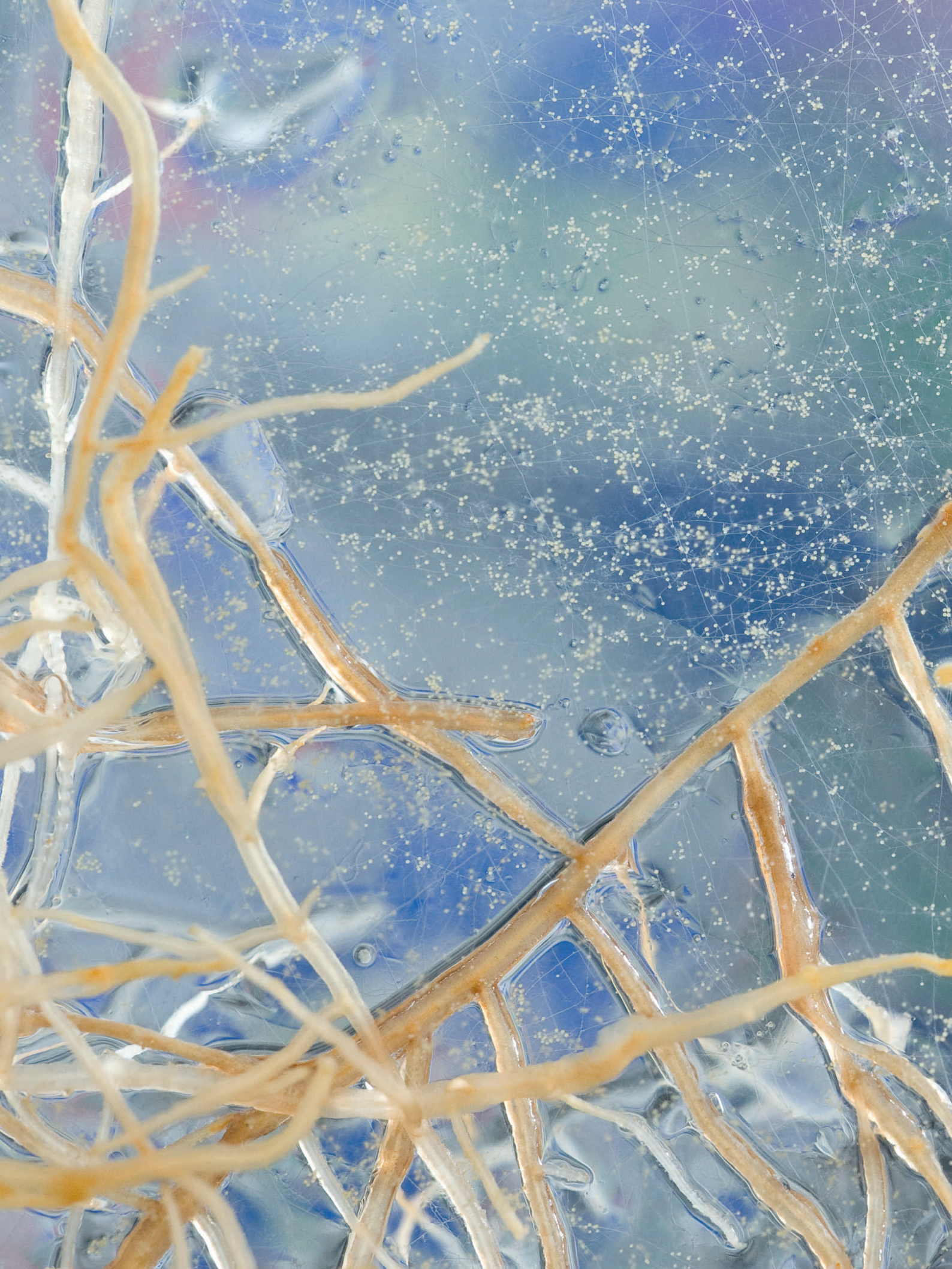


Photo: Norbert Michalke

Getting to the **Root of Things**

Plants have lived in close community with certain fungi for millions of years. The microorganisms provide them with vital mineral salts such as phosphate, and in return, they supply the fungi with carbohydrates. **Franziska Krajinski** from the **Max Planck Institute of Molecular Plant Physiology** in Golm observes how these unequal partners establish contact with each other and exchange nutrients.

