Making Rice Disease-Resistant

For the first time, scientists have used genetic engineering to protect this essential crop from disease

by Pamela C. Ronald

Rice is arguably the world's most important food. Almost two billion people—one third of the world's population—depend primarily on rice for basic nourishment. Rice fields cover more than 360 million acres of land around the globe and yield 560 million tons of grain every year. But farmers plant much more rice than they harvest, because insects, bacteria, viruses and fungi often claim a substantial portion of each crop. One of the most devastating of these pestilences is blight, caused by bacteria common throughout Asia and Africa.

These bacteria—*Xanthomonas oryzae* pv. *oryzae* (known as *Xoo*)—spread rapidly from rice plant to rice plant and from field to field in water droplets. Infected leaves develop lesions, yellow and wilt in a matter of days. In severely infected fields, bacterial blight can wipe out half of a farmer's rice crop.

And yet rice plants possess an amazing assortment of genes that offer protection from a host of diseases, including bacterial blight. The farmers' predicament is that no single variety has every gene and that all plants are vulnerable to some diseases more than to others. Breeders have exploited disease-resistance genes in rice for nearly a century, redistributing this genetic wealth from hardy species to agriculturally useful varieties. But conventional breeding is painstaking and time-consuming; often a decade or more is needed to produce desired traits.

With the advent of genetic engineering, we are now able to introduce isolated disease-resistance genes directly into rice plants, trimming years from the time required to develop a useful variety. My colleagues and I recently cloned the first such disease-resistance

RICE PLANTS are subject to many destructive diseases, including bacterial blight, which causes devastating leaf damage and reduces yield (*left*). Water droplets carry the bacteria into leaf wounds; yellow lesions develop on infected leaves in days. If infected while very young, the entire plant may succumb. Certain plants, however, have a genetic resistance to blight (*right*).

gene from rice—a gene that protects against common forms of bacterial blight. We have used this gene to generate the world's first transgenic disease-resistant rice plants. These new varieties of rice have tremendous potential to aid farmers around the world.

Gene Hunt

The story of the first disease-resistance gene cloned from rice begins in the developing world. *Oryza longistaminata* is a wild species of rice native to Mali, useless as a crop plant because of poor taste and low yields but quite hardy when it comes to resisting bacterial blight. In 1977 researchers in India evaluated this plant for resistance to various strains of *Xoo* and found it could withstand every type they tested.

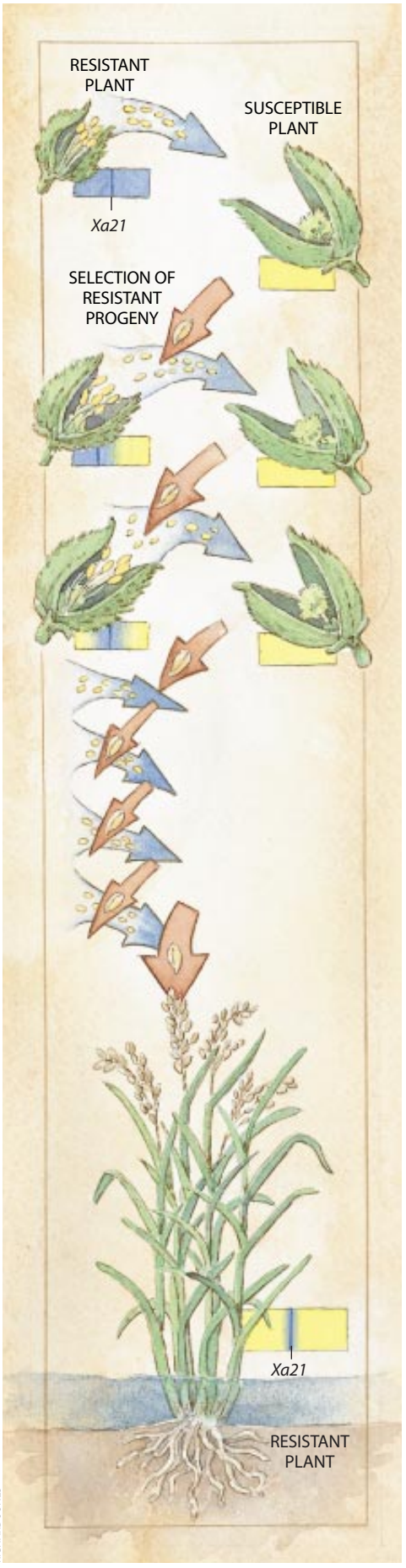
A year later Gurdev S. Khush and his co-workers at the International Rice Research Institute (IRRI) in the Philippines began work with *O. longistaminata.* Their goal was to transfer blight resistance from the wild rice to a cultivated variety, using conventional techniques. Twelve years of intensive breeding yielded one such resistant variety as well as the knowledge that the resistance was conferred by a small region of a single chromosome, perhaps even a single gene, which they named *Xa21.*

