

# Improving Crop Plants Through Genomics

In 1968, genomics breakthroughs at ARS's U.S. Plant, Soil, and Nutrition Laboratory (PSNL) in Ithaca, New York, earned the U.S. Department of Agriculture its only Nobel Prize to date. Biochemist Robert Holley received the award for being part of the team that first determined the structure and nucleotide sequence of transfer RNA.

Today, PSNL scientists are building on Holley's legacy, applying genomics and related sciences such as proteomics and molecular genetics to improve the nutritional value of leading crops. The ARS researchers, whose labs are located on Cornell University's main campus, are also out to boost crop plants' resistance to disease and their tolerance to soils lacking nutrients or containing toxic amounts of metals. And two PSNL scientists are now part of major efforts to map and sequence the genomes of tomato and maize.

## Number Crunchers

A pivotal moment in PSNL's genomics research occurred 5 years ago, when David J. Schneider was hired as one of ARS's

first computational biologists—experts at integrating computer science with biological research. He and molecular biologist Samuel W. Cartinhour have since helped make computational and molecular biology critical components of the lab's work.

"We are combining computational and bench-based biological research to help solve complex problems in agriculture," says Schneider.

Schneider and Cartinhour apply this interdisciplinary approach to their research on plant diseases. Says Schneider, "We're studying disease development from the pathogen's perspective."

They're using the bacterial pathogen *Pseudomonas syringae* DC3000 as a model system for studying virulence-related genes and pathways. "We're relying on statistical physics, computer science, and complex-systems theory to identify regions in a pathogen's genome that help regulate gene expression," says Cartinhour. "We're showing that there's a real role for number-crunching in genomics."



Molecular biologist James Giovannoni examines high-lycopene tomatoes from *HIGH-PIGMENT 1* mutant varieties.

## Tomato's Genes

Among other projects at Ithaca is one led by molecular biologists James J. Giovannoni and Li Li, who are using tomato and cauliflower as models for improving crops' nutritional qualities.

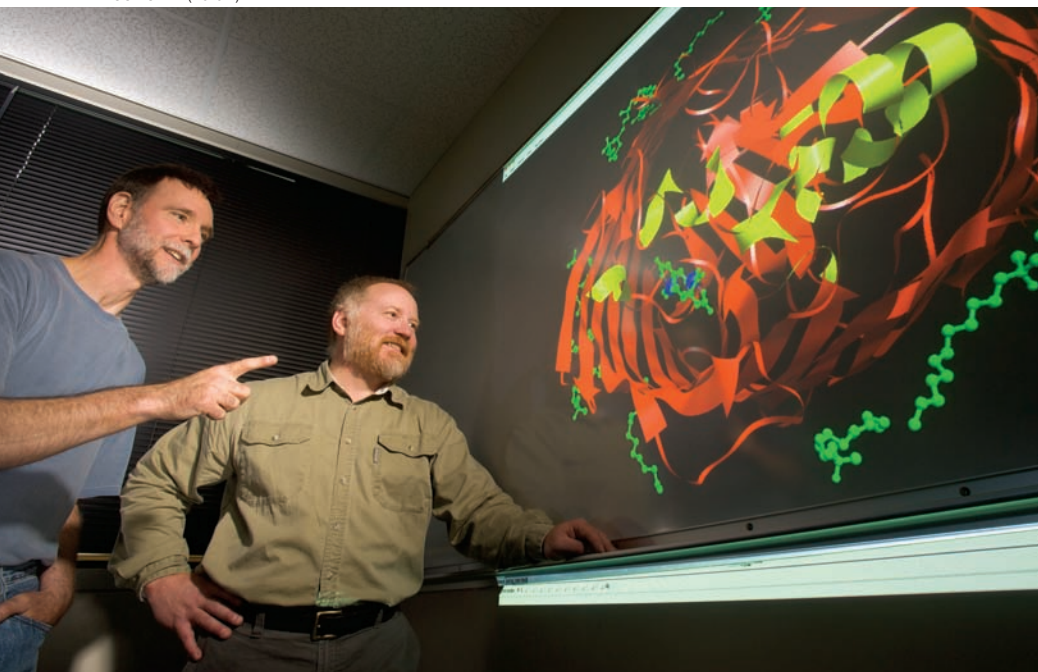
Five years ago, Giovannoni and colleagues reported the discovery of *RIN*, a tomato gene that regulates ethylene, a plant hormone that stimulates ripening. This landmark finding raised the possibility of both growing better tasting tomatoes that meet commercial shelf-life needs and genetically manipulating ripening in other fruits, such as melon and strawberry.

