Zinc and cadmium hyperaccumulator: *Thlaspi praecox*

Metal tolerance in Ljubljana

Plants Against Pollution

Accumulation of metals in soil poses a threat to plant growth and survival. Marjana Regvar from the University of Ljubljana investigates how some tough species deal with the stress. A question which is becoming increasingly important for our own food chain, too.

Pollution of soil and water with metals and metalloids increases as human civilisations grow. Mining and smelting, the burning of fossil fuels, use of pesticides and the disposal of batteries and other municipal wastes are typical sources. The effects are long lasting because metals cannot be chemically degraded. They accumulate and are taken up into plant tissues where they pose toxic stress, leading to growth disturbances and even to death. As a consequence, postindustrial landslides often develop into wastelands. But even only slightly polluted soils may pose a danger, as contaminated agricultural crops increasingly affect human food safety.

Besides costly and technically complex physical procedures to remove metals from soil, scientists are searching for cheap and sustainable solutions. One of these scientists is Marjana Regvar from the University of Ljubljana in Slovenia. “In the last decades it has been discovered that some plants themselves might do the job, using mechanisms developed for their own protection,” she says. In her plant physiology group at the Department of Biology she investigates so-called hyperaccumulating plants that assimilate high concentrations of metals and store them in sites where they cannot do any physiological harm. Such plants may be used for phytoremediation in the future because they extract metals from the soil.

Marjana Regvar

Another strategy is some plants’ ability to form arbuscular mycorrhiza symbioses. Some fungi enter plant root tissue, form branches which are called arbuscules and contact the membranes of the cells, making the exchange of molecules possible. But they do not just help their hosts to gain minerals and water from the soil. They also bind toxic metals and avoid their uptake into the plant’s roots.

Time for applied ecology

Regvar studied Mycorrhiza systems for her masters thesis and her PhD, between 1992 and 1998, but was searching for the signals that help plants to distinguish between parasitic fungi and profitable Ecto-Mycorrhiza symbionts (Ecto-Mycorrhiza fungi do not form arbuscules although they colonise cortical root tissues). In 1998 she changed direction. Her professor, Nada Gogala, retired and Regvar took over the chair for Plant Physiology. At this point she gained a project in the highly polluted Mežica Valley in Northern Slovenia, previously an area crammed with heavy industry.

“It was the time when applied ecology started to be more important for governments and industry than basic research,” Regvar remembers. She and her team of two assistants and one technician switched to Arbuscular Mycorrhiza and metal hyperaccumulating plants. They began to ask how the highly degraded postindustrial soils in Northern Slovenia could be restored. The situation in the Mežica Valley is dramatic, especially for Slovenians, because most of them grow vegetables in their own gardens. “The soil in this valley is really toxic,” Regvar says. “Some people even suggested that we should wear protective clothes while working, and in the same time the inhabitants of the valley grow their food on this soil. A third of the children suffers from heavy diseases and has more lead in their blood than allowed.”

Since 1998, the Regvar group and their partners in Slovenia, Germany and Spain have made good progress. This year, for example, they found evidence for Arbuscular Mycorrhiza colonisation in common and tartary buckwheat, which had been doubted before (Likar *et al.*, *Mycorrhiza* (2008), 18: 309-315). This finding gives rise to the hope that plants could be inoculated with a mixture of the symbiotic fungi, thus benefiting agricultural outcome in the future. A second research focus led to the discovery of cadmium and zinc hyperaccumulation in the *Thlaspi praecox*. This plant belongs to the Brassicaceae family, a group with numerous hyperaccumulating plants, but it shows some special features. “This discovery was very interesting” says Regvar. “That’s why we are still studying this plant today.”

Rare expertise

In order to investigate the mechanisms by which *T. praecox* stores toxic metals in its organs Regvar’s team had to develop a technique that would make it possible to measure metal concentrations in plant tissues. The solution was delivered by Bogdan Povh of the Max-Planck-Institute for Nuclear Physics in Heidelberg, who introduced them to a technology based on so-called micro-proton induced x-ray emission (micro-PIXE). The basis of this technique is a small beam of protons which makes it possible to measure the typical spectral energies of elements against the cellulosis background of plant tissue. Additionally, the researchers gain information about the thickness of element clusters and can compute an element's concentrations.

