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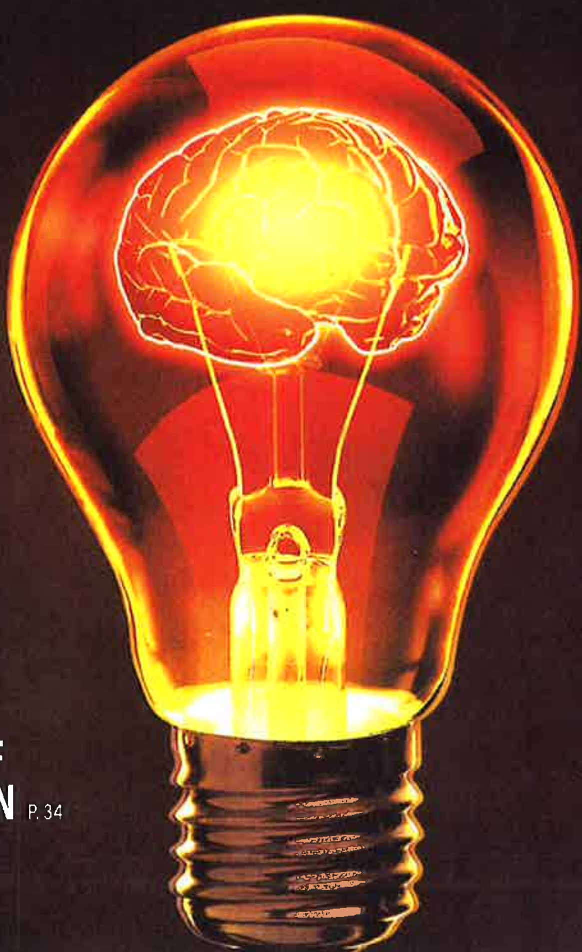

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THE FIX IS IN

Some plants can form a partnership with soil bacteria to get the nitrogen they need. Can we teach modern corn a similar trick?

Could a marriage between corn and bacteria solve the world's fertilizer problem?

BY TRACY STAEDTER

In retrospect, a Wisconsin cornfield in mid-September 2018 wasn't exactly the best place for an academic seminar. It was hot. There were horseflies. The sun cast a glare on the white poster boards, and the metallic chunk-chunk-chunking of a nearby grain elevator made it difficult at times to hear the main speaker. He was Walter Goldstein, a soft-spoken man in his mid-60s who had invited about 30 researchers and farmers to this field to make a point: Corn could thrive with little to no nitrogen fertilizer.

"We're using too much nitrogen," Goldstein said. "It's polluting all of our water ... It's polluting the Mississippi. It's just awful, and yet we need it in order to get the yields."

Goldstein, an agronomist and the founder and executive director of the nonprofit Mandaamin Institute in Lake Geneva, Wisconsin, said he'd been breeding corn under low-fertilizer conditions for decades. He asked one of his assistants to hold a poster board with a blown-up photo of rows of corn. "Can you see the color differences here?" Goldstein asked. On one side of the photo were the rows of corn he'd bred, noticeably more vibrant and deeper green than the rows of commercial corn planted on the other side. This deep color indicated the plants were getting abundant nitrogen, a fundamental element they need to grow and make chlorophyll, the green pigment necessary for photosynthesis.

Although commercial corn typically gets some nitrogen from decayed organic material in soil, it mainly gets it from fertilizer — either organic, such as manure, or inorganic, such as ammonia — spread by the farmer (See "A Century of Ammonia," opposite page). Goldstein said he hadn't added fertilizer to his greener crops, though. Instead, he had cultivated varieties that would team up with microbes that process, or "fix," nitrogen into a form usable by the plants.

Goldstein's microscopic organisms came as a powder that he sprinkled in the soil when the seeds were sowed. These particular bacteria convert nitrogen gas in the air, which plants can't process, into nitrogen-rich ammonia, which they can. In return for the ammonia, plants provide the nitrogen-fixing bacteria with shelter and the sugary carbohydrates they need to survive.

