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Technician Catherine Corr compares a healthy tomato plant (left) to one infected by tobacco mosaic virus (right). (K5994-1)

Boosting Plants' Virus Resistance

Genetic engineering research could yield a safe, natural way to produce hardy new plants.

When a virus attempts to invade your body, your immune system kicks in, mounting a defense to keep it at bay. Plants, too, have "evolved sophisticated tactics to thwart virus enemies," says ARS plant molecular geneticist Barbara J. Baker.

Baker's team at the ARS/University of California Plant Gene Expression Center at Albany has ferreted out and copied a gene that may yield exciting new clues about how plants fend off their attackers.

Their discovery could open the door to new ways for biotechnologists to boost these defense mechanisms in tomorrow's green plants. That, in turn, could reduce growers' and gardeners' reliance on chemical pesticides needed to kill insects that transmit viruses and other pathogenic microbes.

The researchers last year cloned a gene that enables tobacco plants to resist a virus that afflicts not only tobacco, but more than 150 other kinds of plants worldwide. Virus victims include supermarket favorites like tomatoes and bell and chili peppers.

Called tobacco mosaic virus, or TMV, the microbe causes some vulnerable plants to form an unhealthy mosaic of yellow-and-green splotches on their leaves.

The virus can stunt plants' growth and reduce their yields. Besieged tomato plants, for instance, may bear fewer and smaller tomatoes than uninfected ones. And the tomatoes themselves could be blemished and have some browning inside.

Resistant plants, in contrast, kill off some leaf and stem cells where the virus strikes. Dead cells form a small ring, or lesion, with a light-tan center and dark-brown outer circle surrounded by healthy, dark-green tissue. Because the virus can't move through dead cells, these lesions serve to isolate it and stop its spread. By sacrificing lesion cells, others in the plant are saved.

Termed the hypersensitive response, this is "one of the most common forms of plant resistance," according to Baker.

Baker did the work with colleagues Steven A. Whitham, S.P. Dinesh-Kumar, Doil Choi, Reinhard Hehl, and Catherine Corr. More recently, co-researcher Whitham has slipped the new virus-resistance gene into tomato plants. He expects to learn this year if the transfer succeeds in virus-proofing the greenhouse seedlings. Sheila M. McCormick of the Plant Gene Expression Center is collaborating in this test.

The gene shuttle couldn't be accomplished through conventional plant breeding, because tomatoes and tobacco can't be crossed, or hybridized. But biotechnology allows scientists to nudge useful genes from one plant species into another.

Some TMV-resistant tomato varieties already exist. They're the products of conventional plant breeding. But the bioengineered plants might offer added resistance. What's more, the tomato experiment should reveal whether the borrowed gene can perform as well in another plant species. If that happens, the tomato study could lead to similar transfers of the TMV-resistance gene into other susceptible crops.

"This," says Baker, "could be a safe, natural way to produce hardy new plants."

But that's not all.

It turns out that some portions of the protein produced by the TMV-resistance gene match or closely resemble some pieces of proteins produced by two other resistance genes already discovered. All genes make proteins to carry out their instructions.

"If you compare the protein products of these three plant genes," Baker says, "they look very similar." One protein is produced by a gene from a mustardlike plant called *Arabidopsis thaliana*. It protects the plant from a widespread bacterium, *Pseudomonas syringae*.

One-fourth of the pieces, or amino acids, that make up the protein from the *Arabidopsis* gene exactly match those produced by the TMV-resistance gene. And one-half of the pieces—though not exact matches—are strikingly similar.

Another look-alike protein, this one from a gene in flax plants, fortifies flax against a fungus, *Melampsora lini*. If unchecked, the fungus causes rust disease.

"These three totally different plant species—tobacco, mustard, and flax—rely on similar genes to fight a broad range of microbial thugs—viral, bacterial, and fungal," notes Baker. This sameness, or homology, "suggests that the world's plants have some common elements among different patterns of defense."

The commonalities could save scientists years of time in their efforts to bolster plants' innate strategies for resisting assault.