"Using micro-PIXE we were able to scan plant tissues and determine the store sites of essential and non essential minerals and metals," Regvar says. Only a few labs in the world have the expertise assembled in Regvar's group and its collaborating lab at the Josef-Stefan-Institute in Ljubljana. Their experiments showed that *T. praecox* accumulates zinc in the large vacuolated cells of a leaf's epidermis, indicating that it might be stored and detoxified in the vacuoles (Vogel-Mikus K. *et al.*, *Plant, Cell and Environ.* (2008), 31: 1484-96). Cadmium turned out to be stored in the vacuoles of leaf mesophyll cells and in this way was kept away from the mechanisms of photosynthesis. Lead seems to accumulate in the vacuoles of mesophyll cells as well as in cell walls, where it might be bound chemically as a second detoxification mechanism.

Close to the embryo

These experiments affirmed potential metal accumulation strategies that had already been shown for other plants. But they revealed something unexpected, too. "The scholar opinion tells us that metal hyperaccumulating plants keep the toxic metals away from their seeds," explains Regvar. Normally, embryonic cells do not share plasmodesmata with mother cells; they are disconnected from the mother plant and thus saved from toxic materials which could cross borders. "But in *Thlaspi praecox* seeds we found considerable amounts of cadmium in the epidermal cells of the cotyledons," says Regvar. How the metal gets there is uncertain, the plant somehow fails to avoid its transport. Nevertheless, it succeeds in keeping the cadmium from embryonic cells in the deeper layers of the embryo, storing them at the surface. In any case, *Thlaspi praecox* is worth further investigation. And who knows, in future, farmers might use it on their fields as a metal extractor.

With a little help from some fungi

The special nature of *Thlaspi praecox* is highlighted by another fact. Unlike other Brassicaceae the plant is able to form Arbuscular Mycorrhiza symbioses. In another project, Regvar's group investigated the role that fungi play in the plant life cycle and how plants might control them (Pongrac *et al.*, *J. Chem. Ecol.* (2008), 34: 1038-1044). To do this, they determined the degree of colonisation by symbionts in different plant organs and during different phases of the year. At the same time, they measured the concentration of glucosinolates (GS) which are sulphur-containing secondary compounds known to deter pathogens. It turned out that the total GS concentrations and profiles of nine individual GS differed during life cycle stages, with the highest peak of general GS concentration in the rosette leaves and during the vegetative period. That is, when leaves are young and worth protecting. The interesting thing was that some of the GS peaks in the roots coincided with the highest peak of symbiont colonisation: glucotropaeolin reached its highest concentration and glucobrassicanapin its lowest.

"The presence and absence of individual glucosinolates in the roots is likely to affect formation of Arbuscular Mycorrhiza symbioses," assumes Regvar. "Some glucosinolates may inhibit this formation and some may contribute to it." This finding might be important when it comes to the inoculation of agricultural soils with fungi to strengthen the plants' metal resistance and nutrition. And even if it's far too early for manipulating GS concentrations in agricultural crops, it might be at least helpful to understand why some plants fail to form Arbuscular Mycorrhiza systems.

Phytoremediation in sight?

"Today the phytoremediation efforts are still limited; we actually know too little," says Regvar. The knowledge that she and the ecologist community have accumulated is still largely theoretical. It is almost certain that in heavily polluted areas plants might survive better with the help of Mycorrhiza. But inoculation with profitable microbes is far from being easily applicable since not every plant forms symbiosis and not every species of symbiotic Mycorrhiza fungus protects against toxic metals. Much more work needs to be done to understand the molecular and ecological relationships between fungi, the complex microsystems of the soil and plants. The same holds true for the use of hyperaccumulating plants as extractors of metal, but research funding is not easy to come by in a country of just 2 million people. Nethertheless, Regvar and her team persevere to stick at it.

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