That the microbes enhanced the corn's vitality was somewhat of a surprise. For a long time, scientists thought these microorganisms only lived inside the nodules on the roots of legumes — members of the bean family, like soy, peas and alfalfa — not corn. Nodules give microbes a safe place to produce the enzyme nitrogenase, necessary for fixing nitrogen, while shielding them from oxygen, which can shut down the reaction.

But over the years, research teams have found nitrogen-fixers living in the root nodules of other species, too, like red alder trees and certain tropical trees and shrubs. They've also found them in the plant tissues of sugarcane and some trees. And last year, researchers published a report that an ancient corn variety in Mexico was harboring N-fixers, too. Though it once seemed impossible that plants without nodules could team up with bacteria to get this essential nutrient, it's become increasingly clear that that's not the case. Most importantly, it's not the case for corn, one of the planet's most nitrogen-hungry crops.

Nitrogen is an essential nutrient for plant growth. The darker green color of the hybrid corn plants on the left shows they are getting more nitrogen than the commercial variety on the right.

FROM LEFT: ALEN KADR/SHUTTERSTOCK; WALTER GOLDSTEIN/MANDAAMIN INSTITUTE

FROM TOP: JAY SMITH; LIBRARY OF CONGRESS/JOHN VACHON

For decades, scientists have been working to find a way to grow corn with less fertilizer. Some are using genetic engineering techniques to focus on the plant, others have been experimenting with microbes, and still others, like Goldstein, have been using classical breeding methods to tap into the plants' age-old ability to partner with the bacteria.

What these scientists uncover could upend agriculture as we know it by reducing the global use of fertilizer — all while still producing the yields necessary to sustain our civilization.

SKIP THE MIDDLEMAN

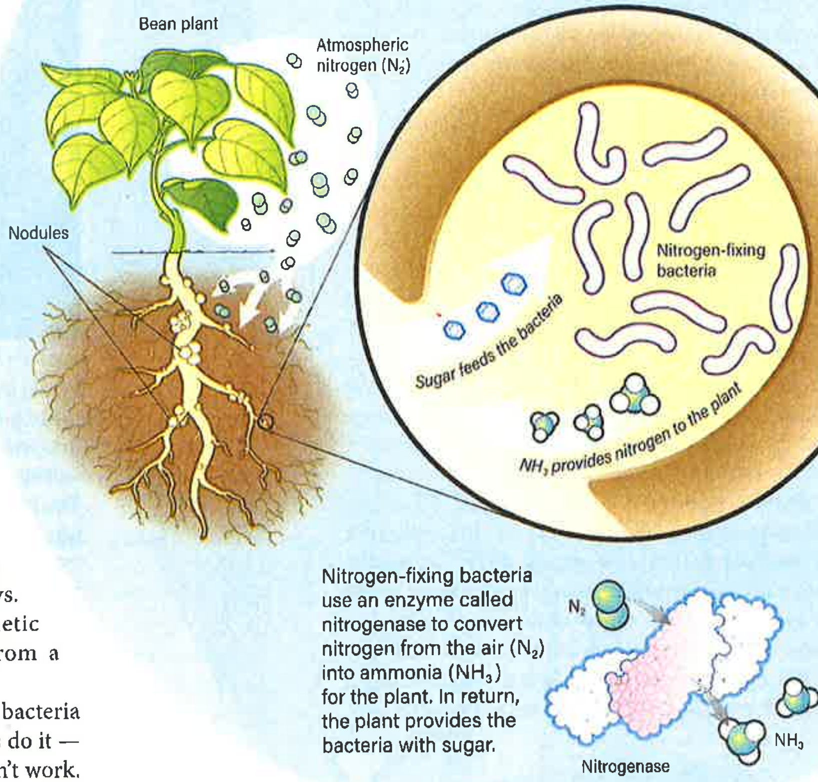
For nearly 20 years, a simple question has nagged Luis Rubio, an associate professor at the Center for Plant Biotechnology and Genomics in Madrid, Spain. Why can't plants fix nitrogen on their own, without the help of microbes?

