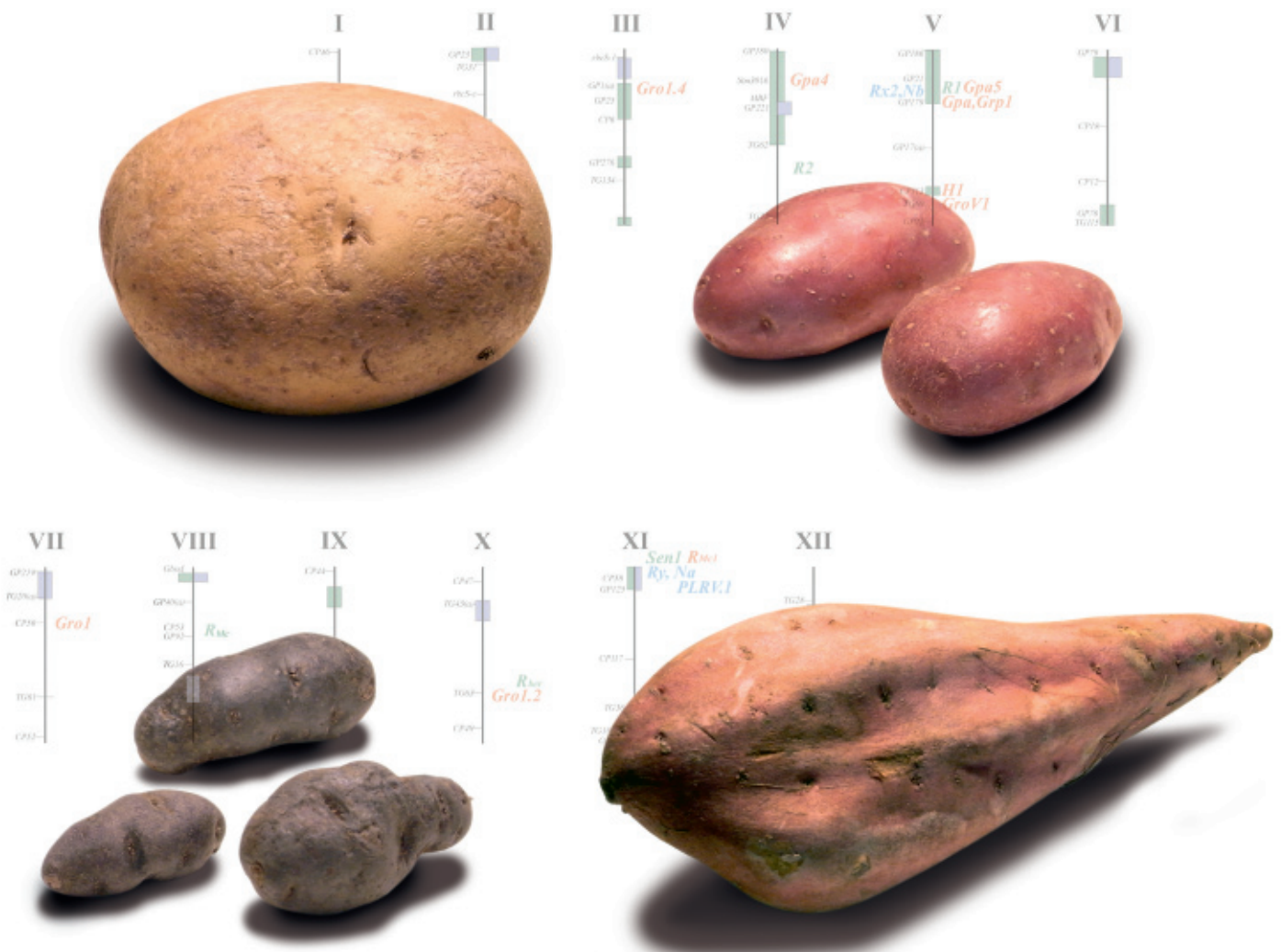


Potatoes à la Genetic Carte

The infestation of crop plants by pathogens can have devastating consequences for world nutrition. **Christiane Gebhardt** and her collaborators at the **Max Planck Institute for Plant Breeding Research** are searching for genes in the potato genome that will make it easier to breed potatoes with disease resistance and other quality traits.

