Genetic duplication governs nitrogen fixation symbiosis between legumes, bacteria

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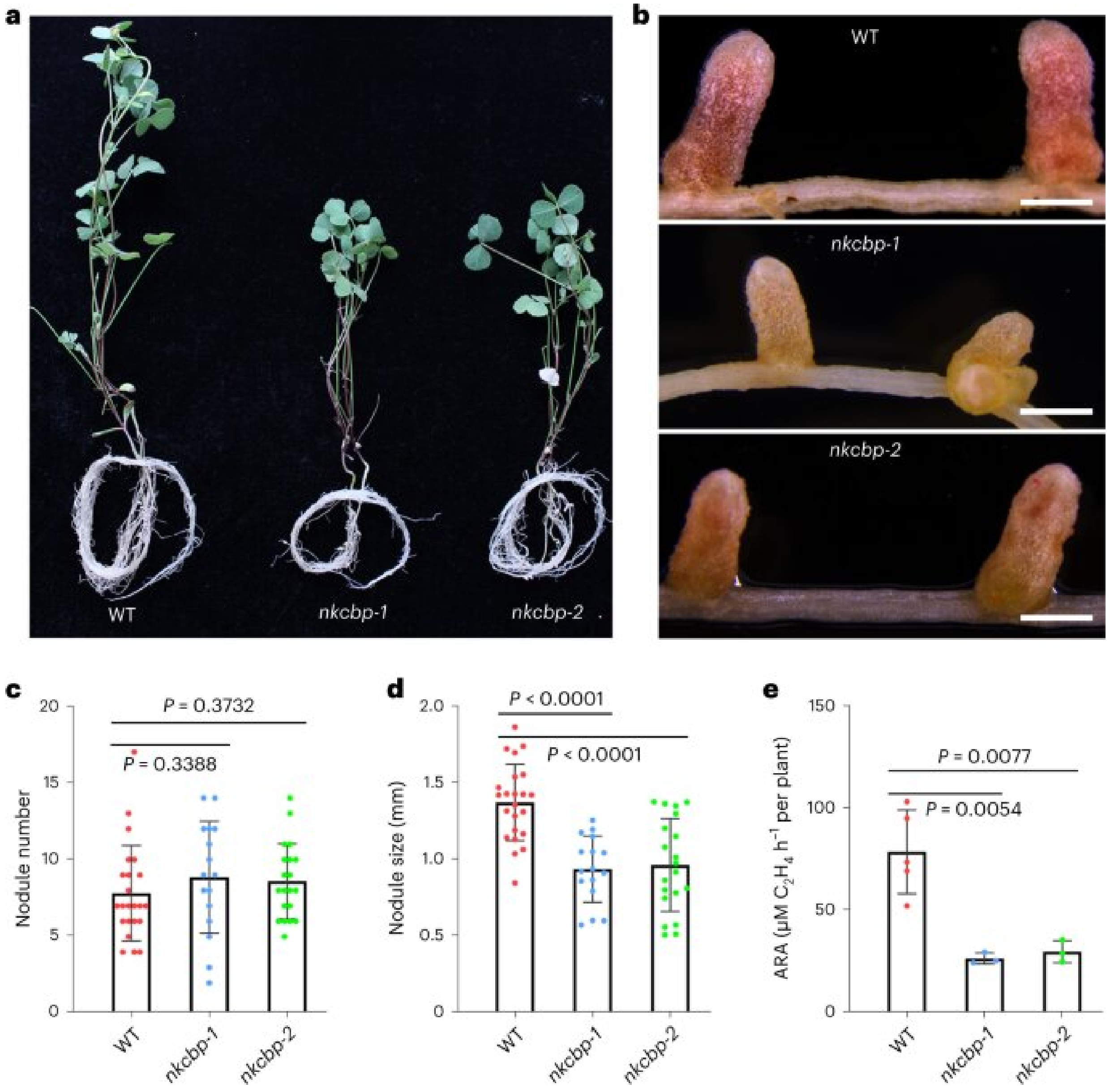

M. truncatula nkcbp mutants exhibited nitrogen starvation symptoms and nodule developmental defects. Credit: *Nature Plants* (2022). DOI: 10.1038/s41477-022-01261-4

The processes that govern the formation of symbiotic structures between nitrogen-fixing bacteria and legumes in the latter's roots remain largely a mystery to science, but researchers have recently discovered that a duplication of the genes is playing a key role.

A paper describing the researchers' findings was published in the journal *Nature Plants* on October 31.

Nitrogen is one of the most important ingredients of life. It is an integral component of amino acids, proteins, and the nucleic acids that make up DNA and RNA—the very building blocks of living organisms. If plants, animals, fungi, bacteria or any other organisms suffer from nitrogen deficiency, they will almost certainly die.

Some 80 percent of the atmosphere is made up of nitrogen gas, so one might think that nitrogen deficiency is unlikely. But nitrogen in this form—molecules composed of two nitrogen atoms bonded together, or N_2–cannot be used by almost all organisms.

Nitrogen-fixing bacteria are the only bacteria in all of nature that can break the incredibly strong triple bond of N_2 and attach the nitrogen atoms to hydrogen to make ammonia (NH_3)—a species of nitrogen-based molecule that organisms can actually take up and use.