He suspects it's because plants can't make the nitrogenase enzyme. "Here comes a challenge: Let's make it possible," he says. For Rubio, that means moving the genetic instructions to produce nitrogenase from a bacterium into a corn cell.

This involves a host of challenges. How bacteria make enzymes differs from how plant cells do it — enough that simply transferring genes won't work. On top of that, the instructions for the nitrogen-fixing reaction are complex, a puzzle of between

NITROGEN-FIXING NODULES

Plants need the element nitrogen to grow, but they can't use the nitrogen that's in the air. Legumes, members of the bean family, get around this problem by providing a home for nitrogen-fixing bacteria in nodules on their roots.



Nitrogen-fixing bacteria use an enzyme called nitrogenase to convert nitrogen from the air (N_2) into ammonia (NH_3) for the plant. In return, the plant provides the bacteria with sugar.

A CENTURY OF AMMONIA

For centuries, growers relied on manure and crop rotation to keep soil fertile. But in 1908, German chemists Fritz Haber and Carl Bosch invented a way to transform nitrogen gas and hydrogen into a liquid chemical — ammonia — that could be sprayed onto soil to make it almost instantly fertile. Relatively cheap to produce and easily mixed with other nutrients like phosphorus, potassium and sulfur, this new way to fertilize sparked an agricultural revolution. Crop yields skyrocketed 30 percent to 50 percent. So did the world's population, which went from 1.65 billion in 1900 to 7.6 billion today.

Fertilizer use comes at a cost, though. Manufacturing it produces 3 percent of the world's carbon emissions. Most commercial farmers in the U.S. apply between 160 and 220 pounds of mainly inorganic fertilizer per acre. Nearly a third to a half of that is lost to the environment.

Rains can soak fertilizer into the soil, where it pollutes groundwater. Soil bacteria process ammonia from both inorganic and organic fertilizer and turn it into nitrous oxide, the third most significant greenhouse gas after carbon dioxide and methane. Washed into rivers, lakes and oceans, nitrogen increases the population of microscopic organisms. Toxic cyanobacteria poison fish and other aquatic animals. Blooms of algae decrease oxygen in the water. The decay from dead marine animals further consumes oxygen in the water, creating dead zones, like those in the Gulf of Mexico and the Chesapeake Bay.

Inorganic fertilizer has become an agricultural catch-22, polluting the world it feeds. Without it, yields would decline by 40 percent, putting millions of people at risk of starvation. But if corn — the most prevalent crop in the U.S. — could fix its own nitrogen, some estimates say fertilizer use on the crop could drop by 25 to 50 percent.

A farmer in Iowa pours fertilizer into a corn planter in 1940.

10 and 20 different genes — and each needs to be assembled in precisely the right order for the overall system to work.

Since 2012, Rubio and his colleagues have been using genetic engineering and computer science to overcome these obstacles. First, they search databases for genes from bacteria cells that might be able to perform a particular function, such as make one part of the nitrogenase enzyme.

Once they pinpoint such a target, the scientists make a synthetic copy of the gene and insert it into yeast, which grows and multiplies quickly, to make other copies. Next, they extract the product of the gene — its protein — purify it, and check that it functions well. If it does, the gene is then combined with another gene that was analyzed the same way to see if the two work well together. If they do, that subgroup is combined with another subgroup and they're analyzed to see how well they function together. Over and over again, genes are copied and tested, first alone and then with others, slowly adding to the chain of genetic command.

So far, Rubio's team has gotten 15 of 20 genes to work well together, in three distinct subsets of just a few genes each. Recently, they started testing a string of working genes in rice, which is more complex than yeast. The crop is similar to corn, but easier to work with.

Piecing together this scientific puzzle and putting it into corn may take another two decades, Rubio says.

A petri dish full of poplar tree cuttings covered in goo led to the discovery that nitrogen-fixing bacteria can exist inside plants other than legumes.

When the researchers succeed, they're aiming to get the engineered enzyme into a corn cell's mitochondria, where energy production naturally occurs; it will be shielded there from reaction-ruining oxygen. This bioengineered corn, if successful, would pull the nitrogen it needs right out of the air.

