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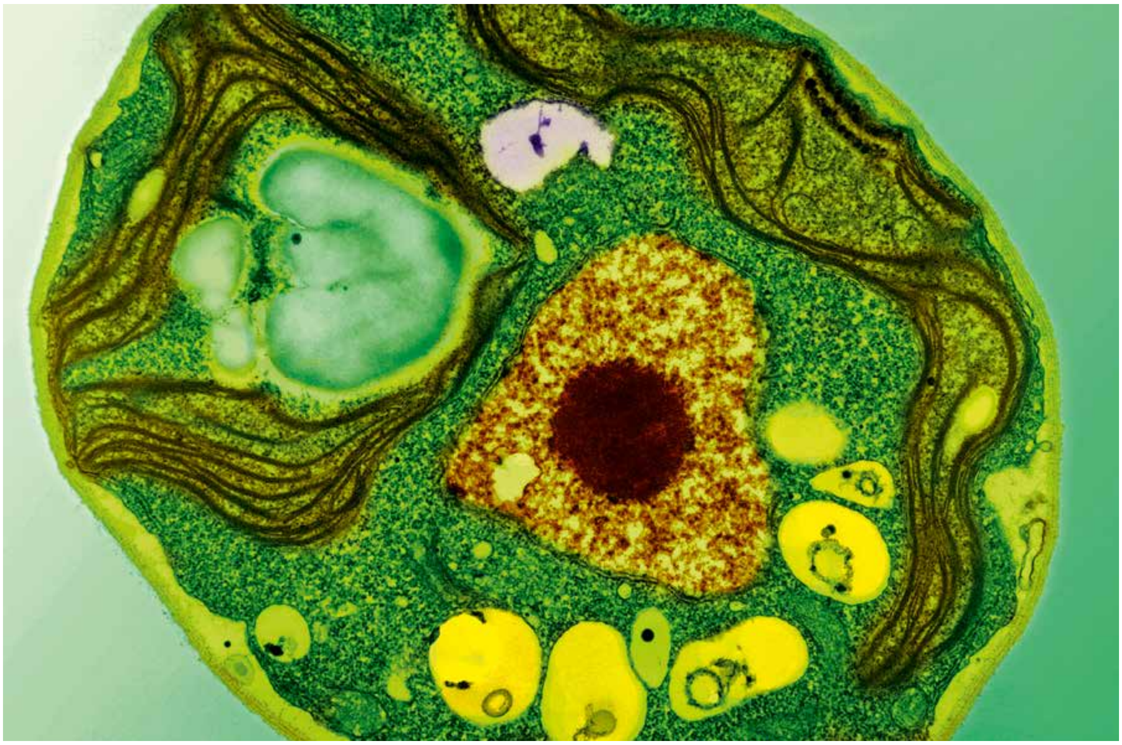
# FIELD FACTORIES

*TEXT: CATARINA PIETSCHMANN*

Factories of the future will be growing in fields – at least according to Ralph Bock and his team at the Max Planck Institute of Molecular Plant Physiology in Golm. The researchers are hoping to turn plants into production sites for substances that would otherwise be difficult and expensive to produce. One plant that has recently been somewhat scorned could experience an unexpected renaissance in pursuit of this goal.

A tobacco plantation in Italy. These plants could also be cultivated for the production of pigments or vaccines in the future.



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Electron microscope image of the alga *Chlamydomonas reinhardtii*. Researchers want to produce the pigment astaxanthin in algae such as these (center: nucleus, black: chloroplast).



Plants don't grow by fresh air and love alone. They can, however, produce an incredible variety of substances from comparatively few initial materials. Carbon dioxide from the air, sunlight, plus water and minerals – these are the ingredients from which plant cells produce carbohydrates in a process known as photosynthesis. These sugars form the basis for a vast range of carbonic substances such as cellulose, starch, fatty acids, amino acids, proteins, hormones, and vitamins in addition to various pigments, odoriferous substances and toxins. Plants produce all of these substances for their own use, but they also incidentally serve as food for humans and animals.

