Good preparation is key -- even for plant cells and symbiotic fungi

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Not only mineral oil and petroleum gas, also phosphorous is a scarce resource. According to well-respected scientists who gathered together for a conference in Cambridge this August, we will face significant problems relating to phosphorous deficiency in just 20 years from now. Phosphorous, this important and essential mineral, is part of our DNA and, therefore, irreplaceable. Many soils are already depleted for phosphorous today. Plants growing on these soils are only able to take up enough phosphorous by living in symbiosis with arbuscular mycorrhizal fungi (AM fungi). Arbuscular mycorrhizal symbiosis (AM symbiosis) can be found in almost all vascular plants and there is strong indication that plants have a special genetic programme for it.

The goal of Franziska Krajinski and her "Plant-Microbe Interactions" group from the Max Planck Institute of Molecular Plant Physiology is to understand which genes are involved in AM symbiosis. This symbiosis is a non-synchronous process, which means that different cells in the root can show different phases of symbiotic interaction with the fungus. For this reason, the scientists tried to analyse individual cells as opposed to whole roots. They managed to excise single root cells with the help of laser capture microdissection and deciphered these cells' specific gene activity.

When scientists are analysing the molecular composition of plant cells they usually assume that different cells from the same tissue are alike. In many cases, this assumption is true. The majority of cells from leaves, stems or roots show similar levels of gene expression and metabolic activity. It gets more complicated when plants undergo symbiosis, because interactions with the symbiotic partner may alter the cell's metabolism. And even cells adjacent to colonised cells that have not yet come into direct contact with the fungus can show drastic changes in their gene expression levels.

The most prevalent plant symbiosis is that between root cells and arbuscular mycorrhizal fungi, called AM fungi. AM fungi make sure that plants can grow on nutrient-depleted soil – unnoticed by most people. These fungi outstretch their filamentary cells, called hyphae, far into the soil and are thereby able to take up more nutrients than plants can absorb with their roots. The fungus takes up mainly phosphate, but possibly also nitrate and metal ions like copper, zinc and iron and gives these willingly to the plant. In return, it is rewarded with sugars that plants produce via photosynthesis.

Interestingly, fungus and plant cell never really merge; they are constantly separated by membranes, the outer boundaries of the cells. To enable the relatively big sugar and phosphate molecules to pass through these membranes, the plant cells insert big protein complexes that resemble tunnels through which the molecules can freely travel from one cell to another. This was already known, and it was not astounding that the scientists around Franziska Krajinski found genes that encode for such transport proteins to be highly expressed in cells that are already colonised by the fungus. A more surprising discovery was, however, that even cells that are in close vicinity of the colonised cells seemed to be already reprogrammed. More than 800 genes showed enhanced activity exclusively in these cells. "The higher transcription rate of genes that are responsible for transport proteins, lipid acid metabolism and gene regulation does not seem to be a result of the colonisation by the fungus," explains Nicole Gaude, first author of the study. "It is more likely that cells are preparing themselves for an imminent colonisation by the fungus."

These very precise and specific results were obtained with the help of laser capture microdissection. In this method, a laser beam is used to excise individual cells from a tissue. At least 5000 cells were cut out by Gaude and her team; a time-consuming manual labour that even Sisyphus would have been proud of. But the time and effort were worth it. "We now know which genes are activated even before a symbiosis is physically established," explains Gaude.

Understanding the symbiotic programme of plants could enable the use of AM fungi in agriculture and reduce the application of expensive, artificial fertilizer in the future.

More information: Nicole Gaude, Silvia Bortfeld, Nina Duensing, Marc Lohse, Franziska Krajinski, Arbuscule-containing and non-colonized cortical cells of mycorrhizal roots undergo a massive, *The Plant Journal*, online advance publication, 6 October 2011, DOI: 10.1111/j.1365-313X.2011.04810.x

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