Recently, Giovannoni and colleagues cloned tomato's green-ripe (*GR*) gene, which inhibits the plant's ripening responses to ethylene. The gene greatly affects fruit development while exerting minimal influence on other plant tissues.

"This may help control ethylene's effects on ripening—and bring about longer shelf-life and better quality—while retaining ethylene's desirable effects, such as disease resistance, on other plant tissues," he says. "It makes it possible to control ripening in fruit while maintaining normal plant vigor."

Giovannoni has also helped discover two genes that regulate fruit's response

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Molecular biologist Samuel Cartinhour (left) and computational biologist David Schneider examine a molecular model of a protein involved in iron uptake by bacteria. Iron availability can regulate interactions between plants and both pathogenic and beneficial bacteria.

to light, and he's found that these genes—*LeCOP1LIKE* and *HIGH-PIGMENT 1*—can be manipulated to alter fruit quality and nutritional value.

Today, his team is using microarray, or gene-chip, technology, which enables quick examination of thousands of genes in a single experiment. One significant study showed how microarrays can help characterize gene expression in tomato-related fruit species, such as pepper and eggplant, for which genomic resources are either currently unavailable or limited.

The fruits studied are part of the plant family Solanaceae, which—with more than 3,000 members—is the most important vegetable family. “We showed that tomato microarrays can be used to characterize gene expression in four of the most important Solanaceae crop species,” says Giovannoni.

Giovannoni is also contributing to the Tomato Sequencing Project. Undertaken by a consortium involving scientists from 10 countries, this effort is part of an even larger initiative: The International Solanaceae Genome Project: Systems Approach to Diversity and Adaptation.

### Cauliflower and Beta-Carotene

Meanwhile, Li is using cauliflower as a model system to identify genes and define molecular mechanisms regulating the content, quality, and availability of nutrients in plant-based foods.

She's focusing on carotenoids, the fruit-and-vegetable compounds that the body converts into essential vitamins and uses as antioxidants for cancer prevention. She's using a cauliflower gene, dubbed “*Or*” for the color orange, to induce accumulation of high levels of beta-carotene in food crops.

The human body uses beta-carotene, the carotenoid that gives carrots their color, to make vitamin A. “Our work is important, as vitamin A deficiency has been reported to affect some 250 million children worldwide,” says Li.

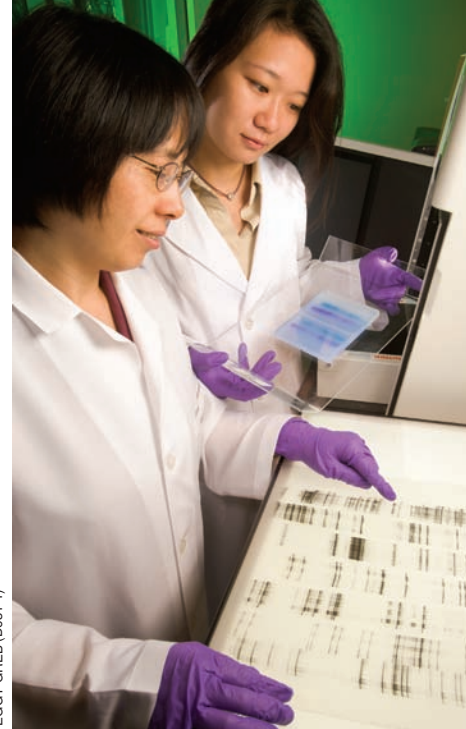
She says the *Or* gene promotes high beta-carotene accumulation in various tissues

in the cauliflower plant that normally don't have carotenoids. “It can help us understand how carotenoid synthesis and accumulation are regulated in plants and in turn can help us better understand the health benefits of carotenoids.”

### The Maize Genome

PSNL's genomics work includes development of statistical and genetic tools for identifying natural variation in agronomically important traits in maize. Scientists are also contributing to the genome sequencing of maize.

Plant geneticist Edward S. Buckler is working with ARS plant geneticists Michael McMullen in the Plant Genetics Research Unit at Columbia, Missouri, and Jim Holland in the Plant Science Research Unit at Raleigh, North Carolina, and Stephen Kresovich, director of Cornell's Institute for Genomic Diversity.



ARS molecular biologist Li Li (left) and Li-Wei Chiu, a Cornell University graduate student, isolate novel genes from cauliflower to improve crop nutritional value.

“We're analyzing many related families of corn as well as unrelated, genetically diverse corn lines,” says Buckler. “We are looking for genes and novel alleles, or variations, that control maize's complex quantitative traits, such as yield, flower development, and seed quality.