These "green factories" work extremely efficiently, sustainably and without producing any environmentally harmful waste. On the contrary, microorganisms decompose dead plant matter completely, and then reintroduce it into the natural cycles of material. All of these properties continue to make every chemist green with envy. But couldn't we put the potential of plants to even better use for humans?

This concept is known as "molecular farming." One particularly promising approach is based on targeted genetic modifications within the so-called chloroplasts, the organelles within the plant cells that are responsible for photosynthesis. Chloroplasts have their own genomes and are therefore able to produce some of the proteins used in photosynthesis themselves.

Ralph Bock is hoping that these changes to the genome will enable his plants to produce substances that are of importance to humans. The pigment astaxanthin, for example, is a substance produced by *Haematococcus pluvialis*, a unicellular marine alga, and is a powerful antioxidant. Fish and crustaceans absorb astaxanthin via their food and use it to form red-colored muscle tissue. In contrast, farmed salmon have to be fed extra pigment, otherwise their meat remains white and cannot be sold at such a high price. As a food supplement, astaxanthin is not cheap: it costs USD 15,000 per kilogram, because it has to be extracted from the

**"Making biofuels from rapeseed is both economic madness and a sin against the environment."**

*RALPH BOCK*

algae using a complex process. Bock and his team are relying on tobacco plants instead: “If,” says Bock, “we integrate the metabolic pathway for the production of astaxanthin into the plants, they’ll be able to produce it at a significantly lower cost.”

The quantity of astaxanthin produced by the leaves of genetically modified tobacco is visible at a glance from their orange hue. Viewed under the microscope, it can be seen that this is due to the chloroplasts which are closely packed within each plant cell: whereas regular tobacco leaves are green, due to the natural leaf pigment chlorophyll, the astaxanthin crystals color the leaves of the modified plants a bright orange. But why are the researchers using chloroplasts as bioreactors instead of the entire plant cell? “After years of intensive research,” Bock explains, “we now know that non-native genes are easier to activate within the chloroplasts. In addition, they are only inherited from the female plant so the risk of modified genes being blown to neighboring fields via pollen and transferred to non-modified plants is minimal.” Another major advantage is that each chloroplast contains up to 100 copies of a given gene; at about 100 chloroplasts per cell, this adds up to 10,000 copies. “So we can produce much larger quantities of a given substance in this way,” says Bock.

Tobacco plant with the astaxanthin genes (left): the pigment formed within the chloroplasts gives the leaves a reddish hue.

PHOTO: MAX PLANCK INSTITUTE OF MOLECULAR PLANT PHYSIOLOGY

Tobacco, which is already one of the model plants used in biology, is ideally suited to large-scale molecular farming; its genome can be altered more easily and quickly than that of other plants, for reasons that are still not understood. It also grows extremely quickly, which means that its leaves can be harvested several times a year.

There is also a social aspect involved: because less and less tobacco is needed for cigarettes, many tobacco farmers in the U.S. are struggling to survive. “Farmers in Kentucky and Virginia are very conservative,” Bock explains: “For them, the idea of switching to wheat, corn or potatoes is completely unthinkable.” So, existing tobacco acreage could continue to be used and the tobacco farmers would gain new opportunities for the future – a classic win-win scenario. But tobacco has another property that almost predestines it for use as a botanical bioreactor: its toxicity. The nicotine within the leaves is so toxic that just consuming a single leaf could kill a human being. “Anyone hoping to produce pharmaceutically active substances in a certain plant species naturally wants to avoid these substances entering the food chain at all costs.” says Bock: “The risk of this is negligible if a plant is inedible from the outset.” So, tobacco that contains astaxanthin is unsuitable as fish food for farmed salmon, because the nicotine would have to be removed from the tobacco extracts. A much better approach would be to equip an organism that can be added directly to fish food with the relevant metabolic pathway, which is why Bock’s team are relying on red and green algae. Both are cheaper and can be added to the fish food in desiccated form.

