PLANT GENETICS

Mapping the Sequence of Disease Resistance

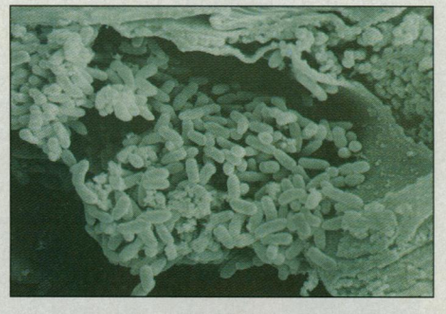
It takes time to breed a hardy crop—too much time, if you ask plant scientists. Identifying a disease-resistant plant variety and breeding it with a commonly grown stock can be spectacularly successful—producing tomatoes that don't keel over in midsummer from a viral infection, for instance—but it can take nearly a decade. When a disease threatens a major crop, that's too long. A fungal blight devastated Midwestern corn in the 1970s, for example, while breeders struggled to create a resistant variety. And even now, breeders are trying to develop a durable strain of rice that resists fungal rice blast, a serious disease that afflicts rice in Southeast Asia, Japan, and the Philippines. The disease costs farmers $5 billion a year.

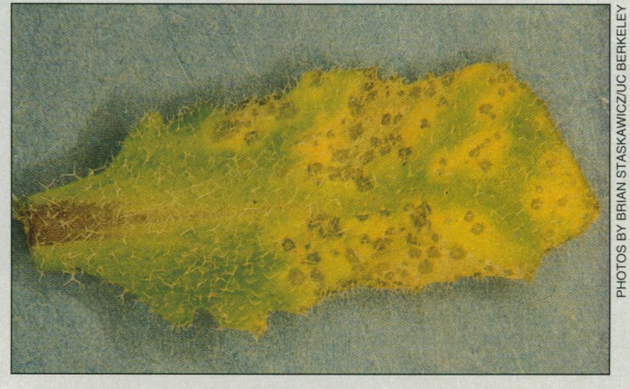
The emergency response time would be greatly improved if scientists could identify genes that resist disease and shuttle them into plants under attack—or even before the attack starts. Scientists have known where many of these genes reside on plant genomes, but not their sequences, making it impossible to move them between diverse species with transgenic techniques. The slow classical breeding process has been the only option, and it only allows the crossing of closely related varieties.

But that situation is changing. Recently, researchers have begun to describe a bumper crop of disease resistance genes. Over the past 2 years, investigators have identified and sequenced genes involved in resistance to fungi, viruses, and bacteria—three of the four pathogens that plague plants in the field (parasitic worms make up the fourth affliction). These discoveries have brought loud cheers from the usually circumspect plant research community. "It is the biggest thing in plant biology since the discovery of chlorophyll," says molecular biologist Jeff Dangl of the Max Delbrück Institute in Cologne, Germany. "People in this field have been praying for these results for 30 to 40 years."

This week alone, scientists are reporting the discovery of two such new genes. On page 1856 of this issue of *Science*, Brian Staskawicz of the University of California, Berkeley, and his colleagues describe a gene called *RPS2* from the small mustardlike plant, *Arabidopsis thaliana*. *RPS2* confers resistance to the bacterial pathogen *Pseudomonas syringae*. In the current issue of *Cell*, Fred Ausubel and his colleagues at Harvard University also describe the *RPS2* gene, and another current *Cell* paper describes the *N* gene from tobacco. The *N* gene, found by Barbara Baker and her colleagues at the University of California, Berkeley, and the U.S. Department of Agriculture (USDA) Plant Gene Expression Center in Albany, California, is the first characterized plant gene that defends against a viral disease, tobacco mosaic virus (TMV).

These results follow on the heels of several discoveries during the past year: The identification of a tomato gene called *Pto* that also confers resistance to *Pseudomonas syringae* was reported last fall (*Science*, 26 November 1993, p. 1432); another tomato gene, *Cf-9*, which helps plants fight off the fungal pathogen *Cladosporium fulvum*, was announced 3 months ago at the Fourth International Congress of Plant Molecular Biology in Amsterdam, the Netherlands; and the L^6 gene in flax, which affects resistance to a fungal rust disease, *Melampsora lini*, was also reported this summer at the International Conference on Plant Microbe Interactions in Edinburgh, Scotland.