In 1990, just as they were reaping the intellectual rewards of this labor, I became a postdoctoral fellow at Cornell University. In an effort to learn about the mechanism of bacterial blight resistance, I decided to try to clone *Xa21* from the IRRI variety. A gene such as *Xa21* is a short stretch of genetic material (DNA) that encodes a protein and often, therefore, a trait (such as blight resistance). Although we frequently study traits in whole organisms, we cannot easily study single genes in the context of an organism's total genetic content, or genome. Nor can we accomplish genetic engineering without isolated genes. The solution is to generate accessible copies of individual genes in the process known as cloning.

To clone *Xa21,* I needed to identify the precise region of the rice genome that bears this gene, transfer this bit of DNA into bacteria where it could be easily copied, insert these copies into susceptible rice plants and then prove that the inserted DNA made these plants resistant to blight. When I began my effort, no one had yet succeeded in cloning a disease-resistance gene from any plant, although many such genes were known and used in traditional breeding. At that time, the barriers to genetic engineering of disease resistance fell into two categories: problems in finding the genes and problems in moving them around.

Finding a gene in a genome is a lot like the proverbial search in the haystack, and the genomes of most plants are particularly huge haystacks. As a rule of thumb, it is harder to locate specific genes in large genomes than in smaller ones; large genomes are also tough to manipulate. A simple yardstick is the tiny genome of the bacterium *Escherichia coli,* from which we can isolate genes quite easily. By this measure the rice genome is large—almost 100 times the size of the *E. coli* genome. A gene hunt in rice is challenging: my own search for *Xa21* would eventually require sever-

RICHARD JONES



RICHARD JONES

TRADITIONAL BREEDING has been used for years to produce disease-resistant rice. Pollen from a resistant plant fertilizes a susceptible plant that also has desirable characteristics—it produces high yields of grain, for instance, or tastes good. Progeny inherit a random mixture of genetic material from both parents (*colored bars*). Resistant progeny are crossed again with the susceptible plant, endowing offspring with more of that parent's valuable traits. Selection of resistant progeny at each cross ensures the continuing presence of the resistance gene—in this case, *Xa21*.

al years and many sophisticated techniques. Still, I was fortunate; researchers seeking to isolate genes from certain other grains face even bigger barriers. The genome of wheat, for example, is almost 3,500 times the size of the *E. coli* genome and fully five times the size of the human genome. Cloning a gene from grains such as rice and wheat is extremely difficult without some prior knowledge of the gene's location or sequence (think of trying to find a friend's house in New York City or Tokyo without an address or description).

In 1990 I felt the time was right for cloning genes from rice, because pioneering work led by Steven D. Tanksley and Susan R. McCouch, also at Cornell, had just produced a key development: a map to guide my exploration of the vast rice genome. The type of cloning I used is known as map-based cloning, and as the name implies, it requires some knowledge of the location of various landmarks, or markers, in the DNA. The genetic map constructed by the Cornell group showed the locations of hundreds of useful markers on the 12 rice chromosomes.

Over a period of a few years, first at Cornell and later at the University of California at Davis, my colleagues and I used this map to track down *Xa21*. During our search, we examined more than 1,000 rice plants to see how often the known DNA markers showed up in conjunction with resistance to bacterial blight. This strategy takes advantage of a certain amount of chromosomal swapping and rearranging that goes on during sexual reproduction: the closer two sites on a chromosome are, the less likely they are to be separated from each other during this process of recombination. In our case, the more often we saw resistance passed to progeny along with a given marker, the closer the resistance gene must lie to that marker.