Artemisinin, an antimalarial drug, is one example of a medicinal substance derived from plants. It is derived from *Artemisia annua* (sweet wormwood) an annual short-day plant grown in China, Vietnam and some countries in southern Africa. However, the plant only contains 0.1 to 0.4 percent of the active ingredient in its dry mass. Even genetically modified plants developed by the researchers in Golm contain no more than that. Tobacco, on the other hand, produces much more biomass than the small wormwood plant. The greater yields would also increase the quantity of artemisinin per hectare that could be harvested many times over.

Tobacco is therefore the model that Ralph Bock uses to test out his ideas. The researchers must first discover which metabolic pathway could best be repurposed for the production of the desired substance and how much of a given molecular intermediary step the plant could live without. “We mustn’t interfere with the metabolic processes too much, or the plant won’t grow enough.”

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Scientists have collected vast amounts of data on the concentrations and activities of metabolic products over the years, so they know precisely what quantity of a given enzyme is required to enable the plant to produce a new substance. They therefore first have to couple the new genes with existing signaling structures, so-called promoters, which determine the extent to which the gene should be activated and transcribed. Next, they coat nanoparticles of gold with different variants of the relevant DNA segments and "shoot" them at pieces of a leaf using a kind of pressure gun. A small quantity of these particles get lodged inside plant cells, which can then read and transcribe the DNA to produce the desired proteins. The genetically modified leaf cells are then grown into complete plants in culture media.

Certain technical questions are also of central importance: is there a simple and inexpensive way of enriching the substance and, if so, could it be scaled up? Substance extraction can be lengthy and costly; the extent to which it is or is not worthwhile depends crucially on the intended use of the molecule. "If, for example, we can only isolate ten milligrams of a given substance from one kilogram of leaves at great expense," Bock explains, "then of course it is not suitable as a fish food supplement. The same quantity may however be sufficient for a highly effective cancer drug."

It is easiest if the desired molecule can be produced in an edible plant. "Tomatoes, for example, are ideal for health-promoting substances, such as vitamins, because you can eat them raw." Tomatoes are rich in the red pigment lycopene, the precursor to vitamin A. Vitamin A deficiency, which is prevalent in large parts of Africa and South Asia, impairs vision, increases susceptibility to infectious diseases and causes growth and fertility disorders. "Using a relatively simple enzymatic conversion process," Bock explains, "we can produce vitamin A from lycopene in tomatoes." Genetically modified plants could also be used to produce edible vaccines. To prevent a loss of efficacy, vaccines used for vaccination campaigns in the tropics usually have to be refrigerated for transportation and storage. But tomatoes, for example, could be cultivated directly on site and could also be stored for a certain period. Cereals, nuts or pumpkin seeds could also serve as "factories" and natural packaging for future vaccines.

A whole host of applications are conceivable. But the term "genetic engineering" still raises hackles among large sections of the population in many industrialized nations. Surveys suggest that 70 percent of Europeans are against so-called green genetic engineering, which involves the genetic modification of fruit, vegetables and cereals, yet 90 percent of soya products currently come from genetically modified plants. And another fact that most consumers are unaware of is that traditional breeding techniques often involve the use of mutagenic chemicals or radiation, which cause a large number of random mutations in the genome.

The situation is exactly the opposite when it comes to using transgenic plants to produce drugs: 70 percent of those surveyed think that these green genetic engineering applications are a good idea. Actually, this is absurd, as scientists are unanimous in their opinion that inserting or modifying genes to protect a crop against insect damage or to make it more resistant to drought is harmless. If, on the other hand, a new, highly potent active substance is produced in a plant, researchers first have to clarify a number of safety issues, because, under no circumstances must the substance be permitted to enter the food chain accidentally. "One would think," says Bock, "that would cause more concern. But this is the very point that the populace views least critically." In his opinion, these contradictory survey results reflect the priorities of Europeans. "Immanent health risks include stress, cancer, cardiovascular diseases and dementia and we need new drugs to tackle them. By contrast, we have no lack of food. Africans, of course, view things quite differently," Bock emphasizes.

