleic acids were detected by an enzyme-catalyzed chemiluminescence reaction using Luminogen PPD as substrate. All buffers used for hybridization and chemiluminescence detection were made according to the instructions of Boehringer.

Enzyme activity was photometrically determined using guaiacol as the substrate (Hammerschmidt et al., 1982). Protein was determined according to Bradford (1976) using BSA as standard.

IEF gels were run by means of the PhastSystem (Pharmacia) using gels with a preformed pH gradient of 3 to 9 according to the instructions of the supplier. Peroxidase was stained as described (Kerby and Somerville, 1989) using o-dianisidin as substrate.
Table I. Induction of a resistance status against the powdery mildew fungus Egh DC. ex Merat, race A6 in primary leaves in response to treatment of barley seedlings with DCINA or INA

- No sporulation; + to ++++, increasing degrees of sporulation. ++++, More than 85% of the leaf surface is covered by sporulating colonies.

<table>
<thead>
<tr>
<th>Compound (Soil drench)</th>
<th>Macroscopically Visible Colony Formation</th>
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<tbody>
<tr>
<td></td>
<td>Time from treatment to inoculation</td>
</tr>
<tr>
<td></td>
<td>2 d</td>
</tr>
<tr>
<td>2 ppm DCINA</td>
<td>+</td>
</tr>
<tr>
<td>20 ppm DCINA</td>
<td>+</td>
</tr>
<tr>
<td>2 ppm INA</td>
<td>+++</td>
</tr>
<tr>
<td>20 ppm INA</td>
<td>++++</td>
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</table>

RESULTS

Infection Types of Powdery Mildew/Barley Interaction in Response to DCINA Treatment

Treatment of susceptible barley seedlings of cv Pallas bearing no known resistance gene for powdery mildew (Kölster et al., 1986) by soil drench with as low as 2 ppm of DCINA induced significant resistance to the race A6 of Egh (Table I). Alternatively, when seedlings were treated with the related dehalogenated compound INA and subsequently inoculated with Egh-A6, a typical compatible biotrophic interaction was observed. This was evident at the macroscopic level as the formation of mildew pustules on the leaf surface. Inspection at early time points of the interaction suggests that fungal development proceeds in INA-treated leaves to the formation of haustoria and elongated secondary hyphae (Fig. 1A), which is typical for an unrestricted growth (Masri and Ellingboe, 1966). At later time points, fungus formed a com-
plex net of hyphae on the leaf surface (Fig. 1B). Quantitative recording of attempted mildew infections in terms of single-cell events in DCINA-treated leaves demonstrates significant levels of whole-cell autofluorescence (HR) in epidermal cells (Fig. 1C). The establishment of fungal haustoria is almost completely abolished (Fig. 2).

It has been previously shown that the timing of fungal establishment in the host proceeds in a cell-type-specific manner in short (<450 μm in length) and long cells of the barley epidermis (Koga et al., 1990). Cell-type-specific evaluation for the DCINA treated leaves revealed that 48 h after inoculation (6 d after DCINA treatment), the HR was essentially confined to the short cell type, e.g. A and B cells in adaxial epidermis and A cells in abaxial epidermis (Fig. 3; cell designation according to Koga et al., 1990). Analogous reactions of these cells have been observed in genetically resistant plants with functionally active Mla12 or Mlg loci (Görg et al., 1993), indicating that DCINA does not modify the natural potential of these cell types to react differentially to an attacking pathogen. The onset of HR in the attacked epidermal cells is detected as early as 21 h after inoculation (Fig. 4). At this time point no premature or differentiated haustoria could be detected in the majority of attacked epidermal cells from 10 leaves.

A comparative kinetic inspection of the onset of HR in Mlg- and Mla12-plants with DCINA-induced plants reveals striking similarity between the Mlg- and DCINA-mediated response: whereas autofluorescence was found in the case of Mla12 mainly in cells with an established haustorium, this plant response predominated in the case of Mlg- or DCINA-mediated response in those cells attacked by the fungus but...
lacking the fungal organ. Hence, on the cytological level the observed mechanism of DCINA-induced resistance is a phenocopy of the mechanism dictated by the Mr locus (Fig. 4).

Because papilla formation has been suggested as a major factor of resistance mediated by the Mr locus (Görg et al., 1993) and in acquired resistance of barley (Sahashi and Shishiyama, 1986), the frequency and quality of this response was recorded and newly calculated as the percentage of total germings that attempted penetration. In accordance with Koga et al. (1990), the total number of papillae formed in response to the attacking pathogen is different in short (44%) and long cell types (95%). These values are not affected by DCINA treatment (data not shown). However, the quality of the papillae has changed in leaves with acquired resistance in that the frequency of strongly fluorescing papillae in short epidermal cells is significantly elevated (Table II). The quality of papillae in long cells was not changed, again indicating that cell-type specificity persists in DCINA-treated leaves.