TEXT **CATARINA PIETSCHMANN**

He worships her and keeps her in a constant embrace. He simply can't live without her. She, on the other hand, can survive without him, but she values his companionship. After all, he gives her strength and helps her to be better and more beautiful than the others who are not lucky enough to have such a caring partner. And she rewards his hard work with sweetness; a truly symbiotic relationship that lasts until they are parted by death.

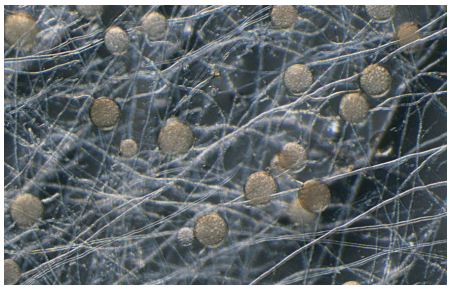
Despite how it sounds, this is not a description of the perfect marriage, but the age-old story of the intimate underground relationship between plants and mycorrhizal fungi. It all began 400 million years ago when the first plants ventured onto dry land. All plants had such helpers in their roots in the beginning. The fungi supply them with nitrogen, phosphorus and other nutrients from the soil. In return, they receive glucose, which the plants produce through photosynthesis. The carbon it contains is the elixir of life for the fungi, the very basis of their existence. There is a perfect balance of give and take.

FUNGI SUPPLY THE PLANTS WITH NUTRIENTS

Even the most dedicated amateur gardeners give little thought to how plants obtain their nutrients. They water them regularly and fertilize them occasionally. "However, the one to two millimeters of soil surrounding the roots quickly becomes denuded of phosphate. It doesn't simply flow through the soil," explains Franziska Krajinski from the Max Planck Institute of Molecular Plant Physiology. "The plant is reliant on help here." The help provided by fungi, for example.

At the Max Planck Institute in Golm near Potsdam, the biologist studies this interaction on the barrel clover *Medicago truncatula*, which is related to alfal-

Symbiosis in the Petri dish: The roots of a carrot plant are surrounded by a network of hyphae from the *Glomus intraradices* fungus.



The mycorrhizal fungus *Glomus intraradices* forms a dense underground network of fungal hyphae (left). Tiny spores emerge from these thread-like cells (right), which enable the fungus to reproduce.

fa, or lucerne, and the fungus *Glomus intraradices*. Despite the fact that their barter system has existed since time immemorial, exactly how it functions at the molecular level remains a mystery. How do the host and symbiont communicate? How exactly does the exchange of substances take place? The fact that some plants, including cabbage-like species, refrain from engaging in such support relationships with fungi doesn't make things any easier. The favorite model plant of biologists, the thale cress *Arabidopsis*, is among the few plant species that do not enter into symbiosis with fungi. "Because of this, mycorrhizal symbiosis was the poor relation of plant research for a long time," explains Krajinski.

VAST DIVERSITY OF ENDOSYMBIOTIC FUNGI

First, however, some difficult terminology needs to be explained. The term mycorrhiza originates from the Greek and means "fungus root." Mycorrhizal fungi thus engage in fungus-root interaction and are very common in the fungal kingdom. In contrast, the AM fungi – AM standing for arbuscular mycorrhizae – form a separate phylum or group known as the glomeromycota. Their approximately 400 species are all endosymbionts, which means that they penetrate directly into the plant cells with the tips of their thread-shaped cells, the hyphae. There they form a tree-shaped apparatus, the arbuscule, for the specific purpose of exchanging nutrients with the plants.

This distinguishes them from Boletus and other typical forest fungi. The latter, which also form an extensive network of hyphae in the soil and, above ground, sometimes tasty, fruiting bodies, are known as ectosymbionts: although they grow in the roots of their host organisms, in many cases trees, unlike the endosymbionts they do not penetrate the root cells. These species developed at a far later stage in evolutionary history than the AM fungi.