PHOTOS BY BRIAN STASKAWICZ/UC BERKELEY

Illness prone. The bacteria *Pseudomonas syringae (top)* can destroy *Arabidopsis* if the plant isn't protected by the *RPS2* gene.

With these genes in hand, researchers are beginning to identify their products: the proteins and other compounds that form a plant's defense system. The immediate payoff should be a better understanding of the mysterious processes of disease resistance. Eventually, scientists hope to manipulate these genes, using genetic engineering to transfer disease resistance across species. "It is a major breakthrough year for plant biology and disease-resistance genes," says plant molecular biologist Alan Bennett of the Department of Vegetable Crops at the University of California, Davis.

The transfer of disease resistance, however, will not be easy. Although scientists now know these gene sequences, this yields few clues to the function of the protein products. In addition, a resistance gene often only offers protection against select strains of a pathogen. This means a gene that fights off a fungus species obliterating a crop in Africa may not work on the same fungus in Asia. Says Steven Briggs of Pioneer Hi-Bred International in Johnston, Iowa: "There will be a lot of trial and error in finding resistance genes that are effective."

The current cornucopia of results is, however, an admirable starting point. The bonanza is the result of the convergence of older plant genome maps and new tools to manipulate DNA. "For some crops, such as tomatoes, pepper, and corn, that have undergone years of selective breeding, we have had good genetic maps but inadequate tools for manipulating the genes," says plant pathologist Richard Michelmore of the University of California, Davis. "And for some plants, such as *Arabidopsis*, we've developed good genetic tools." Now, he says, both are available for many plants. "We're getting the payoff on years of work."

Applied research on crops such as corn and wheat, and basic research on *Arabidopsis*—which, within the past 6 to 8 years, has become a model for the study of plant molecular genetics—has led to the mapping of thousands of genes that code for diverse traits, such as seed yield or disease resistance. Just 8 years ago, Baker and plant geneticist Nina Federoff of the Carnegie Institution of Washington in Baltimore, Maryland, provided a way to pinpoint genes precisely when they showed that so-called "jumping genes," also called transposons, from one plant could function in other plants. When a transposon jumps into another gene, it can inactivate it. Researchers can trail the transposon, because they know its sequence, and this clues them in to the unknown gene's location. Three of the recently discovered genes, *N*, *Cf-9*, and L^6, were found using transposon tools; scientists then sequenced the transposon-interrupted gene.

The sequences have yielded at least one startling discovery. Three genes—*RPS2*, *N*, and L^6—even though they confer resistance to different bacterial, viral, and fungal pathogens, turned out to have common sequence patterns. In particular, they all code for proteins that have "P loops," amino acid sequences that bind phosphates of nucleotides and are involved in energetic reactions. They also have leucine-rich repeats, amino acid segments that have been associated with protein/protein interactions. "We are all extremely surprised," says Staskawicz. "The degree of homology is unexpected because the motifs work in different species responding to different pathogens. It is unbelievable serendipity." Overall, the proteins produced by the *RPS2* and *N* genes have 25% identical and 50% similar sequences.

These homologies suggest to Bennett that "there are underlying mechanisms to disease resistance that could lead to strategies for conferring resistance to a broad variety of pathogens." Most of the newly discovered genes, researchers believe, participate in one such mechanism, known as the gene-for-gene response. In this defense mechanism, the pathogen produces a protein that is recognized by a plant receptor: a protein (or another molecule attached to that protein) that recognizes a protein from the pathogen and then triggers a cascade of defensive reactions such as the release of an enzyme that kills off pathogenic cells. This plant-pathogen recognition, first noted in around 1950 by H. H. Flor of the North Dakota Agricultural Experiment Station in Fargo, is highly specific and is activated by only two complementary genes, one in the pathogen and another in the plant.