Activities of Defense-Related Enzymes in Leaves with Acquired Resistance

In tobacco and Arabidopsis, DCINA induces the accumulation of mRNAs that encode a variety of extracellular enzymes including the hydrolases chitinase and β-1,3-glucanase (Ward et al., 1991; Uknes et al., 1992). Because these enzymes exhibit antifungal activities (Mauch et al., 1988), they represent traits for arresting fungal development alternative to or in addition to HR and papilla formation. Therefore, we measured accumulation of gene transcripts from extracellular defense-related proteins in barley leaves with acquired resistance. Figure 5 shows accumulation of chitinase but not β-1,3-glucanase in response to DCINA treatment. Furthermore, high levels of peroxidase- and PR-1-type transcripts were observed. Since peroxidases have been implicated in lignification, a reaction probably occurring in cereals during papilla formation (Mayama and Shishiyama, 1978; Aist and Israel, 1986; Cadena-Gomez and Nicholson, 1987) and hypersensitive cell death (Beardmore et al., 1983; Tiburzy and Reisener, 1990), they were analyzed in more detail.

Gene Expression and Subcellular Accumulation of Peroxidase Activity and Isozymes

Total RNA was extracted from primary leaves of 7-d-old seedlings (cv Pallas) at different time points after treatment with DCINA and inoculation with Egh-A6. Northern blots

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Table II. Evaluation of cell-type specificity of papilla frequency (percent) and papilla fluorescence in susceptible and induced-resistant barley primary leaves inoculated with Egh

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Fluorescence</th>
<th>Papilla Frequency (%)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>20 ppm DCINA</td>
</tr>
<tr>
<td>Abaxial</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A cells</td>
<td>+</td>
<td>41 ± 6.9</td>
</tr>
<tr>
<td></td>
<td>++</td>
<td>25 ± 4.8</td>
</tr>
<tr>
<td>B cells</td>
<td>+</td>
<td>30 ± 4.1</td>
</tr>
<tr>
<td></td>
<td>++</td>
<td>62 ± 8.4</td>
</tr>
<tr>
<td>C cells</td>
<td>+</td>
<td>32 ± 9.7</td>
</tr>
<tr>
<td></td>
<td>++</td>
<td>62 ± 3.1</td>
</tr>
<tr>
<td>Adaxial</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A cells</td>
<td>+</td>
<td>49 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>++</td>
<td>18 ± 3.7</td>
</tr>
<tr>
<td>B cells</td>
<td>+</td>
<td>43 ± 3.3</td>
</tr>
<tr>
<td></td>
<td>++</td>
<td>33 ± 4.5</td>
</tr>
<tr>
<td>C cells</td>
<td>+</td>
<td>31 ± 2.5</td>
</tr>
</tbody>
</table>

Figure 5. Transcript accumulation 24 and 36 h after soil-drench treatment with 2 ppm of DCINA in primary leaf segments of genotypically susceptible barley cv Pallas. Data shown are representative of three replicate experiments. Each lane contains 10 μg of total RNA extracted from primary leaf segments from 20 individuals. Filters were subsequently hybridized with cDNA probes encoding wheat peroxidase (pPOX381; Rebmann et al., 1991), barley PR-1 (Bryngelson et al., 1994), and wheat class I/II chitinase (pCHIT6.1; Münch, 1994) as well as β-1,3-glucanase (pGLUC3.1; Münch, 1994). No transcript accumulation was observed after INA treatment (not shown).
were prepared and hybridized with RNA obtained from the peroxidase-cDNA clone pPOX381. The level of the transcript is elevated preinfectionally in response to DCINA but not to the biologically inactive INA (Fig. 6). An additional drastic accumulation of transcript is observed after inoculation of DCINA-treated leaves with Egh-A6. An inoculation-related accumulation is also detectable in leaves pretreated with INA but to a much lower extent. To confirm that this result is not influenced by uneven loading of the gels, we hybridized blots with an uninduced transcript of rDNA (Forde et al., 1991; data not shown).