By sheer luck, the first chromosomal landmark that my group and I identified as lying very close to *Xa21* turned out to be incredibly useful. One weekend in May 1994, two years after I set up my own laboratory at Davis, I discovered that the sequence of the marker DNA was similar to that of several disease-resistance genes recently cloned from tobacco, tomato, flax and a mustard plant. Alone in the lab that Sunday morning, I called my longtime friend and colleague John Salmeron of the University of California at Berkeley and asked him to compare my sequence more carefully with his tomato disease-resistance gene. We were thrilled to find very strong similarities among genes from such different plants. I felt confident that I was searching in the right neighborhood.

My group and I spent the next year cloning candidate *Xa21* genes and preparing to insert them into other rice plants. We knew that the crucial test would come when we transferred our isolated rice DNA into a plant normally susceptible to *Xoo*. If we had cloned the right gene, the resulting transgenic plants would be resistant to bacterial blight. We were anxious to begin these experiments, but we faced an undeniable obstacle: we had no experience introducing genes into rice cells. And at that time, only a very few labs in the world were able to carry out this process, called transformation, in rice.

Under the Gun

This problem of transferring genes into plant cells is the second great hurdle in engineering disease resistance. Many types of plant cells, including rice, are refractory to taking up extraneous DNA. The breakthrough came in 1987, when John C. Sanford of Cornell developed a gun that shoots microscopic particles into intact cells [see "Transgenic Crops," by Charles S. Gasser and Robert T. Fraley; Scientific American, June 1992]. Sanford's early versions were propelled by a gunpowder charge; later models are helium-driven and fire pellets made of gold. These pellets, which are less than a hundredth of a millimeter in diameter, can be coated with DNA that they then carry directly into cells.

Researchers did not use this technique in rice until 1991; when we were ready to test our *Xa21* clone, the International Laboratory for Tropical Agricultural Biotechnology (ILTAB) was one of the facilities doing so routinely. It is conve-

niently located in California and, to our delight, agreed to help us.

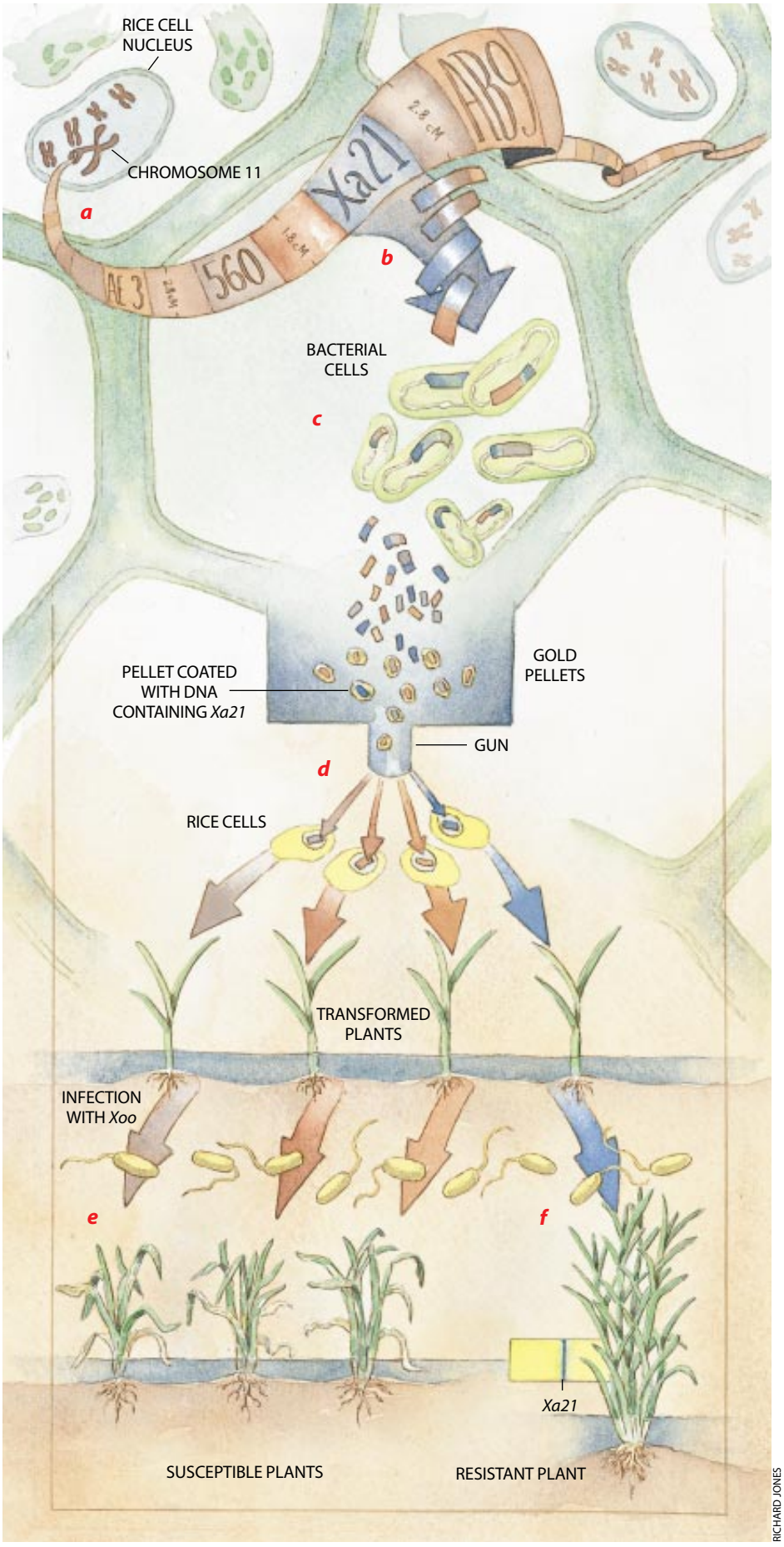
Researchers at ILTAB used the gun to transform our cloned DNA into rice cells of the variety Taipei 309. This is an old variety that is no longer grown, but we chose it because it is easily transformed and susceptible to *Xoo*. We grew 1,500 plants from the transformed cells; each plant had a bit of cloned DNA in every cell. When our plants were six weeks old, it was finally time to test for resistance to bacterial blight.