It appears that there is a lot more going on in the soil than might be supposed. When the 39-year-old scientist from Magdeburg talks about her research, she opens the door to a hidden underground kingdom. Soil bacteria, worms and small insects jostle around between the roots of the trees, bushes, and grasses. But this area is also inhabited by various species of AM fungi that form hair-thin webs with their colorless hyphae, which can extend across entire square meters of soil. "An extensive network is thus formed between different plants and fungi," says Krajinski. The modest fungi offer their services to every reachable plant that appreciates their services. Unlike bacteria, which sometimes like to overrun other species and, if necessary, defend their own territory by means of toxic attacks, AM fungi do not engage in turf wars. "They probably don't have any spare capacity to form toxins," explains Krajinski. "After all, they obtain every single carbon atom from their host plants." Specific plants rarely express a preference for a special type of fungus and vice versa. "All we know is that some symbiotic relationships are more efficient than others."

Over 80 percent of all terrestrial plants today engage in AM symbiosis. Some also avail of the services of rhizobia or root nodule bacteria – also a form of endosymbiosis – which are able to fix nitrogen from the air. The barrel clover can do both, and is not selective. It will barter with any plant that happens to be close by. To ensure that the scientists can study the pure symbiosis with the fungus, it is strictly barred from contact with root nodule bacteria in the Golm-based research greenhouse.

PLANT AND FUNGUS EXCHANGE MESSENGER SUBSTANCES

So how does the life of a so-called obligate symbiont – that is, an organism that can survive only in association with a plant partner – begin? The fungus starts life alone as a tiny spore: a round little survival capsule, a mere one millimeter but chock full of provisions, since it can take a long time until the day arrives when it germinates. It is not known exactly what kind of signal prompts the spore to do this. It could be something as simple as water: when the spores are placed on a watery bed of algal gelatin in the laboratory, the germination process begins and two to three small hyphae emerge from the spore.

With a little luck, a root will grow very close by at that very moment, and the establishment of contact begins. Not through touch, but chemically, through molecules. "I personally believe that the initiative comes from the plant, simply because it has more resources. It gives the first signal," says Krajinski. "The fungus responds only when it can be certain that a host has made contact with it."

This is the exact response that the research group is aiming to track down, the so-called mycorrhizal factors. To

Photos: MPI of Molecular Plant Physiology (2)

The benefit the barrel clover derives from the mycorrhizae is noticeable (above): Without the support of the fungus *Glomus intraradices*, it grows far more slowly (left) than it does with the symbiotic fungus (right). Using a two-compartment system (below left), biologists can analyze which substances the fungus delivers to the plant. The fungus hyphae in the inner compartment absorb radioactively marked substances and transport them across the gap to the plants in the outer compartment. The researchers can then measure them based on their radioactivity: in addition to the mycorrhizae, the barrel clover also forms a symbiosis with root nodule bacteria that can convert nitrogen from the air into compounds that the plant can use.

Photos: Norbert Michalke (top), Catarina Pietschmann (bottom left), MPI of Molecular Plant Physiology (bottom right)

Daniela Sieh (left) and Franziska Krajinski in a phytotron, a research greenhouse in which the scientists can keep plants under precisely defined conditions.

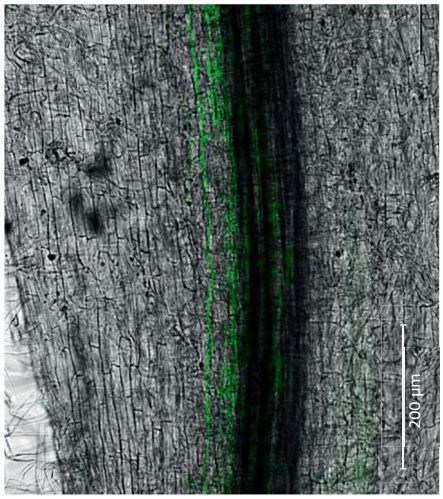
identify them, they must first decode the plant's reaction. To this end, an already colonized root culture in which messenger molecules from the plant and the fungus are present is covered with a membrane. The membrane prevents direct contact with the barrel clover whose seedlings are placed on top of it. "The messenger molecules diffuse through the membrane," says the biologist, "and we examine the means the plant uses to respond." Based on its reaction, which involves the activation of genes, the researchers can then identify the fungus' signaling substances. However, how the plant immune system distinguishes between friend – a potential symbiont – and foe – a pathogenic fungus – remains a mystery.