In principle, an increase in total enzyme activity in response to DCINA treatment could be due to a general increase in peroxidase isoforms or to an increase in a specific subset of isoforms with a specific subcellular or tissue-type localization. In this context it is important to recognize that _E. graminis_ is an ectoparasite whose contact with the host is normally restricted to the apoplast when penetrating through the leaf surface and, subsequently, to the haustorial interface in invaded epidermal cells. Table III summarizes subcellular and tissue-specific peroxidase activities 24 h after inoculation or mock inoculation of seedlings that had been treated with DCINA or INA, respectively, 4 d before inoculation. The ICF shows the highest specific enzyme activity (1239 mkat/kg protein). Specific activity is enhanced 4.1-fold in DCINA-treated leaves and even more (8.2-fold) after inoculation. Enzyme activity in abaxial epidermal strips removed from detached primary leaves increased 2.1-fold upon DCINA treatment and 5.5-fold upon subsequent inoculation. Cell-wall-associated peroxidases are present in ICF. However, a certain amount of extracellular activity (56 mkat/kg protein) is released from isolated, extensively washed cell walls only by extraction with 1 M LiCl, demonstrating that the walls contain ionically bound peroxidases. Salt-extractable activity is slightly enhanced in DCINA-treated leaves. Only traces of activity were detected in mesophyll protoplasts or microsomal vesicles (data not shown).

To address the question of whether enhanced peroxidase activity is related to the appearance of new specific isozymes, extracellular proteins were isolated and separated by IEF gel electrophoresis. The isozyme profile showed a number of distinct enzymes (Fig. 7) with prevailing bands corresponding to pi values of 5.7, 5.9, 6.5, 6.8, 7.7, 8.3, 8.5, and 8.8 as well as seven-day-old leaves were treated with the respective isonicotinic acid. They were subsequently inoculated or mock-inoculated at d 4 and peroxidase activity was measured 24 h after inoculation.

### Table III. Subcellular accumulation of peroxidase activities (mkat kg⁻¹ protein) in barley primary leaves after treatment with DCINA and inoculation with Egh

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Treatment</th>
<th>INA</th>
<th>DCINA</th>
<th>Inoculation</th>
<th>DCINA + inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extracellular (ICF)</td>
<td></td>
<td>1,239</td>
<td>5,067</td>
<td>4,547</td>
<td>10,154</td>
</tr>
<tr>
<td>Cell wall-bound</td>
<td></td>
<td>56</td>
<td>110</td>
<td>70</td>
<td>98</td>
</tr>
<tr>
<td>1 M LiCl-extractable</td>
<td></td>
<td>240</td>
<td>510</td>
<td>390</td>
<td>805</td>
</tr>
</tbody>
</table>

* Seven-day-old leaves were treated with the respective isonicotinic acid. They were subsequently inoculated or mock-inoculated at d 4 and peroxidase activity was measured 24 h after inoculation.
as several minor, not consistently present isozymes with PI values of 4.7, 4.8, and 4.9. DCINA treatment increased the activity of isozymes with PI values of 5.2, 6.8, 8.3, and 8.5. The same isozymes were also induced by inoculation. Extraction of isolated, extensively washed cell walls with 1 mM LiCl released two constitutively formed isozymes with PI values of 5.8 and 6.1. The slight increase in activity in the cell walls from DCINA-treated leaves is related to an isozyme of pl 6.8 that is probably identical with the induced pl-6.8 isozyme from the ICF. The epidermal tissue constitutively contained a major enzyme of pl 7.5. Two new epidermal isozymes of pl 6.6 and 8.1 appear in response to DCINA treatment.

**DISCUSSION**

Soil-drench treatment of susceptible barley seedlings with DCINA results in systemic expression of resistance against the fungal pathogen *Egh*. Our data demonstrate that DCINA mediates an arrest of fungal growth prior to the developmental stage of haustorium formation. The establishment of the haustorium is the committed step for fungal development in the epidermal cells, since this organ serves to feed the pathogen. Thus, preventing haustoria formation is a very effective mechanism of resistance. Single-cell autofluorescence at the attempted penetration sites in epidermal tissue is reminiscent of the HR, which occurs in a genetically incompatible plant-pathogen interaction in the presence of a functionally active *Mlg* or *Mla* resistance locus (Kita et al., 1981; Koga et al., 1990; Aist and Bushnell, 1991). These loci govern resistance in a typical race-specific and semidominant manner. A comparative analysis of the resistance responses revealed that *Mlg* and *Mla* mediate an arrest of fungal growth at different developmental stages of the pathogen (Görg et al., 1993). Although autofluorescence was found in the case of *Mla* mainly in cells with an established haustorium, this plant response predominated in the case of *Mlg* in those cells attacked by the fungus but lacking the fungal organ. Therefore, on the cytological level the observed mechanism of DCINA-induced resistance is a phenocopy of the mechanism dictated by the *Mlg* gene (Fig. 4). A time-course analysis of the early infection process combined with gene dosage experiments provides evidence that the hypersensitive cell death is a secondary consequence that is not causally required for *Mlg*-mediated arrest of fungal growth (Görg et al., 1993). In the light of these data the significance of the HR as the primary defense reaction in barley with acquired resistance is not unambiguously elucidated. Alteration in quality but not in frequency of papillae formation can be an additional or an alternative mechanism to arrest fungal growth in leaves with induced resistance (Table II). Irrespective of the resistance mechanism elicited by DCINA, our data demonstrate that the short epidermal cells are the primary targets of this compound. Furthermore, our data provide evidence that there is not a common mechanism for acquired resistance in cereals. For instance, a microscopic analysis of the mode of action of a factor isolated from culture filtrates of *Bacillus subtilis* with the ability to induce resistance in barley against *Egh* demonstrated oversized papillae but not HR to be correlated with the onset of resistance (Ebrahim-Nesbat et al., 1983).