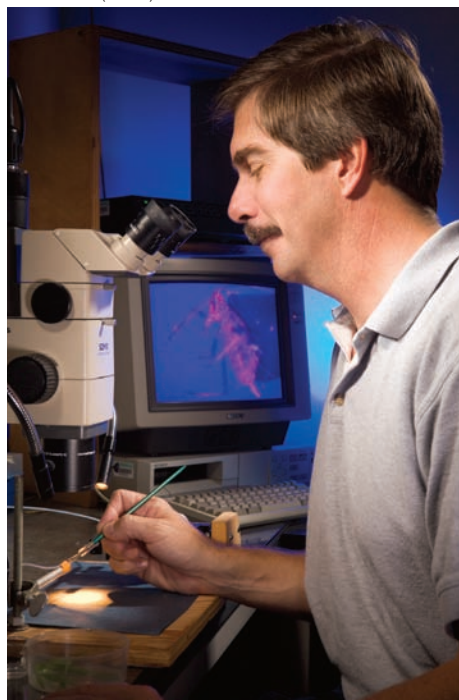
“By using this approach, the best genetic variants can be discovered, and their position within the genome can be resolved to a single gene,” he adds. “This can help us identify genes that can spur a wide array of traits, such as kernel quality, nutritional content, and tolerance of soil-related stresses.”

PSNL computational biologist Doreen H. Ware, who works at the nonprofit Cold Spring Harbor Laboratory in New York, is contributing genome annotation and bioinformatic tools to the sequencing of the maize genome. This project is being funded by the National Science Foundation (NSF), USDA, and the U.S. Department of Energy.

### Tolerating Bad Soil

Plant physiologist Leon V. Kochian, research leader of PSNL's Plant, Soil, and Nutrition Research Unit, is using similar genomic and molecular genetic techniques in work—partially funded by

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Plant pathologist Stewart Gray prepares to microinject a virus into an aphid to determine which insect tissues are not able to recognize the virus, thereby preventing the aphid from transmitting the virus.

NSF—to improve crop-plant cultivation on marginal, and even highly acidic, soils that limit crop production worldwide.

With the genomic tools used on maize and rice—some of which are being developed by Buckler and Ware—Kochian and his team have identified genes and associated mechanisms that help plants tolerate soil acidity and toxic metals.

“We’ve zeroed in on aluminum tolerance in maize and sorghum,” Kochian says. “Aluminum is what limits root-system growth in acid soils. These crops are ideal for this project because in sorghum, aluminum tolerance is a simple trait, while in maize, the tolerance is complex.”

Kochian’s group and researchers at Brazil’s EMBRAPA Maize and Sorghum Research Center have cloned *Alt<sub>SB</sub>*, the major sorghum aluminum-tolerance gene. And recently, he and colleagues confirmed the importance of a gene called *AtALMT1* to aluminum tolerance in *Arabidopsis*. They also found that a second, still unidentified, gene plays a major role in that plant’s aluminum tolerance in acidic soil.

### Targeting Insect Vectors

In PSNL’s Plant Protection Research Unit, plant pathologist Stewart Gray is using genomics to find genes that regulate plant virus transmission by insect vectors.

He’s focused on how aphids transmit barley yellow dwarf and potato leafroll, the most economically important viruses of wheat, barley, oats, and potatoes worldwide. “We want to identify both the virus genes and the aphid genes that regulate transmission of the virus between insect and host,” says Gray.

Recently, Gray identified and characterized the two virus genes that regulate how a virus moves through its aphid vector. Now his group is out to identify the corresponding genes in aphids regulating the insects’ interaction with the virus.

Also, Gray and Iowa State University scientists are determining the complete nucleotide sequences of up to 100 biologically important barley yellow dwarf



Plant geneticist Edward S. Buckler uses high-throughput robotics to efficiently sample the DNA variation of thousands of genes in maize.

and cereal yellow dwarf isolates from around the world. His lab is also part of a scientific consortium that’s sequencing the aphid genome.

Meanwhile, Holley’s legacy will continue on into the lab’s future. Planning is under way to transform the PSNL into the Robert W. Holley Center for Agriculture and Health. This center would house all PSNL scientists within a new, \$40 million building.—By **Luis Pons**, ARS.

*This research is part of Plant Genetic Resources, Genomics, and Genetic*

*Improvement (#301), Plant Biological and Molecular Processes (#302), Plant Diseases (#303), Crop Protection and Quarantine (#304), and Crop Production (#305), five ARS National Programs described on the World Wide Web at [www.nps.ars.usda.gov](http://www.nps.ars.usda.gov).*

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Progress in phytoremediation studies is limited by the complexity of metal transport and tolerance mechanisms. Here, Cornell University research associate Miguel Pineros (left) and ARS plant physiologist Leon Kochian study some of these mechanisms in corn.