THE ROOT OF THE MATTER

It was white. It was slimy. It was frustrating. For the umpteenth time that month, Sharon L. Doty, then a post-doctoral researcher in the biochemistry department at the University of Washington, peered into a glass petri dish at yet another small tree cutting contaminated by gobs of goo. Bacteria. It was mucking up her labwork. She sent the slime out to have its DNA sequenced, thinking that if she could identify the bacteria, she could kill it.

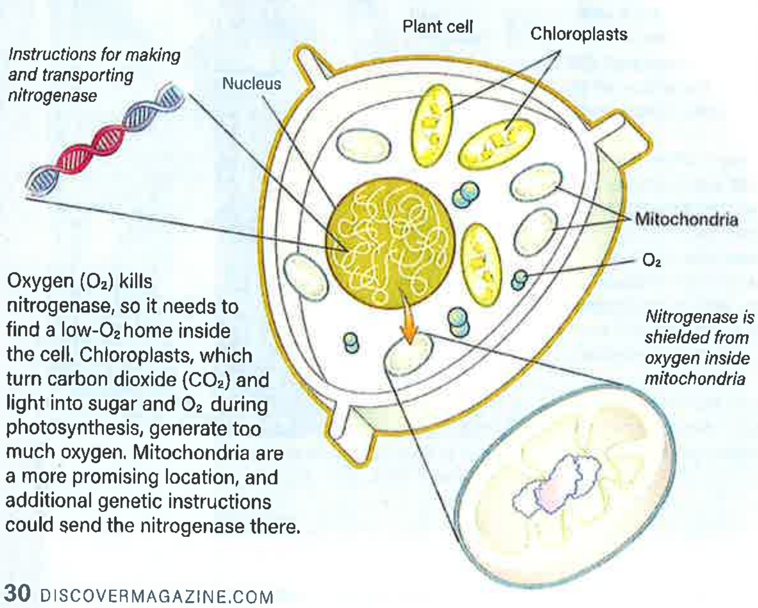
But Doty was surprised to find that the slime was made of common soil bacteria called *Rhizobium tropici*, the kind that fixes nitrogen in the low-oxygen environment of legume root nodules. No one had ever found *Rhizobia* living inside the tissue of the stems, branches or leaves of trees outside of that plant family. Doty went on to discover several different kinds of these endophytes, or bacteria that live inside plant tissues, in other poplars and willow trees.

She spent the next 18 years trying to convince the scientific community that she'd found endophytes that fix nitrogen. Many shrugged her off because the research went against the dogma that nitrogen-fixing microbes only lived in root nodules of specific plants. "It's only through my stubbornness that I kept pushing it and pushing it," says Doty.

It paid off. In 2016, she published a report in *PLOS One* about the nitrogen-fixing endophytes she'd found in poplars. By then, she'd also exclusively licensed the microbes to the ag-biotech firm, Intrinsyx Bio, which is

GENETIC ENGINEERING

If plants could make their own nitrogenase, could they fix their own nitrogen? One research team hopes to find out by piecing together a synthetic version of the enzyme that will move into the plant cell's mitochondria.



FROM TOP: SHARON L. DOTY; JAY SMITH

CLOCKWISE FROM TOP: JAY SMITH; THE UNIVERSITY OF NOTTINGHAM; AZOTIC NORTH AMERICA developing them as a microbial product for crops, called an inoculant.

This year, distributor Unium Bioscience released the first commercial product based on Intrinsyx's formulation. Called Tiros, it's available in the United Kingdom as a liquid to apply as a seed coat, and the group is working on powder and small pellet forms that could be applied straight onto a field during sowing. As the seeds sprout, the microorganisms grow with the plant, and migrate into plant tissue through cracks at the junctions of branching roots or root hairs. Once inside, the endophytes fix nitrogen.