Along the Path to Understanding

"We have a few clues about strategies plants use, like tobacco's lesion-forming response," says Baker. "But the pathway of steps and signals that make up this biochemical activity is mostly a mystery. We'd be in a better position to rework the pathway if we knew more about it."

The TMV-resistance gene that Baker seized, dubbed simply *"N,"* was known since the 1930's to be hidden inside tobacco's genetic makeup. Without the benefit of to-

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Despite its battered look, this leaf has done its job, according to plant molecular geneticist Barbara Baker. It has successfully stopped tobacco mosaic virus by surrounding the virus with dead cells. (K5992-1)

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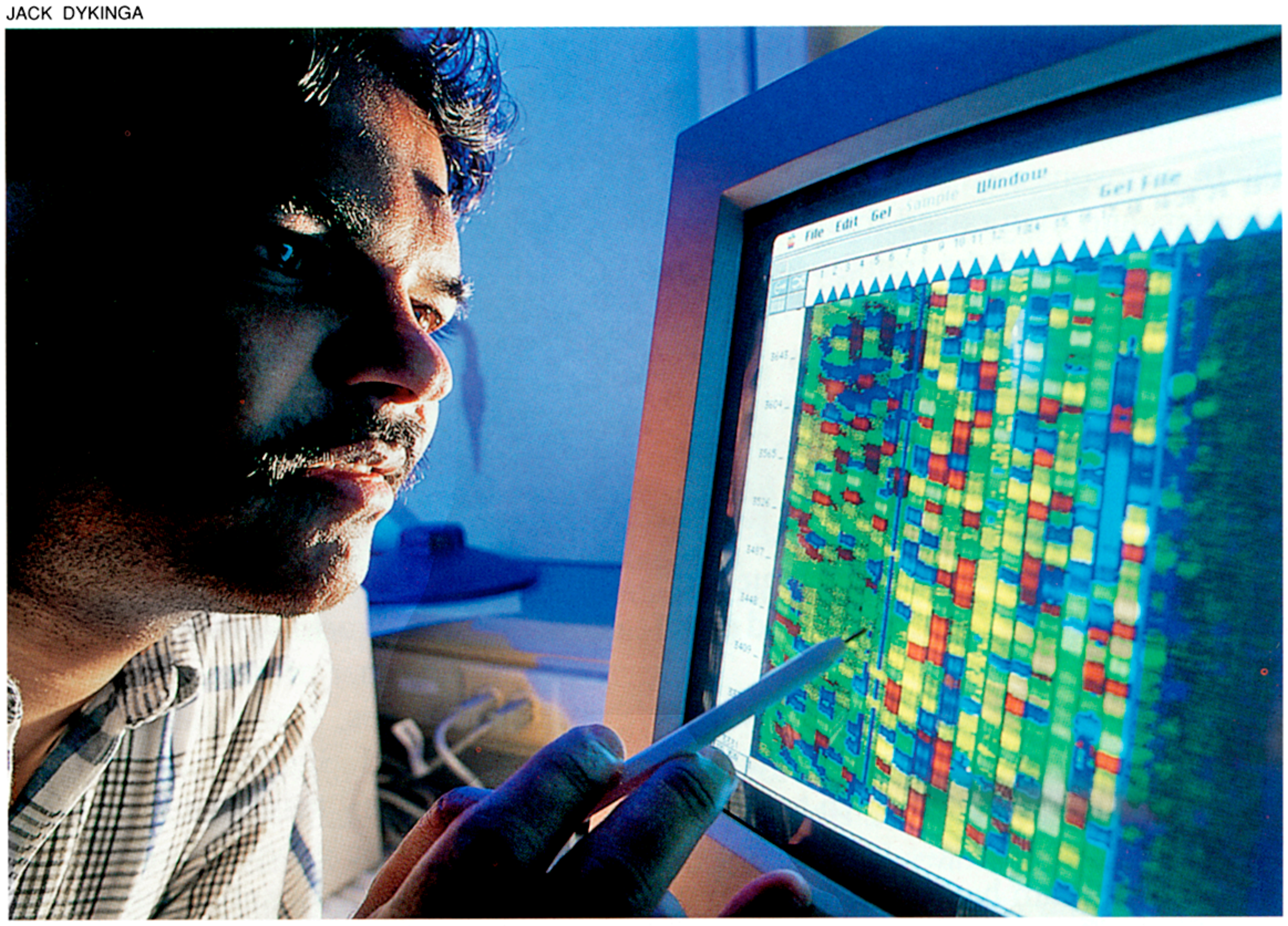

Molecular biologist S.P. Dinesh-Kumar views the DNA sequence of a newly discovered gene that may enable many plants to resist tobacco mosaic virus. (K5994-7)

day's biotech tools, genes like *N* couldn't have been further identified or copied in the laboratory.