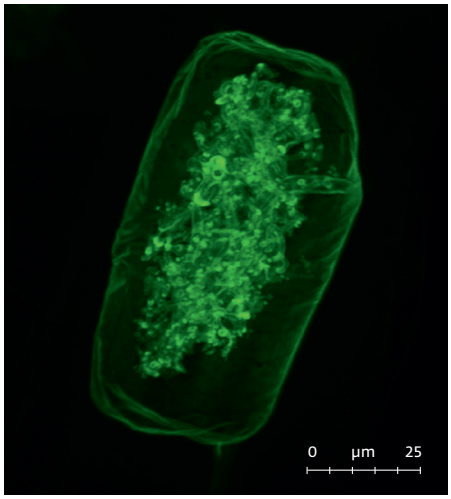
ROOT CELLS CLEAR THE WAY

If there is no root there, the spore retracts its hyphae. The energy reserves in the capsule are sufficient for several attempts. Some time later, it tries again; it may get lucky with a new seedling. And if it works this time, and plant and fungus have recognized each other, the first physical contact soon takes place. The hyphae branch out and place a small foot on the root. They adhere to it using tiny round plates. Finally, the fungus penetrates the root.

The cells of the root skin actively prepare and voluntarily make space for the fungus. "They reorganize their entire cellular skeleton and form a tunnel through which the fungus hypha can grow." The web-like threads meander between the cells and further on to the middle of the root. Their destination is the cells inside the root cortex that surround the central cylinder. This contains the vascular bundles that convey water and nutrients to the aboveground parts of the plant. The glucose that is vital to the fungus also arrives here from the opposite direction.

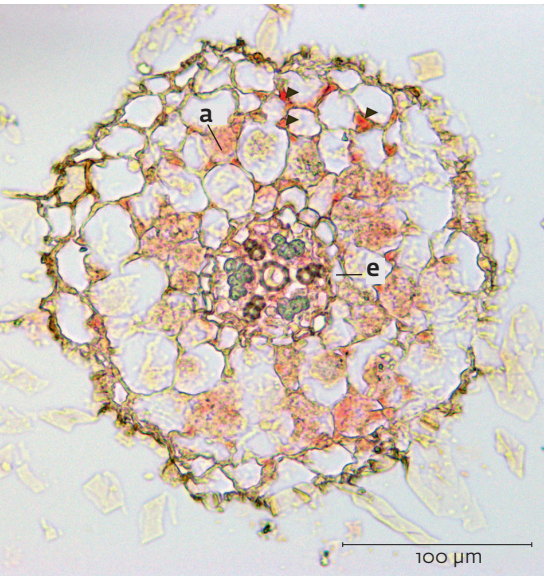
The formation of the arbuscule starts now: initially, there are just two to three protrusions, but more form subsequently. The hypha soon looks like a ball of inflated miniature rubber gloves. More and more protrusions form until the plant cell is almost completely filled with the arbuscule. The fungus never comes into direct contact with the interior of the plant cell. It is always surrounded by the plant cell membrane and simply creates inward dents in it. The more branches it forms, the better, as the surface will be larger





left: Barrel clover roots with mycorrhizal fungus (green). The fungus penetrates the cells of the root cortex around the central cylinder. This contains the vascular pathways for water and nutrient transport. right: Fungus bodies inside a root cell. The plant cell wall, the fungus's point of entry (above right), and the fungus's tree-like structure (arbuscule) are stained with green fluorescent dye.