In early tests conducted on corn fields in Iowa, growers who applied a typical amount of fertilizer plus Tiros got six additional bushels per acre (state average is 196.) More recent field trials that reduced fertilizer by up to a third also showed promising results, but Intrinsyx Bio CEO Ahsan Ali says that more tests are needed. In May 2019, the company received a grant to work with scientists at the Donald Danforth Plant Science Center near St. Louis to test their microbes on 250 corn varieties, and, among other things, figure out how to get the highest yield with the smallest amount of nitrogen fertilizer.

START SMALL

"One of the blessings in research is not getting funding," says Ted Cocking, a plant scientist and professor emeritus at the University of Nottingham.

For decades, Cocking believed, as other scientists did, that nitrogen fixation could only occur in the root nodules of legumes. Trained in plant physiology, cell biology and bacteriology, he originally set out to breed corn that grows root nodules of its own, with the notion they would attract nitrogen-fixing bacteria already living in the soil.

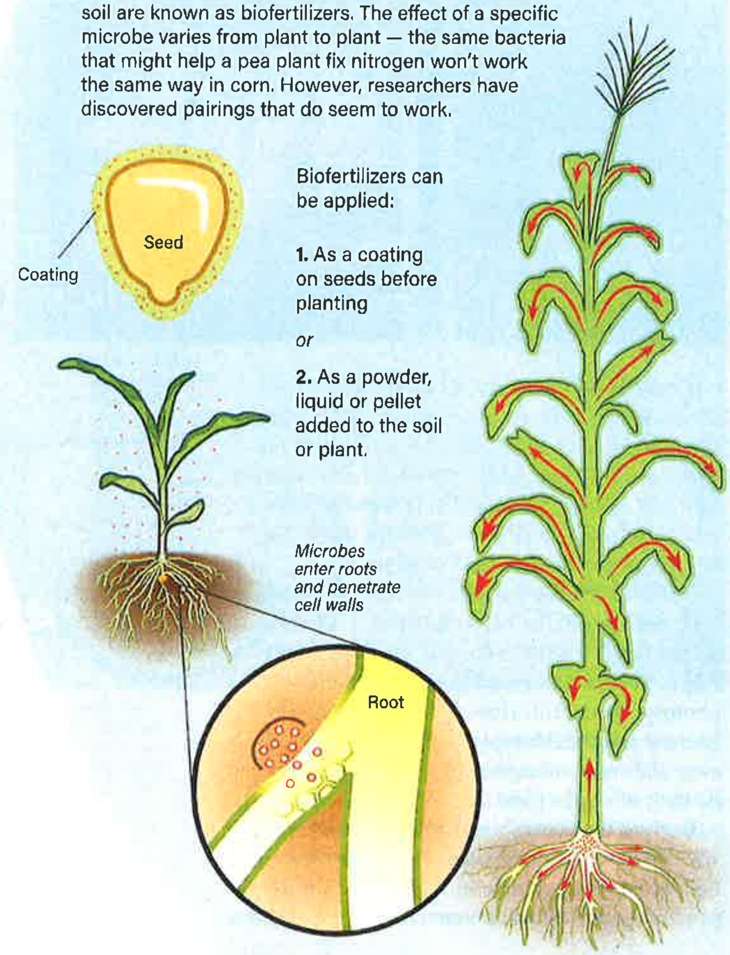
He got the nodules to sprout, and the bacteria to move in — but only into dead tissue. The critical symbiosis with the living plant never formed. With his funds drying up, he realized he had to take a different approach. Instead of trying to get the bacteria to live in root nodules, he wondered if they could live inside the plants' root cells.

His new proposal was approved, and the new funding allowed him to move forward. But he faced a double challenge. Cocking required a bacterium that could fix nitrogen, even in the presence of some oxygen, and make its way past a plant's tough cell wall. He eventually hit the jackpot with *Gluconacetobacter diazotrophicus*, a bacterium found in Brazilian sugarcane.