We exposed each of our transgenic plants to *Xoo* by trimming their leaves with scissors dipped in a bacterial suspension. Ten days later we examined the plants for lesions caused by the bacteria. We found that of the original 1,500 transgenic plants, 50 plants were highly resistant to infection with *Xoo:* each had lesions between 75 and 90 percent shorter than those in the original susceptible plants. In these 50 plants, the transformed piece of DNA contained an intact blight-resistance gene.

Pièce de Résistance

We had succeeded in cloning *Xa21*. Subsequently, we showed that *Xa21* was passed on to the next generation through self-fertilization, giving rise to seedlings that were also resistant to bacterial blight. We challenged our transgenic plants with 31 different *Xoo* strains from eight countries spanning Asia and as far flung as Colombia. The plants resisted infection by 29 of these strains, exactly replicating the disease-resistance profile of their wild African predecessor. For the first time, we were able to engineer rice for resistance to bacterial blight.

Our current goal is to insert *Xa21* into varieties that, unlike Taipei 309, are agriculturally important. In collaboration with ILTAB, we have successfully



GENETIC ENGINEERING of disease resistance requires isolation of individual genes that confer resistance. To isolate *Xa21*, the author determined its approximate location on rice chromosome 11 (*a*) and then generated pieces of DNA that spanned the region (*b*). Next, the DNA was copied in bacteria (*c*), and the replicas were placed on gold pellets that were shot into cells of disease-susceptible rice plants (*d*). Some cells took up the foreign DNA but not the gene for resistance (*e*); a few cells, however, did receive intact *Xa21* and grew into resistant plants (*f*).

introduced *Xa21* into two popular varieties—IR64 and IR72—which are grown on about 22 million acres in Asia and Africa. Our ongoing studies show that the transgenic plants are blight-resistant. And recently we have also engineered resistance in Ming Hui 63, a variety of rice widely grown in China.