It is currently virtually impossible to carry out field experiments with genetically modified plants in Germany. "We have almost given up on that entirely," says Bock. Whilst researchers can apply for the regulatory approval of the release of transgenic plants for research purposes, the relevant experiments are barely feasible in practice. "Although we were very candid about our experiments and invited public participation, activists cut fences at night and destroyed the plants." The experiment had to be repeated a year later under stringent surveillance. "That protected field experiment cost us EUR 30,000. After that, we decided only to perform release experiments when the expected knowledge gain would be so great that the expense would be justified." As a result, Bock and his team usually only study their


## SUMMARY

Researchers are transforming plants into "green factories" by inserting genes into the chloroplast genomes.

Plants can use the new genes to produce such things as vaccines and vitamins.

Because they can produce large quantities of any given substance, tobacco plants are particularly well suited to being used as "green factories." But other crops as well as unicellular algae can also be converted into "green factories" in bioreactors.

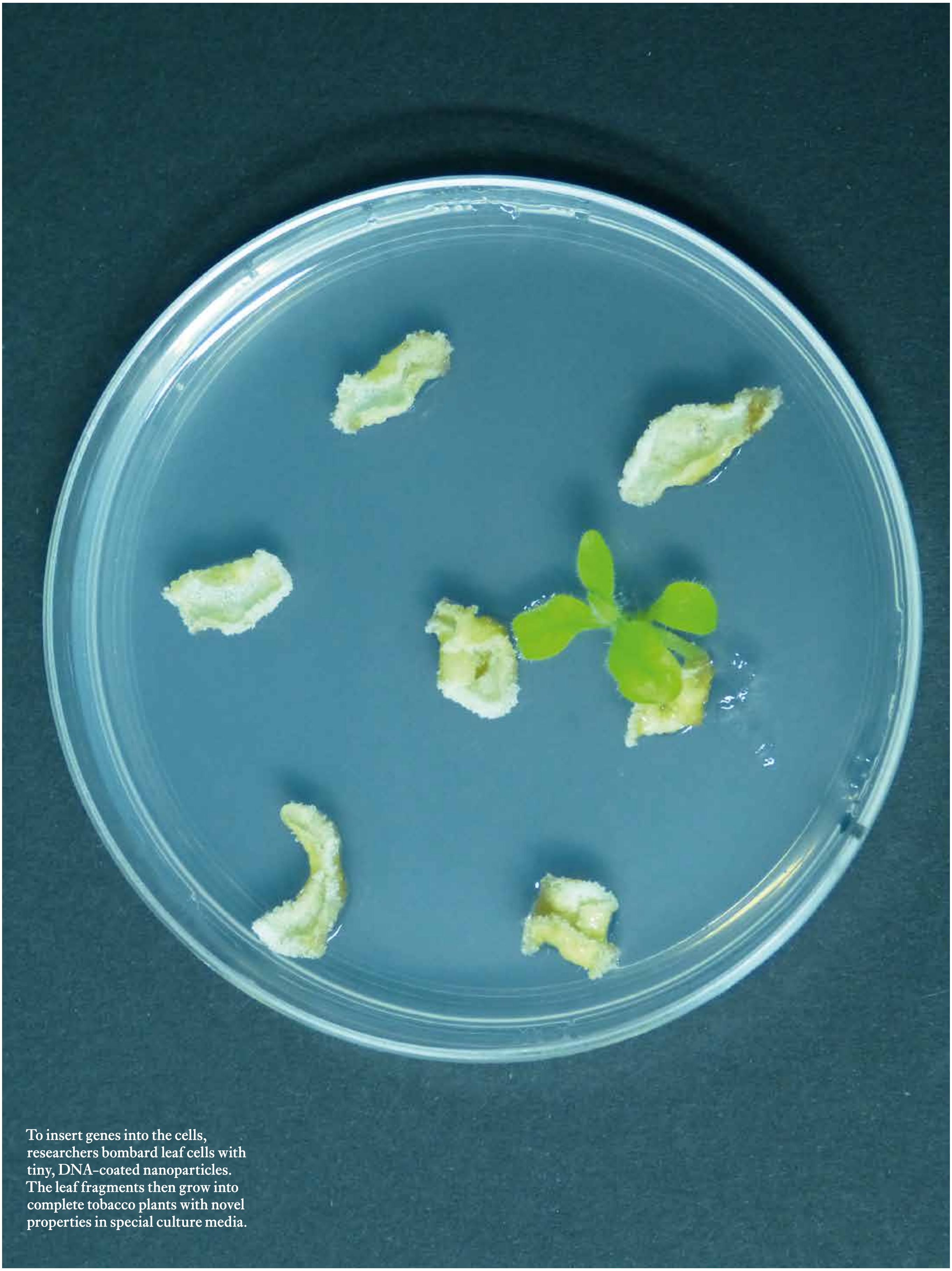

PHOTO: MAX PLANCK INSTITUTE OF MOLECULAR PLANT PHYSIOLOGY

To insert genes into the cells, researchers bombard leaf cells with tiny, DNA-coated nanoparticles. The leaf fragments then grow into complete tobacco plants with novel properties in special culture media.

plants in the greenhouse. Whenever the results look promising, researchers from both the Max Planck Institute of Molecular Plant Physiology and Knowledge Transfer of the Max Planck Society seek partners in industry to continue the development to market maturity.

Yet regardless of how environmentally friendly and sustainable molecular farming may sound, could it not suffer a similar fate to that of plant-based biofuel production? After all, the initial enthusiasm for energy crops quickly subsided once the world became aware of the consequences of immense cornfields and palm oil plantations, which included the replacement of species-rich meadows by monotonous arable land and tropical rainforest clearances. Whilst biofuels may be good for climate protection, they can have disastrous consequences in terms of biodiversity. "Bioenergy from the field" is something