He brought *Gluconacetobacter* into his lab and got the bacteria to break through. The plant cells then surrounded them with a thin membrane, in a natural process called endocytosis, to form little

BIOFERTILIZERS

Beneficial microbes introduced to plants, seeds or soil are known as biofertilizers. The effect of a specific microbe varies from plant to plant — the same bacteria that might help a pea plant fix nitrogen won't work the same way in corn. However, researchers have discovered pairings that do seem to work.



Ted Cocking, right, worked with a strain of bacteria found in Brazilian sugarcane that led to the development of a commercial product called Envita. The widespread tiny black dots of bacteria in corn cells, above, show how they spread through the plant.

FROM TOP: SHARON L. DOTY; JAY SMITH

CLOCKWISE FROM TOP: JAY SMITH; THE UNIVERSITY OF NOTTINGHAM; AZOTIC NORTH AMERICA

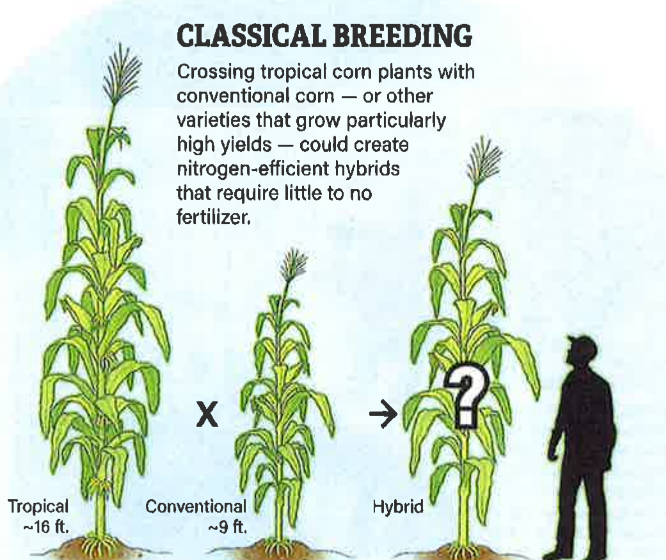
The gelatinous goo oozing from these corn plants hosts nitrogen-fixing bacteria. Discovered in the tropics of southern Mexico, these ancient varieties of maize are considered some of the oldest in the world.

self-contained bubbles. The bacteria survived off sugars the plant made during photosynthesis, and tapped into the cell's own energy molecules — adenosine triphosphate (ATP) — as a power source for their nitrogen fixation. And *Gluconacetobacter*, unlike *Rhizobia*, can fix nitrogen even when there's a little oxygen around — like in a plant cell.

"We have now, for the first time in the world, a plant with nitrogen-fixing bacteria [sitting] side by side, almost fused, with chloroplasts," says Cocking, referring to the structures inside plant cells responsible for photosynthesis. This close relationship between the bacteria and the chloroplasts allows them to directly swap the sugar, nitrogen and ATP that each need to do their job in the plant cell.

In 2012, the Lancashire, England-based company Azotic Technologies formed to develop an inoculant based on Cocking's science. Called Envita, the product began selling commercially this spring as a liquid applied with the seed when it's sowed. Nolan Berg, Azotic North America's president and general manager, says it will be added this year to more than 100,000 acres of cropland. In 2020, Azotic plans to break a million acres.

In the U.S., farmers who used a normal amount of fertilizer plus Envita got up to a 20 percent boost in yield. Tests indicated they could reduce fertilizer as much as 27 percent and get the same yield as before. "Farmers don't have to choose between productivity and sustainability," says Berg. "They can have both."



NATURE'S SOLUTION, HIDING IN PLAIN SIGHT

In the montane forests of Sierra Mixe (pronounced *MEE-hay*), in the Mexican state of Oaxaca, grows a corn — or maize — with clear, gelatinous film oozing from fingerlike structures protruding from its stems.

Here, in the birthplace of corn, indigenous peoples domesticated maize from the wild grass teosinte. Over millennia, the local farmers preferentially planted the corn that grew best at different altitudes and in fields that were sometimes poor in nutrients. In doing so, they produced a wide variety of plants with different ear sizes and kernel shapes, colors and textures. Today, these local cultivars, or landraces, are considered some of the oldest varieties of maize in the world.