This process is called biological nitrogen fixation. Many plants are able to take up the ammonia synthesized by the nitrogen-fixing bacteria, and other organisms, including animals, can eat those plants or eat animals that have eaten those plants, and in this way gain their nitrogen "fix".

There are also a small number of plants, in particular legumes such as peas, beans and lentils, that enjoy a symbiotic relationship with rhizobia in which the bacteria are incorporated into some of the cells in the roots of the plants, forming small nodule-like growths. The symbiotic bargain between bacteria and plant involves the rhizobia trading some of their ammonia for other types of nutrients needed for development.

But lots of crops do not enjoy this symbiotic relationship with rhizobia. And in these cases, farmers need to spread manure or synthetic fertilizer on their fields so that these crops can access life-giving nitrogen from ammonia.

For sustainable agriculture, this poses two major problems. Synthetic fertilizer is produced via the Haber-Bosch process, one of the most important chemical reactions in the modern world. It uses high temperatures and pressures to combine atmospheric nitrogen to hydrogen to artificially produce ammonia.

But the easiest, cheapest way to source the hydrogen ingredient necessary for this ammonia recipe is by breaking apart the methane molecules that make up natural gas, in turn producing carbon dioxide as a byproduct. This makes fertilizer production one of the leading causes of global warming within agriculture.

In addition, application of both manure and synthetic fertilizer to fields results in ammonia agricultural runoff into rivers and streams. This "nitrogen pollution" causes deadly algal blooms that suck out oxygen offshore, resulting in vast underwater dead zones.

"So, if scientists can learn more about how the rhizobia-legume symbiosis happens, maybe we can engineer other types of plants than just legumes that can form such a symbiosis, or even fix nitrogen directly," said Kong Zhaosheng, a microbiologist with the State Key Laboratory of Plant Genomics at the Chinese Academy of Sciences in Beijing and a co-author of the paper.

"This could radically reduce our dependence on manure and synthetic fertilizer, or even eliminate their need entirely. This has long been the Holy Grail of sustainable agriculture."

While a lot is known about this symbiosis, a great deal remains mysterious, in particular the biochemical process that governs endosymbiosis—how the bacteria incorporates itself into the plant's root nodule cells. In most legume species, the rhizobia become entrapped in the host through curly hairs on the outside of the roots.

The bacteria then "infect" plant cells, proliferating inside them, via tubular infection threads. These threads are in turn enveloped by a membrane produced by the host plant, forming a structure akin to organelles (the "organs" that perform different functions inside a cell). This nitrogen-fixing organelle-like structure is called the symbiosome, which has in effect radically reorganized the cell to accommodate the rhizobia bacterium.

It was known that the plant-derived symbiosome membrane provides an interface for exchange of nutrients and "signals"—chemical directives—between the two symbionts, plant and bacterium, and that the plant-cell cytoskeleton (the filament-like internal scaffolding within cells) plays a key role in this interface.

In addition, researchers suspected that as the central vacuole—the large, water-storage organelle in plant cells—exerts force on the cell and cell wall to maintain pressure equilibrium, thus helping to coordinate the internal organization of the cell, it likely plays some role in the symbiosome.

But the underlying mechanisms of how all of this might work remained largely unknown.

In the common liverwort, Marchantia polymorpha—one of the earliest plants to conquer land some 400 million years ago, there is a protein, the kinesin-like calmodulin-binding protein, or KCBP. Kinesins are "motor" proteins that work to transport molecules throughout the cells of many different types of organisms by "walking" along internal microtubule structures.

KCBP however is unique to plants and in liverworts is essential to the growth of their rhizoids, root-like structures of these early plants. The protein is thought to be one of the key evolutionary developments that allowed plants to adapt to land.

Tantalizingly, in the barrelclover plant (a type of legume), the genes that are responsible for production of this KCBP are activated just about everywhere in root hairs at the infection-thread stage.

So the researchers focused on the KCBP-encoding genes, using BLAST (Basic Local Alignment Search Tool) analysis, a program that compares genetic or protein sequences of specific organisms to databases of such sequences to find similar regions.

They found that in the barrelclover's genome, there is a duplication of them. And where this duplication of KCBP-encoding genes occurs, their activity appears solely related to interactions between the barrelclover and the rhizobia bacteria that enable the formation of the symbiosome.

A separate phylogenetic analysis—an evolutionary history of genetic changes in ancestral species over time—found that this duplication of

KCBP-encoding genes only occurs in the legumes that form symbiosomes.

The researchers believe that the rhizobia are hijacking the plant's duplicate KCBP to direct a cross linking of microtubules within the cell to control how the central vacuole in symbiotic cells forms. In this way, it governs symbiosome development.

There remain many unresolved questions. The team now aim to identify what is driving the activation (expression) of the duplicate KCBP genes, to find the genes that act in concert with those governing the duplicate gene set to regulate rhizobia accommodation in the cell, and to explore how chemical signaling works across these two very different kingdoms of life, plant and bacteria, to govern the symbiosis.

More information: Xiaxia Zhang et al, A legume kinesin controls vacuole morphogenesis for rhizobia endosymbiosis, *Nature Plants* (2022). DOI: 10.1038/s41477-022-01261-4

Provided by Chinese Academy of Sciences

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