Photos: MPI of Molecular Plant Physiology (2), Norbert Michalke (right)



Cross-sectional view of a barrel clover root: Mycorrhizal fungi penetrate the cells of the root cortex and form arbuscules there (a). The endodermis cells (e) can't be penetrated by the fungal hyphae. The triangles show areas that are stained dark red by the microRNA 5229. The activity of the plant cell genes there has been modified.

for the exchange of nutrients. In the plant cell membrane, completely new proteins are now formed that the plant doesn't normally produce. Among other things, they act as a transport vehicle for phosphate and nitrogen.
Arbuscules have their own dynamics. They last for just ten days and then degenerate. Nobody knows why this is so. A cell can, however, accommodate a new arbuscule. Microscopic images of the longitudinal and cross sections of roots show young arbuscules next to fully grown ones.

LASER BEAM SEPARATES CELLS FROM THE TISSUE

It doesn't take long until the complete inner root cortex is colonized. At first glance, the process resembles an invasion, like a hostile takeover. However, it is actually a process of peaceful coexistence. Both sides adapt perfectly to one another to optimally benefit each other – a symbiosis in the truest sense of the word: living together.

A very old genetic program starts up in the infiltrated cells and completely reprograms them for the symbiosis. How does the gene activity in these cells differ from that in neighboring cells without arbuscules? To find this out, Franziska Krajinski's team studied both cells separately. They were the first scientists to succeed in doing this using a technique known as laser capture microscopy. A fine laser beam cuts the cells out and a second catapults the tissue into a test tube. The development of a rapid process for the collection and analysis of the individual cells alone took four years.

With a steady hand, a focused eye and an awful lot of patience, the researchers isolated approximately 13,000 cells. This was enough to study the RNA and proteins in these cells. And just about sufficient to also identify the amino acids and sugars that are formed by cells with arbuscules. Together with other research groups at the institute, Krajinski and her colleagues extracted the cell fluid from the cell sections and analyzed its composition. They found that it contained a lot of sucrose, a lot of amino acids containing nitrogen and some still unknown compounds. "These are particularly fascinating, as they are formed only during symbiosis!" It isn't yet possible to say, however, which metabolite originates from the fungus and which one from the plant.

The comparison of cells with arbuscules, neighboring cells without arbuscules and cells from non-colonized roots shows that around 800 genes change their activity as a result of the symbiosis. Surprisingly, the neighboring cells undergo similarly extensive reprogramming. But why? It is possible that they are preparing for their own colonization. It is also possible that their neighbors assume functions that they can no longer fulfill, as the colonized cells are completely filled by the arbuscules. Or they help to provide glucose reserves. The activity of certain genes in the neighboring cells would suggest that more starch in these cells is decomposed to transportable sugar.

The reprogramming of genes is propelled by transcription factors that regulate the activity of genes. These are the triggers that prompt cells to form different proteins from the usual ones. But are they the only source of control? It has been known for some time now that, in addition to the transcription factors, microRNAs also fulfill this task in many organisms. These RNA sections, which weren't discovered until 1993, are just 21 to 23 nucleotides long and also influence gene activity. Krajinski's team thus examined whether plant-fungus symbioses also have their own microRNAs.

MICRORNAS CONTROL THE EXCHANGE OF NUTRIENTS

Plants can control the extent to which they provide for the fungus with the help of the RNA snippets. This protects them against overfeeding, but lets the poor dependent fungus go hungry as the symbiont receives less glucose in exchange. "For instance, if a plant has enough phosphate, it can curb further consumption using microRNAs from group 399. These molecules could thus be ideal candidates for boosting or slowing down the symbiosis." Although the researchers were unable to confirm this function, they discovered many new microRNAs that are now undergoing further examination.

The molecular logistics of AM symbiosis are highly complex, but the processes are reminiscent of an everyday phenomenon: the fungus acts as the plant's wholesaler, in this case a wholesaler dealing in minerals. It has an extensive supply network – its hyphae. And it uses utility vans – transport proteins in the arbuscule membrane – to supply its plant customers. The plants are ready on the opposite side with wheelbarrows – their own transport proteins – waiting to load up with pallets of nutrients and distribute them.