But there is something special about the varieties that produce gelatinous film. Starting in the 1990s, Mexican scientists began to study the goo and found that it harbored nitrogen-fixing bacteria. And last year, a team from the University of California, Davis, confirmed that the corn was indeed taking advantage of the nitrogen the bacteria produced. Plus, they discovered, the ancient teosinte from which corn descends had this ability all along.

By actively selecting maize varieties for their resilience, indigenous farmers in Sierra Mixe had inadvertently amplified the natural ability of their crop to partner with nitrogen-fixing bacteria. Meanwhile, farmers and breeders elsewhere in the world accidentally did the opposite, fertilizing corn until it lost this natural ability. Now, it seems the secret to corn's future may lie in its ancient past. Researchers successfully planted the goopy corn in Wisconsin and continue to explore how to bring this ancient nitrogen fixation to commercial farms in the future.

THE EARS HAVE IT

Back in the Wisconsin field in September, Goldstein held up an ear of corn. He told the crowd he had had the kernels of his greener plants analyzed, and that the data indicated the presence of nitrogen fixation.

His classical corn breeding method involves crossing a broad range of cultivated varieties, including ancient landraces from Argentina, Peru and Mexico's Sierra Mixe. He grows the plants under stressful,

FROM TOP: JOHN-MICHEL ANÉ (2); JAY SMITH

ALISON MACKEY/DISCOVER

Walter Goldstein kneels in a field full of experimental corn varieties, organized in numbered rows. A nitrogen-efficient hybrid on the right outperforms the conventional corn planted on the left.

nutrient-limited and sometimes waterlogged conditions, and then selects for ones that flourish, which is unlike contemporary methods that rely on selecting plants based on genetic analysis. To date, Goldstein has cultivated seven hybrid varieties that do very well without fertilizer, he says.

"His breeding efforts are very interesting and very useful," says crop scientist Martin Bohn, an associate professor at the University of Illinois Urbana-Champaign.

In 2018, Bohn and his team planted Goldstein's hybrids along with a handful of others in cornfields in Indiana, Illinois and Iowa, as well as in greenhouses under more controlled conditions. He says Goldstein's hybrids, especially one in particular, consistently did well in soil with low, medium and high fertilizer, and even in the presence of weeds. Bohn thinks it has something to do with the roots, which are always large, well-developed and very dense. Roots from conventional plants tend to stay small in the presence of abundant nitrogen or grow large in nitrogen-deficient soil, he says. Normally, when plants use energy to grow larger roots, their yields can suffer. But Bohn says he didn't see this with Goldstein's best hybrid.

"This root system is not only structurally different ... but it might be possible that these plants cultivate a microbial community that favors bacteria that fix nitrogen," says Bohn, who has long studied how root systems manage microbial communities to make nutrients more available to corn.

Goldstein only recently published his evidence on the presence of nitrogen fixation in some of his corn hybrids, and he has not analyzed the genetic makeup of the microbes. This dearth of published data rankles some scientists. One university research professor, who didn't want to be named, says Goldstein doesn't understand how nitrogen fixation works or how to measure it.

But Abdullah Jaradat, a research agronomist at the USDA who has collaborated with Goldstein on grant-funded research, says Goldstein "looks at the larger scale, which does not necessarily reflect the fine relationship between the plants and the microbiota, but an overall outcome of several physical, chemical, biochemical and environmental factors."

Jaradat doesn't think the approach has any flaws in a general sense, and says Goldstein brings value to research on maize because he has collected and crossbred many varieties, reshuffling genetic sources of corn using techniques that were once a mainstay of North American farming. In Jaradat's view, there is room for both the modern analytical approaches as well as the more traditional methods of breeding.

"There must be a hybrid between the old and the new," Jaradat says, "to bring about a change to the current state of affairs." D

__Tracy Staedter__ is a science journalist based in Milwaukee who covers innovations in sustainability.

FROM TOP: JOHN-MICHEL ANÉ (2); JAY SMITH

ALISON MACKEY/DISCOVER