With these exciting results in hand, I have sent *Xa21* to scores of scientists throughout Europe, Africa, Asia and the U.S., with the objective of introducing bacterial blight resistance into locally important rice varieties. Because growing conditions vary greatly from place to place, farmers often prefer to plant a variety of rice that is well adapted to their particular region. These varieties possess valuable traits such as drought resistance, cold tolerance, short stature (for wind resistance) or resistance to indigenous pests and diseases. The genetically engineered versions will be identical to the original plants except for the addition of the single cloned gene conferring resistance to bacterial blight.

JEFF HALL *University of California at Davis*

RICE LEAVES exposed to *Xoo* resist infection when the gene *Xa21* is present, both in our transgenic Taipei variety (*pair on left*) and in traditionally bred plants (*pair on right*). Infected leaves from susceptible plants develop extensive, yellow lesions (*center pairs*).

Once we have generated these new varieties, we need to field-test the plants for yield, taste and hardiness to establish that the useful traits of the original varieties remain unchanged. In the next few years, researchers in California, Asia and Africa will field-test transgenic rice containing *Xa21*. If these lines perform as well as locally adapted varieties, national breeding programs will distribute seed to farmers in developing countries. Because the disease-resistance transgene is passed on to progeny, farmers can grow their own seed for the next season.

Crops of the Future

Compared with conventional breeding, genetic engineering is quick and flexible: we can shuttle individual cloned genes between plants in a matter of months. Donor and recipient need not be compatible for breeding; we can share genes among disparate species, even among different crops.

Thus, scientists should be able to harness cloned resistance genes to control disease in many crops besides rice. Species of *Xanthomonas* that cause blight, for example, infect virtually all crop plants. In Florida, 99 percent of the citrus crop is susceptible, and growers must closely monitor bacterial infections to prevent epidemics. In the mid-1980s more than 20 million orange trees were burned to thwart a suspected outbreak of this disease. State and federal governments spent more than $40 million on eradication alone, and hundreds of growers lost even more in unrealized produce. Scientists may someday be able to protect citrus and other lucrative crops by manipulating the rice bacterial blight-resistance genes and transferring them into susceptible species.

Genetic engineering may also help us cope with the problems any disease-resistant plant faces once it is in the field. In particular, pathogens may mutate and overcome the protection a given resistance gene confers. Breeders must therefore continually identify and introduce useful genes in order to minimize susceptibility to disease, whether through conventional methods or genetic engineering. Fortunately, many resistance genes are known and are ripe for cloning. Combinations of these genes may further enhance disease resistance, much the same way that combinations of antibiotics or antiviral drugs combat microbes such as tuberculosis bacteria and human immunodeficiency virus.

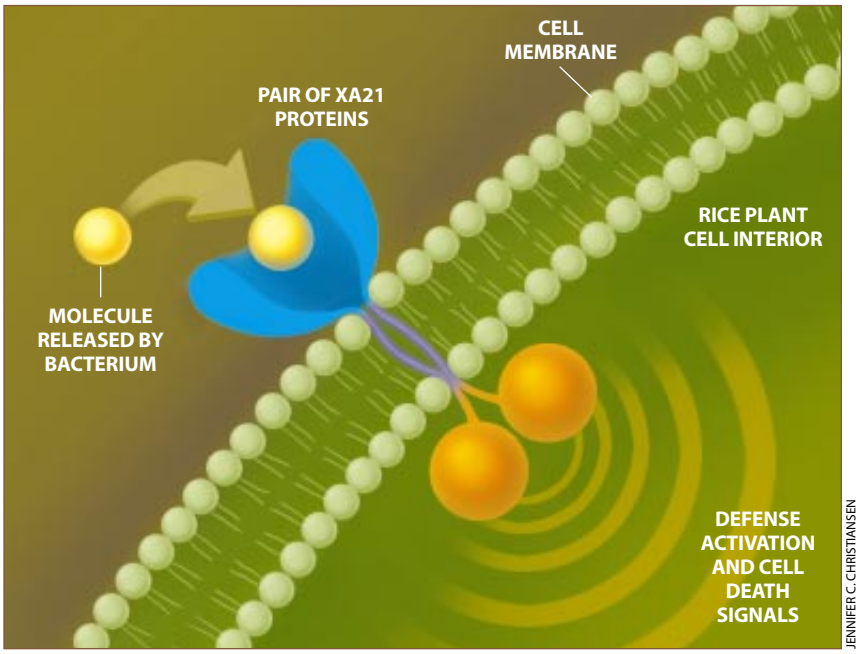
We also hope to incorporate resistance to more than one pathogen in a single transgenic line. In some instances, farmers cannot use rice varieties bred for resistance to bacterial blight, because they lack resistance to other pathogens and pests. The most serious of these pests is the brown plant hopper, an insect that causes severe damage to rice plants as it feeds. It also transmits damaging pathogens such as the grassy-stunt and ragged-stunt viruses. In an early effort at engineering resistance to multiple threats, we are collaborating with colleagues in China and England to incorporate resistance to both bacterial blight and the brown plant hopper into several important varieties of rice, using cloned genes, including *Xa21*. As more and more resistance genes are cloned, the number of available combinations will increase exponentially.