Photos: MPI of Molecular Plant Physiology

Using a fluorescence microscope, Anja Branscheid examines where microRNAs, which influence the activity of genes, are formed in the roots.

The payment process also takes a similarly business-like form. If customers are unable to pay their bills, the wholesaler goes broke – if the plant is short of sugar, the fungus suffers.

Researchers have long focused on phosphate exchange in particular. They have since not only encountered specific transport proteins for this mineral but, among other things, also for ammonium, nitrate, and possibly also for copper. The fungus clearly takes everything from the soil that it can get its hands on and pumps it, using different transport proteins, into the tiny gap between the arbuscule membrane and the membrane of the root cortex cells. The plant transporters then take over the nutrients.

But what is the situation regarding the balance between the nutrients? On which mineral is the plant keenest, and which one can it do without, if necessary? A current cooperative project is researching this using sulfur transporters. "For a long time, sulfur was viewed as not being very important, as it was actually available excessively in the soil," explains Krajinski. Using radioactively marked sulfur, the biologists are seeking to discover how sulfate or a lack of it affects symbiosis.

They administered the marked sulfate to the fungus and measured the proportion of it that arrived in the plant. Sometimes the plant had previously received sufficient phosphate and too little sulfate, and sometimes it was given sufficient sulfate but too little phosphate. "We wanted to understand the conditions under which the symbiosis is suppressed." Their findings: the barrel clover expresses a clear preference – phosphate is the main thing! Even if it is practically flooded with sulfate, it suppresses the exchange of sulfur only if it has sufficient phosphate inside it.

Soil that has no mycorrhizal fungi is practically nonexistent. Some species grow everywhere – from the Siberian Tundra to the Caribbean. It would therefore appear to be ideal for agricultural use. AM fungi could render the use of chemical fertilizer superfluous by making the soil nutrients accessible.

"The large-scale application of AM fungi as a growth aid is still pretty much impossible," says Franziska Krajinski. "The over-fertilization of fields with phosphate and nitrogen for many years slows the fungus down." There are far fewer colonized roots in over-fertilized soil and the fungus can't reproduce well. And because spores are formed only during symbiosis, the soil slowly becomes impoverished. "However, the global phosphate resources used in fertilizer production are almost depleted. So AM fungi could offer a possible alternative in the distant future." The research being carried out by the Max Planck scientists in Golm could literally be preparing the ground for this development.

But for amateur gardeners, there is already an alternative to the use of fertilizers. A renowned British rose breeder now provides a mycorrhizal fungus spore mixture in bags. It can be used to lend a helping hand to garden and pot plants: all it involves is simply powdering the roots, planting them, and then watering them. ◂

GLOSSARY

Arbuscule
Tree-shaped growth from fungus hyphae in the interior of root cells in the arbuscular mycorrhiza. It penetrates the cellulose cell wall, but not the cell membrane. The growths always fork into two branches of the same size. The large surface of the arbuscule facilitates the exchange of substances between the plant and fungus.

Root nodule bacteria
Symbiotic bacteria of legumes (peas, beans, clover). They bind nitrogen from the atmosphere and make it available to the plant, from which they obtain carbohydrates in return. The plants form tuber-like thickenings in the roots; and the bacteria arise in the cells of these nodules.

Mycorrhiza
Symbiotic association between fungi and plants. Around 80 percent of terrestrial plants form mycorrhiza. There are three main types of mycorrhiza: in endomycorrhiza, the oldest form, the fungal hyphae penetrate the plant's root cells. The fungus mainly supplies the plant with phosphate. In ectomycorrhiza, the root is surrounded by a dense network of fungal hyphae that remain outside the root cell. These help coniferous trees, for example, to cover their nitrogen requirements. In the case of ericoid mycorrhiza, many of the fungal hyphae remain outside the root cell, but individual hyphae penetrate the cell. This form arises in particular in moors and heaths, and mainly supplies the plants with nitrogen.

Photo: Norbert Michalke