Improving Plant Disease Resistance

Recent advances in understanding both plant defense mechanisms and pathogen action are opening the door to designing new strategies for helping plants ward off infections

Plants may seem to be passive creatures that will wilt under the slightest attack by predators and disease-causing microbes, but they're tougher than they appear. Indeed, in the face of pathogenic onslaughts, they can deploy a wide range of defensive weapons, similar in some respects to the immune defenses that humans and other higher animals use to fight off infections. But, while immunologists have been pinning down the genetic basis of human immunity for more than a decade, only within the past 6 months or so have a few research groups begun to make significant strides in characterizing the genetic origins of disease resistance in plants.

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"Immunized" plant. The left plant resists infection by tobacco mosaic virus because a previous exposure induced its defense mechanisms. (The blue color is due to the type of film used.)

This work, coupled with other recent advances toward understanding the molecular genetics of some of the plant pathogens themselves, is transforming the discipline of plant pathology from a parochial crop-by-crop study of what makes plants sick to a search for broad principles that may be used to protect the health of hundreds or thousands of species. Most important, says plant pathologist Richard Michelmore of the University of California, Davis, the new knowledge is reinvigorating efforts to improve resistance to plant diseases, which lay waste, on average, to 12% of crops worldwide.

Mounting a counterattack

In the past, plant breeders were often put in the awkward position of having to develop new, resistant varieties (which can take 8 to 10 years) only after a pathogen wrecked a crop. That's what happened, for example, in the case of the corn blight that ravaged the Midwest in the 1970s. But now that plant scientists are uncovering the molecular basis of both disease resistance and pathogen action, says Michelmore, they are beginning to map out strategies to prevent plant infections by using modern genetic engineering techniques, together with more conventional plant-breeding methods, to bolster plant defense mechanisms before disaster strikes.

Though researchers have only recently begun to work out the details of plants' "immune systems," the roots of this work go back to the 1960s, when plant pathologists first discovered that plants can mount a broad-based defense that helps ward off infection by many different kinds of pathogens. When invaded by bacteria, viruses, or fungi, plants produce a complex of proteins, belonging to at least five unrelated families. Some of these so-called pathogenesis-related (PR) proteins degrade the cell walls of disease-causing microbes, for example, but for the most part their activities aren't fully understood. Researchers do know, however, that the PR protein response resembles human immune responses in that once it's activated by a pathogen, it protects the victim against subsequent fungal or viral attacks, and it operates not just at the point of invasion but throughout the organism.

DUPONT

Engineered resistance. A bean chitinase gene helps the two lower tobacco plants ward off a soil fungus.

That much has been known for decades, but just last fall researchers got a look at its genetic heart, and they believe they now have the key to unlock the mysteries of exactly what all the proteins do. A group of researchers from CIBA-GEIGY's research labs in Basel, Switzerland, and Research Triangle Park, North Carolina, working with a team from Calgene Inc., a plant biotech firm in Davis, California, found that the onset of this inducible plant response correlates with the production of nine classes of messenger RNAs, which presumably direct the synthesis of the PR proteins. That idea was further buttressed by the group's discovery that salicylic acid, the aspirin-like plant chemical identified in the late 1970s as an internal signal that triggers general resistance in plants, induces expression of the same messenger RNAs. "We pulled together a lot of the dangling data concerning the role of salicylic acid in triggering the onset of general disease resistance in plants," says molecular biologist John Ryals of CIBA-GEIGY's North Carolina lab.

Ryals says he and his colleagues in Switzerland now have two prime goals. They want to identify chemicals that could be sprayed on plants at the first sign of infection to turn up PR protein synthesis and prevent a more serious infection from taking hold. In fact, the group has already taken a step in that direction by showing that a synthetic compound, methyl-2,6-dichloroisonicotinic acid, triggers the production of the same set of messenger RNAs as invading pathogens do.

The group also plans to use the messenger RNAs for the PR proteins to work backward to find the corresponding genes. Determining the sequences of those genes may then reveal something about what the PR proteins do. In as yet unpublished work, for example, the Ryals group has just isolated the gene corresponding to a messenger RNA that is made in large amounts during the plant defense reaction. It helps protect against downy mildew, a fungus that Ryals describes as "one of the world's worst agricultural pathogens" because it attacks a wide variety of crops. Once the other PR protein genes have been cloned and their functions identified, the information should aid plant breeders and genetic engineers in their efforts to develop new plant strains with increased disease resistance built right into their genomes. Such strains might have extra copies of resistance genes or the control of the genes might be altered so that they work at a higher level.

If the production of PR proteins is akin to the general immune response of higher animals, a second defense mechanism in plants can be likened to the more specific antibody response: Plants, it turns out, have

the capacity to produce select compounds that combat individual pathogens, much as humans make select antibodies to deal with specific viral or bacterial attacks. But unlike the very large animal antibody repertoire, the pathogen recognition repertoire of plants may be limited in size, and it appears fixed for life by the plant's collection of resistance genes. About a dozen academic and industrial teams are now hot on the trail of these genes because they, too, may be used to develop plant strains with improved disease resistance. The researchers have already turned up a handful of these genes, which serve to illustrate the wide variety of ways that plants defend themselves.