Transgenic disease-resistant plants hold great commercial promise. Although no farmers are actually growing such plants yet, U.S. companies are leading the commercialization of other transgenic crops. The Flavr Savr tomato, developed by Calgene for increased shelf life, was the first commercially available genetically engineered food. Soybeans resistant to the herbicide Roundup came on the market in 1996;

Sounding the Alarm

How do our blight-resistant rice plants sense bacterial intruders? We think the protein encoded by *Xa21* acts as a kind of receiver; pairs of the proteins most likely span the cell membrane, picking up external signals sent by the bacteria and relaying them inside the cell. A protective response ensues: the alerted cell signals its neighbors to mount a defense and then dies; groups of dead cells prevent further spread of the invader. The antennalike part of the protein outside the cell resembles proteins from animals that recognize and bind to other molecules. We have not yet found the bacterial molecule that tips off the rice cells to their enemy's presence, but my group is looking for it.

The portion of protein inside the rice cell is also familiar: it appears to be a kinase, a common type of enzyme responsible for prodding cells to action. We think this enzyme switches on in response to the bacterial signal, trumpeting a message throughout the cell to activate defenses. —*P.C.R.*



XA21 PROTEINS appear to consist of three parts: a section that detects signals from bacteria (*blue*), a section that spans the membrane of the rice cell (*purple*) and a section that generates a message inside the rice cell (*orange*).

maize engineered for resistance to herbicide was recently approved for sale in the U.S. and Canada.

Industrial nations will probably benefit most from the currently available transgenic products. In the developing world, for instance, farmers often cannot afford technologies that require input of expensive herbicides. In contrast, both developing and industrial countries are likely to find disease-resistant transgenic grains useful. These grains may also enjoy more acceptance than certain other new transgenics (such as controversial insecticide-producing plants that some fear will lead quickly to insecticide-resistant insects). Transgenic disease-resistant plants may eventually have a significant effect on the economics of crop growth, promoting more efficient land use, better global food supply and environmentally safer methods of disease and pest control.

With the commercial promise of transgenic disease-resistant crops comes a social responsibility. In 1996 Davis established the Germplasm Resource Recognition Fund to acknowledge the contributions of developing nations to the success of the university's programs, including *Xa21,* for which the university has filed a patent application. Financed by income from commercialization of genetic materials obtained in the Third World, the fund will provide fellowship assistance to researchers from developing nations. Farmers in these poor regions will also be able to obtain seeds of our transgenic lines at the same cost as the traditional parent lines. The fund provides a means for University of California scientists to patent their inventions and make them into commercially viable products while recognizing and fostering contributions from the developing world.

The potential of genetic engineering in rice and other grains will not be exhausted with disease resistance. The future will undoubtedly bring the cloning of many more genes responsible for other valuable traits (cold tolerance, perhaps, or drought resistance). Ultimately, breeders and farmers will be able to choose from a whole toolboxful of cloned genes—genes that will let them reap more of what they sow. SA

The Author

PAMELA C. RONALD received a Ph.D. from the University of California, Berkeley, in 1990. Her graduate work in the laboratory of B. J. Staskawicz focused on the genetic basis of disease resistance in tomatoes and peppers. She began her work on rice disease resistance as a postdoctoral fellow in Steven D. Tanksley's laboratory at Cornell University. This work continues at U.C.-Davis, where Ronald is an associate professor. When not working on the problems of the world's crops, she enjoys the harvest from her husband's organic farm.

Further Reading

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