Staskawicz and Ausubel believe the *RPS2* gene may code for the protein that acts as the receptor for the signal from the pathogen or, at least, for a molecule that acts in concert with the receptor. Staskawicz says that comparison of the RPS2 protein's amino acid sequence with that of receptors in a computer database shows that "our protein has a putative membrane-spanning domain," a region of the protein that can stick out through a cell wall. To the researchers, this suggests it may respond to a signal from outside the cell.

Baker, who found the *N* gene, also believes the protein produced by this gene may be a receptor that interacts directly with a protein produced by TMV, triggering a defense mechanism. Although the N protein's amino acid sequence suggests it has no transmembrane segment, Baker notes that because TMV appears to find its way through cell walls, a receptor with an extracellular component may not be needed to get the defensive system going. Indeed, she points out that there are precedents for such intracellular signaling in the human inflammatory and immune response pathways. For example, the gene *MyD88*, sequenced in 1990 by Dan Lieberman and his colleagues at the University of Pennsylvania, codes for a protein that seems to have no transmembrane receptor even though,

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Virus blocker. The *N* gene can prevent tobacco mosaic virus from destroying tobacco leaves *(top and above)*.

Baker says, "it is a primary response gene" for immune system components known as myeloid cells. In fact, Baker suggests, there may be important parallels in the way both plants and animals identify pathogens: "The N protein might trigger an intracellular signal transduction cascade similar to that triggered by the interaction of interleukin with the interleukin receptor."

Other disease-resistance genes, however, provide few hints to their defensive roles. For example, "the amino acid sequence of the L^6 gene doesn't immediately suggest a possible mode of action," says molecular biologist Jeff Ellis of the Commonwealth Scientific and Industrial Research Organization (CSIRO) in Canberra, Australia, who found the gene. For similar reasons, *Cf-9* is also a bit of a mystery, says the molecular biologist who characterized it, Jonathan Jones of the Sainsbury Laboratory in Norwich, England.

Some plant researchers are urging caution in speculating about a protein's functions by extrapolating from sequence data and preliminary structural analysis. The Max Delbrück Institute's Dangl, for example, points out that, for the most part, experimental proof of these functions does not yet exist. "We may not have the receptors yet," he says. "We can look at computer databases, compare gene sequences, and can imagine we know where the action takes place, but we don't know. We don't have evidence yet of resistance gene products binding pathogen signaling molecules. It's all computer biology." Adds Indiana University plant molecular biologist Roger Innes, "I feel the jury is still out on whether these (disease-resistance genes) are things that interact with a pathogen's signal."

There is a practical problem as well: If researchers do catch a disease-resistance gene's protein in the act of responding to a pathogen's signal, that might sway the jury, but it wouldn't necessarily bring researchers any closer to transferring disease resistance across species lines. That's because gene-for-gene resistance is based on a tight genetic link between a pathogen and its host. If the *N* or *RPS2* gene, for example, is transferred to another plant species, it will be useful only if the old pathogen attacks the new plant—a possible but unlikely scenario. A few years ago, Pierre J. G. M deWit of the Agricultural University in Wageningen, the Netherlands, suggested a way around this problem. He proposed that plants be genetically engineered to have both the receptor gene from the plant and the elicitor gene from the pathogen. The elicitor can be regulated to work only early in the plant's life cycle, thus keeping the defensive system on a general alert for pathogens when the plant is young and vulnerable.

Jones and his colleagues have recently tested one aspect of this idea, genetically engineering a tomato to contain both the *Cf-9* gene and its complementary *Avr9* gene from *C. fulvum* under the control of a promoter gene. The promoter can be induced to turn on or off by the presence of a new pathogen, thus switching the plant defense system into readiness. In a paper soon to be published in the *Proceedings of the National Academy of Sciences*, the scientists report that these engineered tomato plants do defend themselves by killing off the cells infected by the pathogen. Unfortunately, too much of the plant is sacrificed, and the young seedlings die.

Jones is working on ways to better manage this reaction. And he's optimistic. He and other researchers, for the first time, have actual disease-resistance genes with which they can tinker to find out the biochemical steps that lead to disease resistance. "By cloning these things, we can begin to get a grip on how they work," says Dangl. This, he says, offers an opportunity "to go from computer biology to real biology."

–Anne Simon Moffat