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Plant Disease Susceptibility Genes?

A recent search of the ISI Web of Science identified 524 documents related to “plant disease resistance” and just 1 match for the phrase “plant disease susceptibility.” This does not mean that scientists are not engaged in the study of what makes plants susceptible to disease. Resistance and susceptibility are opposite sides of the same coin, and research on disease resistance cannot be conducted without reference to susceptibility. Fortunately, plant disease resistance is more than just a coin toss; plants use a variety of mechanisms to achieve both broad-range and pathogen-specific resistance. Research within the last 10 years has greatly increased our knowledge of the genetic mechanisms of plant disease resistance. However, it is worth remembering that semantics often makes a difference (i.e., it matters what we choose to name things), and it may prove enlightening to examine the “susceptibility” side of the plant disease coin from the perspective of plant genetics. Are there plant genes that are required for susceptibility to certain pathogens? This was the approach taken in this issue of *The Plant Cell* by Vogel et al. (pages 2095–2106), who identified a gene, *PMR6*, that is required for susceptibility to powdery mildew in *Arabidopsis* Col-0 (Figure 1).

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Although plant disease resistance is a complex phenomenon involving a multitude of genes and several interconnected signaling pathways, a reasonably clear picture has begun to emerge, based largely on research conducted in *Arabidopsis* and supported by work in various other species (for recent reviews, see Glazebrook, 2001; Jones, 2001). Resistance to fungal and bacterial pathogens often involves the induction of the hypersensitive response

(HR) and the development of systemic acquired resistance (SAR) via the salicylic acid (SA) signaling pathway. HR involves localized expression of pathogenesis-related (PR) proteins and causes localized host cell death and callose deposition at the site of infection, thereby restricting fungal growth and limiting the spread of disease. SAR is characterized by systemic broad-spectrum resistance to virulent pathogens and involves the transcriptional activation of *PR* genes at sites distant from the site of initial infection.

Resistance genes (of which there are many) typically encode Leu-rich repeat (LRR) receptors of several classes that recognize specific pathogen-encoded avirulence (Avr) proteins. These pathogen-specific “gene-for-gene” interactions feed into the SA signaling pathway to elicit the defense response. Broad-spectrum disease resistance, either against multiple isolates of a particular pathogen or multiple types

of pathogen, is associated with a number of other genes that also often impinge on the SA signaling pathway or downstream effectors. SA signaling depends on *NDR1* and *EDS1*, and, farther downstream, *NPR1*, ultimately causing the induction of *PR* genes (*PR1*, *BGL2*, etc.) and the development of SAR. A jasmonate (JA)/ethylene signaling pathway, which operates independently of (but is connected with) SA signaling, also is involved in the response to numerous pathogens and to wounding. The hallmarks of this pathway include dependence on *COI1* and *ETR1* for JA and ethylene perception, respectively, and the induction of the *PDF1.2* and *Thi2.1* defense response genes (Turner et al., 2002).

This picture has emerged, in part, through genetic screens for mutants with either enhanced susceptibility or enhanced resistance to pathogen-induced disease. Most of these mutants define host defense responses that are associated



Figure 1. *PMR6* Is Associated with Susceptibility to Powdery Mildew in *Arabidopsis*.

Arabidopsis seedlings inoculated with *Erysiphe cichoracearum*. The wild type (right) shows characteristic symptoms of powdery mildew disease. The *pmr6* mutant (left) is completely resistant to infection and does not develop disease symptoms, even though it does not exhibit any of the well-defined characteristics of plant defense (such as host cell death), suggesting that *PMR6* encodes a host susceptibility factor.

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with the SA or JA/ethylene signal transduction pathways, and the mutant phenotypes are associated with cell death (i.e., spontaneous or enhanced formation of chlorotic lesions indicative of the HR) and/or the constitutive expression of downstream response genes such as *PR1*. For example, *Isd* (Dietrich et al., 1994), *acd* (Greenberg et al., 1994), and *cpr* mutants (Bowling et al., 1994, 1997) show enhanced resistance to virulent strains of *Pseudomonas syringae* and/or *Peronospora parasitica* and exhibit spontaneous lesion formation and constitutive expression of *PR* genes.