Today, one of the most important biotech tools Baker and co-researchers have at their command is jumping genes. These nimble pieces of genetic material are also known as transposons. [See "Jumping Genes Make Genetic Leaps," *Agricultural Research,* January 1994, pp. 12-14.]

A world authority on transposons, Baker discovered in earlier studies that transposons can be "recruited from one plant species to find genes in another."

In this case, her team employed the transposon *Activator* from corn to seek *N* in laboratory tobacco plantlets. Tobacco is widely used in research because it is relatively easy to manipulate genetically.

Essentially, the researchers moved the transposons into cells of tobacco plantlets and then raised them into seedlings that had the transposon inside. They worked with a type of tobacco known to resist the virus.

When the plantlets began succumbing to the virus, the researchers figured that the transposon had likely hit the *N* gene and disabled it. Because they knew the genetic sequence of the transposon, they could find it in the *N* gene.

Though seemingly straightforward, this quest required scrutinizing more than 93,000 seedlings known to contain the *N* gene.

Once the team had isolated what appeared to be the *N* gene and cloned it, Whitham placed it in cells of a unique line of tobacco plants known to be extremely susceptible to the virus. With the new gene inside, some of the plantlets produced from these cells resisted Whitham's attempt to infect them with the virus. This and other tests confirmed that the Albany researchers had the *N* gene in hand.

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The damage caused by tobacco mosaic virus can be readily seen in this comparison displayed by molecular biologists Steven Whitham (center) and S.P. Dinesh-Kumar. (K5993-1)

This year's experiments, with tomato seedlings, rely on what Baker describes as "a very TMV-susceptible" line of tomatoes. "If we can protect these unusually vulnerable tomatoes by inserting the *N* gene," she says, "we may be able to protect other species as well."

Baker and co-researcher Whitham have applied for a patent on the *N* gene and its potential future use in other plants. And Baker has entered into a joint patent with the three other teams that, working independently, captured other promising plant-protection genes. These patent partners are Brian J. Staskawicz, who led the UC-Berkeley team that found the bacteria-fighting gene in *Arabidopsis;* Frederick M. Ausubel and coworkers at Harvard, who nailed the same gene as the Staskawicz team; and Jeffrey Ellis and colleagues with the Australian research organization, CSIRO, who pinpointed the flax gene that combats the *M. lini* fungus.

Like Baker, these investigators are intent on decoding the cascade of signals that make up a plant's defense system. Baker, for example, wants to find out more about the signals the *N* gene may receive and send and to perhaps rewire it. *N* may prove to be a TMV receptor—a molecule that recognizes the mosaic virus' arrival. The TMV receptor alerts cells to start the lesion-forming response.

In addition, cells elsewhere in the plant—untouched by the virus particles—are alerted to the virus' presence by some molecular signal. They then become resistant—without forming visible lesions. The phenomenon is called systemic acquired resistance.

"If we knew how the plant relayed this molecular signal to these other, uninfected cells," Baker says, "we could reprogram the molecular circuitry—including perhaps that of the *N* gene—so that cells could harmlessly acquire resistance to other pathogens, not just to tobacco mosaic virus.

"We could insert this modified system into other plants, to eventually produce plants with enhanced disease resistance. And we could do this much sooner than if we relied on conventional breeding alone."—**By Marcia Wood,** ARS.

Barbara J. Baker and colleagues are with the USDA-ARS/University of California at Berkeley Plant Gene Expression Center, 800 Buchanan St., Albany, CA 94710; phone (510) 559-5900, fax (510) 559-5678. ◆