TEXT **INGA RICHTER**



Although genetic engineering methods have scored several successes in the area of plant breeding, further research efforts are required to secure adequate food supply for the world's population: "In view of the increasing global population, it is essential that the production of crop plants become even more effective," explains Maarten Koornneef, Director at the Max Planck Institute for Plant Breeding Research in Cologne.

More than 6.7 billion people currently live on planet Earth, with an average of five people being added every second. To keep pace with this increase, global food production must expand. However, only around one fifth of the Earth's surface is available as potential agricultural land. In fact, due to the expansion of residential settlements and transport routes, the land available per capita for food production has been declining for years. It is thus crucial that we increase crop yields per unit of area.

VISIONS FOR THE FUTURE OF POTATO CULTIVATION

One future scenario on which scientists are working could look like this: All attention at the plant-breeding company SaKa Pflanzenzucht GbR in Windeby, Schleswig-Holstein, is focused on *Solanum tuberosum*, the potato. The latest success of variety breeding is called "Sternstunde" ("sidereal hour"). Not only does this new variety have a significantly higher nutrient content, but it is also resistant to the potato's main threat, the fungus *Phytophthora infestans*. *Phytophthora* causes the tu-

bers and above-ground parts of the plant to rot, which is why it is also known as foliage and tuber late blight.

Jens Lübeck, plant-breeding expert at SaKa, examines the harvest reports: the yield in 2020 was increased by 40 percent, and the use of pesticides significantly lowered. Whereas some 8 to 14 pesticide applications were still required just a few years ago, only two were needed this year. Working in collaboration with Christiane Gebhardt's Max Planck research team, Lübeck succeeded in tracking down important genes for resistance to foliage and tuber late blight and in propagating potato plants with these genes ... In reality, however, research still has quite a way to go.

The potato is the world's fourth most important agricultural crop after wheat, rice, and maize. Although it is by no means a cure-all for the world's nutrition problems, potato cultivation does contribute to the resolution of food supply problems in many countries throughout the world that suffer from widespread food shortages and malnutrition. Approximately 325 million tons of potatoes of different varieties are currently harvested annually. This is the result of centuries of selective breeding. The potatoes originally cultivated by the native peoples of South America were already highly developed, adapted to a wide variety of environments and different uses, and far removed from the highly toxic wild forms of the plant with tiny tubers.

The potato became the main food source in Europe in the 19th century because, in addition to the necessary calories, it is also a source of trace elements, vitamins and high-quality pro-

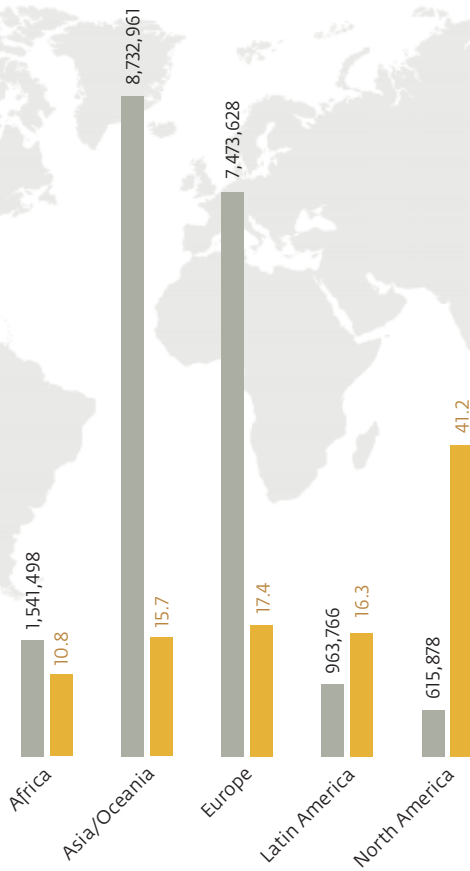
tein. Potato diseases introduced from America in the mid-19th century resulted in disastrous harvests. Millions of people died of starvation, particularly in Ireland, where dependence on the potato was very high. "Even today, without the use of a wide range of fungicides, the harvest loss due to foliage and tuber late blight can be as much as 100 percent," says Jens Lübeck. In Germany alone, this represents an annual cost to farmers of 88 million euros. Moreover, the chemical pesticides used to protect potatoes pollute the ground water and soil and have a negative impact on biodiversity.