Most well-characterized enhanced disease susceptibility mutants also define components of the host defense response. The *eds1* mutant exhibits enhanced susceptibility to *P. parasitica*, and EDS1 has been shown to be a key component of the SA-dependent pathway induced by several *RPP* (for resistance to *P. parasitica*) genes (Parker et al., 1996). The *dth9* mutation in Arabidopsis defines a locus that seems to represent a novel component in disease resistance (Mayda et al., 2000). Interestingly, the mutant is more susceptible than wild-type plants to virulent strains of *P. syringae* and *P. parasitica* and is compromised in the development of SAR, but it shows normal SA metabolism and activation of *PR* genes. The mutant was isolated as a constitutive expressor of the β -glucuronidase transgene under the control of the *CEVI-1* gene promoter, which shows SA-dependent induction after viral infection in susceptible tomato varieties. Thus, *DTH9* appears to act downstream of SA action in the development of SAR, but it is independent of *NPR1* and the induction of *PR* genes (Mayda et al., 2000; Glazebrook, 2001).

RESISTANCE TO POWDERY MILDEW

Powdery mildews are among the most common, conspicuous, and widespread plant diseases, and the losses in plant

growth and yield among all crops combined attributable to this disease are possibly greater than the losses caused by any other single family of pathogens (Agrios, 1988). The disease is caused by the ascomycete fungi *Erysiphe* and related genera in the Erysiphaceae family, which infect a wide variety of monocots and dicots with their characteristic grayish-white powdery growth on leaves, fruits, and other organs. Wheat and barley are two of the most severely affected crops, but many other vegetable, fruit, and ornamental crops and trees are affected similarly. Powdery mildew fungi are biotrophic, obligate parasites that require a living plant host to grow and reproduce. They seldom kill the host, but their drain of host nutrients causes increased respiration and transpiration and decreased photosynthesis, plant growth, and yields.

A number of plant genes have been characterized that confer resistance to powdery mildew. In barley, mutations and duplications of the *Mla* locus confer dominant race-specific "gene-for-gene" resistance against powdery mildew caused by *Blumeria graminis* f. sp. *hordei* (formerly *Erysiphe graminis* f. sp. *hordei*), whereas the *Mlo* locus is associated with broad-spectrum resistance against all isolates of the fungus tested (reviewed by Schulze-Lefert and Vogel, 2000). The *Mla* genes *Mla-1* to *Mla-32* are classic *R* genes that encode nucleotide binding site LRR proteins, each of which presumably allows recognition of a race-specific fungal Avr protein, an interaction that leads to the induction of HR and the development of SAR.

In contrast to the dominant-acting, race-specific *Mla* genes, broad-spectrum resistance to *B. graminis* f. sp. *hordei* in barley is controlled by recessive, loss-of-function mutations in a single gene, *Mlo* (Jørgensen, 1992; Büschges et al., 1997). *Mlo* encodes a plant-specific integral membrane protein that contains seven transmembrane domains and is similar to G-protein-coupled receptors in metazoans (Devoto et al., 1999). *mlo* appears to function as a negative regulator of cell death, and loss-of-function

mutations at this locus confer resistance to powdery mildew via an enhanced cell death response and the deposition of a callose-rich barrier at the site of infection (Wolter et al., 1993). It is important to note that both the race-specific resistance conferred by *Mla* genes and the broad-spectrum resistance conferred by *Mlo* appear to operate via the control of cell death.