Throughout this project we used the compound INA as a proper control for the specific effectiveness of DCINA. As shown in Figures 1, 2, and 3, INA has no resistance-inducing activity. In the biochemical experiments (accumulation of PR protein transcripts, Figs. 5 and 6), no differences were observed between soil-drench treatment of seedlings with INA or water (data not shown). Furthermore, in experiments with DCINA and INA using a direct injection method (Kogel et al., 1985), the efficacy of the compounds was not qualitatively altered (Wasternack et al., 1994). This indicates that the ineffectiveness of INA is not due to problems in uptake or access.

The close association of acquired resistance and the expression of specific peroxidase isozymes, especially in tissue in close contact with the invading pathogen (epidermal layer and extracellular matrix), underlines the significance of peroxidase in the resistance mechanism. Four out of more than 10 isozymes in ICF were induced by DCINA. Two of them (pl 5.2 and 8.5) were previously shown to accumulate during an incompatible barley/Egh interaction in the presence of the resistance gene *Mla* (Kerby and Somerville, 1992). Our results demonstrate that all DCINA-induced isozymes found in ICF also accumulated in the compatible cv Pallas/Egh-A6 interaction. However, higher amounts of isozymes were found when seedlings were treated with DCINA prior to inoculation.

Peroxidase was identified among six cDNA clones locally induced in wheat as a response to the nonpathogen *Egh* (Schweizer et al., 1989) and in a similar study in barley inoculated with *E. graminis f. sp. tritici* (Thordal-Christensen et al., 1992). A comparative analysis of a leaf peroxidase and a seed-specific peroxidase from barley demonstrated the pathogen-induced genes to be more related in sequence to each other than to other barley peroxidase genes, suggesting a comparable function in defense reactions. Because of the unambiguous relevance of the epidermal layer for the establishment of the powdery mildew disease, further work must concentrate on a comparative analysis of the peroxidases from this tissue.

Peroxidases have been implicated in HR and papilla formation (Aist and Israel, 1986; Smedegaard-Petersen et al., 1991). In the presence of hydrogen peroxide they produce the free radical species of monomeric lignin, which then spontaneously polymerize to the three-dimensional network of lignin (Grisebach, 1981; Walter, 1993). Furthermore, peroxidase has been implicated in the cross-linking reactions of cell-wall-associated proteins like Hyp or Gly-rich glycoproteins (Cassab and Varner, 1988). Oxidative cross-linking of lignin and structural proteins may result in strengthening of cell walls, thus presenting physical barriers encountered by invading pathogens (Bradley et al., 1992).

High-level transcript accumulation of chitinase and of the leaf thioun JIP-6 (Wasternack et al., 1994; Kogel et al., 1995) by DCINA may result in a resistance reaction different from HR or papilla formation. Both belong to distinct classes of induced antifungal proteins (Fernandez de Caleya et al., 1972; Bollier, 1987; Bohlmann et al., 1988). We are aware that final proof for the causal resistance mechanism can be provided only via a mutagenesis approach or via experiments...
including inhibitors able to specifically disjoin HR and expression of antifungal compounds after fungal attack.

ACKNOWLEDGMENTS

We are indebted to our colleagues, in particular Drs. Paul Schulze-Lefert and Regina Görg, for their critical comments and encouragement. DCNA was kindly supplied by Dr. H. Kessmann (Ciba-Geigy). We are grateful to Dr. Robert Dudler, Zürich, for permission to use the cDNA clone pPOX381 and for critical comments on the manuscript. We thank Dr. Hans Thordal-Christensen for the PR-1 cDNA. We thank M. Hermanns and A. Schlichtherle for technical assistance.

Received May 16, 1994; accepted September 5, 1994.
Copyright Clearance Center: 0032-0889/94/106/1269/09.

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