Maize researcher Steven Briggs of Pioneer Hi-Bred International Inc. in Johnston, Iowa, has recently cloned a gene that allows corn to fight corn blight. The fungus that causes this disease kills infected cells by producing a toxin. And Briggs, working with Jonathan D. Walton of Michigan State University in East Lansing, has discovered that his new resistance gene counteracts the corn blight fungus by producing an enzyme that inactivates the fungal toxin.

Plant pathology is being transformed to a search for broad principles that may be used to protect hundreds of species.

But sometimes it's the plant that makes the toxin, to the detriment of the attacking organism. Molecular biologist Alfons Gierl of the Max Planck Institute for Plant Breeding in Cologne, Germany, has recently cloned another corn gene, known as the BX1 gene, which confers resistance to larvae of the European corn borer, a major insect pathogen. Because it acts by producing an enzyme that catalyzes the production of dimboa, a toxin that is also lethal to bacteria and fungi, Gierl suggests that the BX1 gene may be manipulated in plants "to add to their defensive strategies." He says, however, that its most likely first use would be in ornamental plants, rather than in food crops, where there would be concern about dimboa's possible toxic effects on humans.

Still more firepower

Still another defensive strategy is illustrated by a gene cloned by a group at the agricultural biotechnology company Mogen International NV in Leiden. The protein encoded by this gene, which goes by the name "osmotin," inhibits fungal reproduction by rupturing the spore cases. The researchers have preliminary results showing that when the osmotin gene is transferred into plant cells in lab culture, they are protected against the fungus *Phytophthera infestans*, best known as the pathogen that caused the notorious "potato famine" in Ireland during the 1840s.

And that may be just the beginning. Michelmore, who himself is tracking down the gene responsible for resistance to downy mildew in lettuce, predicts that within 2 to 3 years many other disease-resistance genes will be identified. "Cloning resistance genes will have a high impact on agriculture," he says.

While many groups are trying to understand and enhance a plant's own genetic capacity for fighting pathogens, others are attempting to bolster disease resistance by a different approach. They are genetically transforming plants with genes from the pathogens themselves, a strategy somewhat akin to improving immunity in humans by using vaccines of weakened virus. One of the first to test this idea was Roger Beachy of Washington University in St. Louis. Beachy was struck by earlier observations of plant pathologists who noticed that plants already infected by a virus are resistant to subsequent infection by the same virus, or others. So in 1986 Beachy, who has since moved to the Scripps Research Institute in La Jolla, introduced a gene encoding the coat protein of tobacco mosaic virus into plants. The result: The plants with the new gene were much less susceptible than control plants when they were later exposed to the virus.

Researchers still don't know exactly how the transferred gene increases disease resistance. But even though the mechanism is unresolved, the strategy has already been used to protect tomato, alfalfa, tobacco, melon, and the world's most important food crop—rice—from a broad spectrum of plant viruses. Within the past year, Milton Zaitlin of Cornell University, in a variation of this scheme, has found that plants transformed with gene sequences for an RNA replicating enzyme from tobacco mosaic virus or cucumber mosaic virus have resistance to those viral diseases. He says that this type of protection may not be as fragile as coat-protein induced resistance, which may break down with high concentrations of virus.

Yet another strategy for using genes from pathogens themselves to protect plants comes from Hiroyaki Anzai and his colleagues at the Institute of Chemical and Physical Research in Wako, Japan, who expressed an acetyltransferase gene, taken from the bacterium *Pseudomonas syringae*, in tobacco, making the plant resistant to the "wildfire" disease caused by this microbe. The enzyme seems to destroy a toxin produced by the pathogen.

And even nonpathogens can provide useful genes for increasing plant resistance to infection. Two teams, one led by molecular biologist Pam Dunsmuir of DNA Plant Technology's lab in Oakland, California, and the other by Richard Broglie and Kay Broglie at the Agricultural Products Division of E.I. DuPont de Nemours in Wilmington, Delaware, have shown that the gene for a chitinase enzyme, which Dunsmuir obtained from the harmless soil bacterium *Serratia marescens*, and the Broglies from a bean plant, protects tobacco from the fungal soil pathogen *Rhizoctonia solani*, which destroys young seedlings. The chitinase enzyme apparently works by breaking down the chitin in the cell walls of invading fungi.

Only the beginning

With knowledge of the molecular basis of disease resistance growing daily, yet more novel schemes for ensuring the good health of crops can be expected. Beachy, for example, has proposed that it might be possible to use a mutant form of the "movement proteins," which pathogenic viruses use to spread from cell to cell in plants, to contain viral infections (*Science*, 17 January, p. 291).

But while plant researchers are encouraged by their initial lab results, many of those working to enhance disease resistance in plants, based on improved understanding of the molecular genetics of the disease itself, say it is unlikely that the goal will be easily achieved. It is a long road from successful lab studies to success in open field trials, a far more complex situation than a controlled laboratory, they caution.

And along the way, some of the more elegant, molecular-based schemes for protecting plants will likely be abandoned. "Field work is different from molecular biology," says Dunsmuir. "It takes a lot more effort to show we have a real effect in bolstering a plant's immunity." Still, plant scientists like Dunsmuir take comfort in knowing that some of the strategies now used to deflect human disease, which are based on detailed, genetic understanding of the disease itself, seemed like scientific long shots only a decade ago.

–Anne Simon Moffat

Additional Reading

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E.R. Ward, *et al.*, "Coordinate Gene Activity in Response to Agents that Induce Systemic Acquired Resistance," *The Plant Cell* **3**, 1085 (l99l).