POWDERY MILDEW RESISTANCE IN ARABIDOPSIS

Although Arabidopsis is not recognized as a common host for powdery mildew fungi, many Arabidopsis accessions are susceptible to powdery mildew caused by *Erysiphe* species, including *E. cichoracearum*, *E. cruciferarum*, and *E. orontii* (Schulze-Lefert and Vogel, 2000). There is considerable natural variation to powdery mildew in Arabidopsis (Adam et al., 1999), and resistance often appears to be polygenic (Schulze-Lefert and Vogel, 2000; Schiff et al., 2001). Adam and Somerville (1996) identified five loci called *RPW1* to *RPW5* (for recognition of powdery mildew), each of which appeared to confer monogenic resistance to *E. cichoracearum* in a distinct resistant Arabidopsis accession. Subsequently, the assignment of these loci was called into question (perhaps because of low inoculation densities in the experiments) when further studies found that resistance mapped to two or three loci in some of the same resistant accessions (Schiff et al., 2001; Wilson et al., 2001). For example, Xiao et al. (1997) characterized three loci, *RPW6*, *RPW7*, and *RPW8*, that control the resistance of Arabidopsis accession Ms-0 to two powdery mildew *Erysiphe* species, and Wilson et al. (2001) mapped resistance to powdery mildew in accession Kas-1 to three loci named *RPW10*, *RPW11*, and *RPW12*. Adam et al. (1999) collected data suggesting that *RPW4* and *RPW7* might be identical or closely linked.

Xiao et al. (2001) determined that the *RPW8* locus, which mapped to chromosome 3, is a key locus for broad-spectrum

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resistance to powdery mildew (and possibly is the same locus as those that elsewhere were named *RPW7*, *RPW10*, and *RPW13*). Xiao et al. (1997) mapped *RPW6* and *RPW7* to chromosomes 5 and 3, respectively, and found that both were required for resistance to *E. cruciferarum*, whereas resistance to *E. cichoracearum* was dependent on a single locus, called *RPW8* (but which mapped to the same position as *RPW7*). Xiao et al. (2001) identified and analyzed the sequences of two powdery mildew resistance genes at *RPW8*, called *RPW8.1* and *RPW8.2*, and confirmed that these genes conferred broad-spectrum resistance in the Ms-0 accession to powdery mildew caused by a number of different isolates of *E. cruciferarum*, *E. cichoracearum*, and *E. orontii*.

They further showed that the resistance of two other accessions, Kas-1 and Wa-1, also mapped to the *RPW8* locus and that the DNA sequences of the *RPW8* alleles in these accessions were identical to those of Ms-0. Three other accessions that were moderately susceptible to powdery mildew, Ler, Nd-0, and Ws-0, contain *RPW8* alleles that are different from those of Ms-0, and they are predicted to encode proteins with 90 to 95% similarity to the Ms-0 proteins. No *RPW8* alleles were detected by DNA gel blot hybridization in the Col-0 accession, which is extremely susceptible to powdery mildew, and sequence analysis of the Col-0 region corresponding to Ms-0 *RPW8* showed a single gene predicted to encode proteins having only 50 to 52% similarity to the Ms-0 *RPW8* proteins. Furthermore, transformation of Col-0 with cDNA corresponding to *RPW8.1* or *RPW8.2* under the control of the 35S promoter of *Cauliflower mosaic virus* was sufficient to confer resistance similar to that seen in the Ms-0 accession.

The protein sequences of *RPW8.1* and *RPW8.2* did not give much clue to their functions, but they showed some similarity to the N terminus of a predicted nucleotide binding site LRR resistance-like protein. As for most other *R* genes, resistance associated with *RPW8* is characterized by SA-dependent defense responses.

PMR6 DEFINES A POWDERY MILDEW SUSCEPTIBILITY LOCUS

Vogel et al. (2002) sought to identify mutants with enhanced resistance to powdery mildew that did not constitutively activate known defense responses. In the original screen (Vogel and Somerville, 2000), mutants were excluded that constitutively expressed *PR1* or formed lesions spontaneously or after pathogen inoculation. Vogel et al. (2002) analyzed the *pmr6* mutant identified in this screen and showed that it exhibits strong recessive resistance to *E. orontii* as well as *E. cichoracearum*, but it is susceptible to the bacterial pathogen *P. syringae* and to the fungal pathogen *P. parasitica*. This mutant is in the Col-0 background, the wild type of which lacks powdery mildew resistance specified by the *RPW8* locus and is susceptible to the disease.

Resistance to powdery mildew in *pmr6* was not correlated with a cell death phenotype or with the expression of *PR1* or *PDF2.1*. Furthermore, double mutants produced from crosses of the *pmr6* mutant with plants expressing *NahG* (which encodes a salicylate hydroxylase that degrades SA), *npr1* mutants (which are blocked in the SA response pathway and do not express *PR1*), *coi1* mutants (which are blocked in JA perception), or *etr1* mutants (which are blocked in ethylene perception) all retained complete resistance to powdery mildew, indicating that the SA- and JA/ethylene-dependent pathways and known host cell death responses are not involved in resistance caused by the *pmr6* mutation.