that even Ralph Bock is skeptical of. "For example, in contrast with tobacco plants containing active substances, only a fraction of the biomass of rapeseed can be used. Only a few liters of biodiesel are extracted from the seeds at huge expense and at the cost of severe environmental pollution. This is both economic madness and a sin against the environment."

There is another reason that the area required for molecular farming is significantly smaller than that of energy and fodder crops: "We've done the math on artemisinin," Bock explains: "We would need a cultivation area the size of the city of Boston to cover the global demand with our tobacco plants." Not a great deal, when one considers the fact that this could be used to treat the more than 200 million people who become infected with malaria every year. Transgenic plants are, therefore, an elegant and cost-effective alternative for the production of active pharmaceutical ingredients compared with traditional pharmaceutical production methods, which often still rely on the use of petroleum, particularly because it is even possible to produce several active substances in parallel in a single plant, for example as combination vaccines.

In one sense, molecular farming represents a return to the roots of medicine, as, for thousands of years, people have been treating their ailments with natural active agents. Medicinal plants have always been mankind's pharmacy, and could now become so once again, but with the benefit of 21st-century know-how.

*https://www.mpg.de/podcasts/biooekonomie (in German)*



In Ralph Bock's view, genetically modified tobacco plants offer a more sustainable way to produce substances in the future.

PHOTO: SEVENS+MALTRY, MAX PLANCK INSTITUTE OF MOLECULAR PLANT PHYSIOLOGY

## GLOSSARY

*CHLOROPLASTS*
... are the organelles responsible for photosynthesis in plant cells. Whereas some unicellular algae have just a single chloroplast, more advanced plant species can have several dozen of them per cell. With the aid of the leaf pigment chlorophyll, chloroplasts convert solar energy into chemical energy, which enables them to form carbohydrates from carbon dioxide and water. Chloroplasts evolved from microorganisms that were originally independent and therefore have their own distinct genomes. Their ring-shaped DNA molecule, which contains around 100 genes, is considerably smaller than the genetic material within the cell nucleus.