Discovery in Legumes Could Reduce Fertilizer Use, Aid Environment, Say Stanford Researchers

Escalating use of nitrogen fertilizer is increasing algal blooms and global warming, but a discovery by Stanford researchers could begin to reverse that. They have revealed a key step in how symbiotic bacteria living in legumes turn nitrogen into plant food, which could be used to improve the process in some plants, reducing the need for chemical fertilizers.

BY LOUIS BERGERON

Nitrogen is vital for all plant life, but increasingly the planet is paying a heavy price for the escalating use of nitrogen fertilizer.

Excess nitrogen from fertilizer runoff into rivers and lakes causes algal blooms that create oxygen-depleted dead zones, such as the 6,000 to 7,000 square mile zone in the Gulf of Mexico, and nitrogen in the form of nitrous oxide is a potent greenhouse gas.

But new findings by Stanford researchers that reveal the inner workings of nitrogen-producing bacteria living inside legumes such as soybeans could enable researchers to blunt those negative effects and aid efforts to make agriculture more sustainable.

"We have discovered a new biological process, by which leguminous plants control behavior of symbiotic bacteria," said molecular biologist Sharon Long. "These plants have a specialized protein processing system that generates specific protein signals. These were hitherto unknown, but it turns out they are critical to cause nitrogen fixation."

The ability of legumes to capture nitrogen from the air and turn it into plant food, or "fix" it, also leaves the soil enriched through the plant matter left after harvesting, creating a natural fertilizer for other crops, which is the basis for crop rotation. Alternating legumes with other crops has been a major component of agriculture around the world for thousands of years. Yet until recently, little was known about how nitrogen fixation worked, or why some legumes are efficient at fixing nitrogen and others poor.

The key part of the process that Long's research group uncovered is a plant gene that triggers a critical chemical signal. Without the signal, no nitrogen gets fixed by the bacteria. Dong Wang, a post-doctoral scholar in Long's lab who pinned down the gene, is first author of a paper describing the work, published February 26 in *Science*. Long, a professor of biology, is senior author.

Do-it-yourself nitrogen fixing

The beneficial bacteria in question reside inside the nodules of legumes such as peas, beans, alfalfa and clover, where they pluck molecules of nitrogen from air in the soil and turn it into ammonia, which feeds the plant. It sounds simple, but it is a complicated and poorly understood process. Only bacteria that contain a special enzyme are capable of this sort of "nitrogen fixing" using airborne nitrogen—no other type of living organism can do it. All other plants have to get their nutrients from using already fixed nitrogen in the soil.

Stanford molecular biologist Sharon Long's discovery could reduce the need for harmful chemical fertilizers.

This special ability allows legumes to flourish in nitrogen-poor soils, whereas other plants require applications of manufactured nitrogen fertilizer to grow well. But even legumes can't flourish without the right symbiotic bacteria.

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Sharon Long

"When you deal with a natural soil, you are dealing with a lot of complexity. Everything we learn about what makes symbiosis work gives us a tool to understand why, sometimes, symbiosis fails." Long said. "Plant breeders who are trying to help develop better-adapted plants can now analyze traits such as this. We've given them a new tool."

The more efficient that legumes can be made and the wider the range of environments they can thrive in, the more they can help reduce the need for chemical nitrogen that runs off into water or sinks into the groundwater or decomposes into a gaseous form, Long said.

The gene's the thing

The legume that Long's team worked with is called barrel medic, a forage plant similar to alfalfa. They tracked down the newly discovered gene by studying mutant plants that were failing to produce healthy nodules on their roots.

While bacteria inside normal nodules will thrive, in the defective nodules of this plant those bacteria can't provide the benefit they are wired to deliver. Long said that the mutant "contained perfectly good bacteria, but was making these lousy nodules."

Wang found that the mutant plants generated the proper precursor to the protein needed to nudge the bacteria into fixing nitrogen. But the critical enzyme for processing that precursor into the final signal was missing. So the bacteria simply sat, the nodules didn't develop, and no nitrogen was fixed.

By comparing the genome of the mutant plants with normal plants, the group found a gene that was missing from the mutants. Suspecting that gene might be the culprit, the researchers took a functional version of the gene from normal plants and put it into the mutants. The mutant legumes then began fixing nitrogen the same as normal ones, "proving that we found the right gene," said Wang.

How less is more

Since 1960, the use of nitrogen fertilizer in the United States has roughly quadrupled, as has the price per ton, according to the U.S. Department of Agriculture. Prices have been driven up by the rising cost of natural gas used to manufacture the fertilizer.

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"That might make things more expensive for American farmers and increase food prices for consumers, but this is going to wipe out people in developing countries, whose soils are perhaps most in need of fertilizers," Long said. "This is a crucial issue. And nitrogen fixation is a key to sustainability."

Costs aside, the production of chemical fertilizer also adds to the problem of global warming, both by way of the fossil fuels used in production of chemical fertilizer and through the impact of leftover fertilizer that degrades into nitrous oxide, a highly potent greenhouse gas.

With the planet's ever-growing population, Long said there is going to be increased need to keep productivity going on lands that are starting to become marginal because of drought, temperature or salinity problems, among others.

"The rhizobium bacteria are a critical partner in whether that kind of extension of serviceable land can occur," she said. "In order for us to take existing symbioses and help make them better, optimize them for being productive even when conditions start to deteriorate, tools such as understanding how to improve nitrogen fixing in legumes are crucial."

Joel Griffitts and Colby Starker, also authors on the paper, contributed to the research when they were graduate students or postdoctoral scholars in Long's lab. Griffitts is now an assistant professor at Brigham Young University. Starker is a research associate at the University of Minnesota. The research was supported by the Howard Hughes Medical Institute, the Helen Hay Whitney Foundation and the National Science Foundation.

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