The *edr1* mutation in *Arabidopsis* defines another locus that confers recessive broad-spectrum resistance to powdery mildew. Like barley *mlo*, and unlike *pmr6*, resistance is associated with an enhanced localized cell death phenotype. The *edr1* mutant does not show constitutive expression of *PR* genes, as do a number of other disease resistance mutants, but multiple defense responses, including cell death and *PR* gene expression, are induced more strongly in the mutant relative to wild-type plants after inoculation with *E.*

cichoracearum (Frye and Innes, 1998). Thus, *EDR1* is believed to encode a negative regulator of plant defense responses.

PMR6 was cloned and found to encode a pectate lyase-like protein, but its specific function and whether or not it has pectate lyase activity remain to be determined. Cell wall analysis using Fourier transform infrared spectroscopy suggested that the cell walls of the mutant are enriched in pectin, supporting the idea that *PMR6* is a pectin-degrading enzyme. The protein sequence contains a predicted N-terminal endoplasmic reticulum transport domain and a predicted C-terminal glycosyl-phosphatidylinositol modification, which in other proteins has been shown to function as an anchor to the plasma membrane surface (Ferguson and Williams, 1988).

A lack of pectate lyase activity and the consequent increase in pectin in epidermal cells might be expected to inhibit the penetration and/or function of fungal haustoria, and alteration of the host cell wall resulting in decreased fungal penetration could be labeled a form of plant defense. But the most interesting points in this regard are as follows: (1) numerous other characteristic host defense responses, such as cell death and the expression of SA-dependent or JA/ethylene-dependent stress response genes, were not observed in the *pmr6* mutant; (2) *PMR6* does not resemble genes found previously to be involved in plant defense; and (3) resistance is associated with a recessive, loss-of-function mutation in *PMR6*. These points suggest that *PMR6* might function as a plant disease susceptibility factor rather than as a component of host defense responses. Vogel et al. (2002) did not observe differences in fungal penetration efficiency on wild-type versus *pmr6* mutant plants. However, after the initial penetration, haustoria must develop a functional channel across the host cell plasma membrane for the uptake of host nutrients, and perhaps increased pectin (or otherwise altered composition of the membrane attributable to the *pmr6* mutation) inhibits haustorial function in some way.

It is tempting to speculate that an activity

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of *PMR6* related to the mechanism of penetration and growth of the fungus (e.g., haustorial function) could explain the specificity of the *pmr6* mutation for powdery mildew resistance. Bacteria such as virulent strains of *Pseudomonas* penetrate plants via wound sites, stomata, and hydathodes and multiply on host cell walls, which collapse after disruption of the cell membrane. The bacteria typically move and multiply intercellularly and through the xylem and subsequently cause cell collapse and cavity formation. *Peronospora* oomycete fungi, which cause downy mildew disease on host plants, become systemic and infect mesophyll as well as epidermal cells. By contrast, *Erysiphe* produces fungal mycelia only on the leaf surface. The fungus penetrates epidermal cells with haustoria, from which host nutrients are retrieved, but rarely invades other cells. Future experiments on the function of *PMR6* will include determining the subcellular localization of the protein in both infected and uninfected cells, determining if it is anchored to the plasma membrane, and examining the accumulation of pectin in the extrahaustorial matrix (the space between the fungal cell wall and the plant membrane surrounding the haustoria) (J. Vogel, personal communication).

The characterization of *PMR6* muddies the waters of plant disease resistance research—hopefully to make them clearer upon further investigation. Does *PMR6* encode a “susceptibility factor” that is required for the establishment and growth of powdery mildew fungi, or does it form part of another, as yet uncharacterized host defense pathway? Is there sequence variation at *PMR6* that correlates with susceptibility among other *Arabidopsis* accessions, and are there functional homologs in other plant species, such as barley? Finding answers to these questions may bring us a deeper understanding of the development of powdery mildew disease and of plant–pathogen interactions in general.

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