MENDEL REVOLUTIONIZES PLANT BREEDING

Against this background, the successful breeding of high-yielding and simultaneously late-blight-resistant potato varieties, like the above-mentioned imaginary "Sternstunde," would represent real progress. The traditional method of plant breeding remains rather cumbersome, although it is no longer as drawn-out as it was around 10,000 years ago, when our ancestors started to settle and cultivate wild plants. Over time, people learned to separate tasty specimens from bitter-tasting ones, and high-yielding varieties from those that failed to yield, and they discovered that the further propagation of individuals with good traits gave better harvest results.

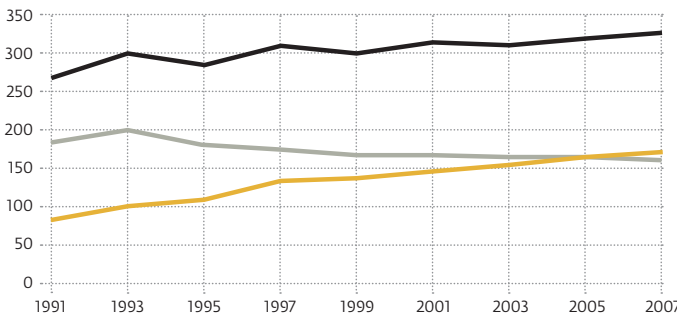
"Scientific plant breeding and, as a result, the increased yields achievable in agriculture, were first revolutionized by Mendel," reports Christiane Gebhardt. When Johann Gregor Mendel transferred the pollen of purple-flower-

POTATO CULTIVATION AREA & CROP YIELD



■ Cultivation area (hectares) ■ Crop yield (tons/hectare)

GLOBAL POTATO PRODUCTION (in million tons)



■ Developed countries ■ Developing countries ■ Global

ing pea plants to the stigmas of white-flowering pea plants in the gardens of the Abbey of St. Thomas in Brno, his experiments were focused on the external characteristics of the plants – the phenotypes.

Mendel, who would later be known as the father of genetics, knew nothing about the molecular secrets of the nucleic acids, their structure and effect. Nevertheless, he formulated the first rules about the function of the true masters of life, namely the genes, and thus provided a new impetus for breeding: the properties of many different plant individuals could finally be combined in a single offspring with the help of systematic cross-breeding.

INDIVIDUALITY: THE SMALL BUT SIGNIFICANT DIFFERENCE

However, the offspring that display the desired combination of their parents' traits still have to be selected over several breeding cycles. In the case of selection for resistance, this can be done by picking out the plants that have not been affected by pathogens in the greenhouse or the field – the trained eye can identify infected plants based on wilted foliage or dead parts. Other characteristics, such as nutrient content, are not as easy to select. Therefore, it would be ideal if the predisposition of the plant could be read directly from its genes – in other words, without the need for a detour via the phenotype. A large part of the time-consuming work involved in the selection process would then be avoided.

Genes determine the characteristics of a plant, such as whether it grows quickly or slowly, whether it is tolerant to drought periods or resistant to certain plant diseases, and which substances it contains. However, even after 30 years of plant genome research, genetic diversity does not give up its secrets readily. Of course, it has long been known that the cards of heredity are constantly reshuffled through the

novel combination of parental egg and sperm cells, and that there are different forms of all genes – the alleles.

However: “Potatoes have sex only once in a lifetime,” says Lübeck. “After that, reproduction takes place vegetatively through the tubers,” he explains. With clones of this kind, there is no new combination of the chromosomes. Why, then, do tubers of one variety contain a lot of starch while a sister clone produces less? And why is one clone more resistant to *Phytophthora* than another?

Gebhardt explains this phenomenon as follows: “The small differences between the individuals of one species are point mutations.” The scientific term for this is single nucleotide polymorphism (SNP). This involves individual random base substitutions that repeatedly arise when the DNA sequence is replicated, even when the organism reproduces asexually. “In the human genome, around one in 1,000 bases are changed; the corresponding figure for the potato is around one in 50,” explains the researcher.

MARKERS FOR THE SEARCH FOR SUPERIOR GENES

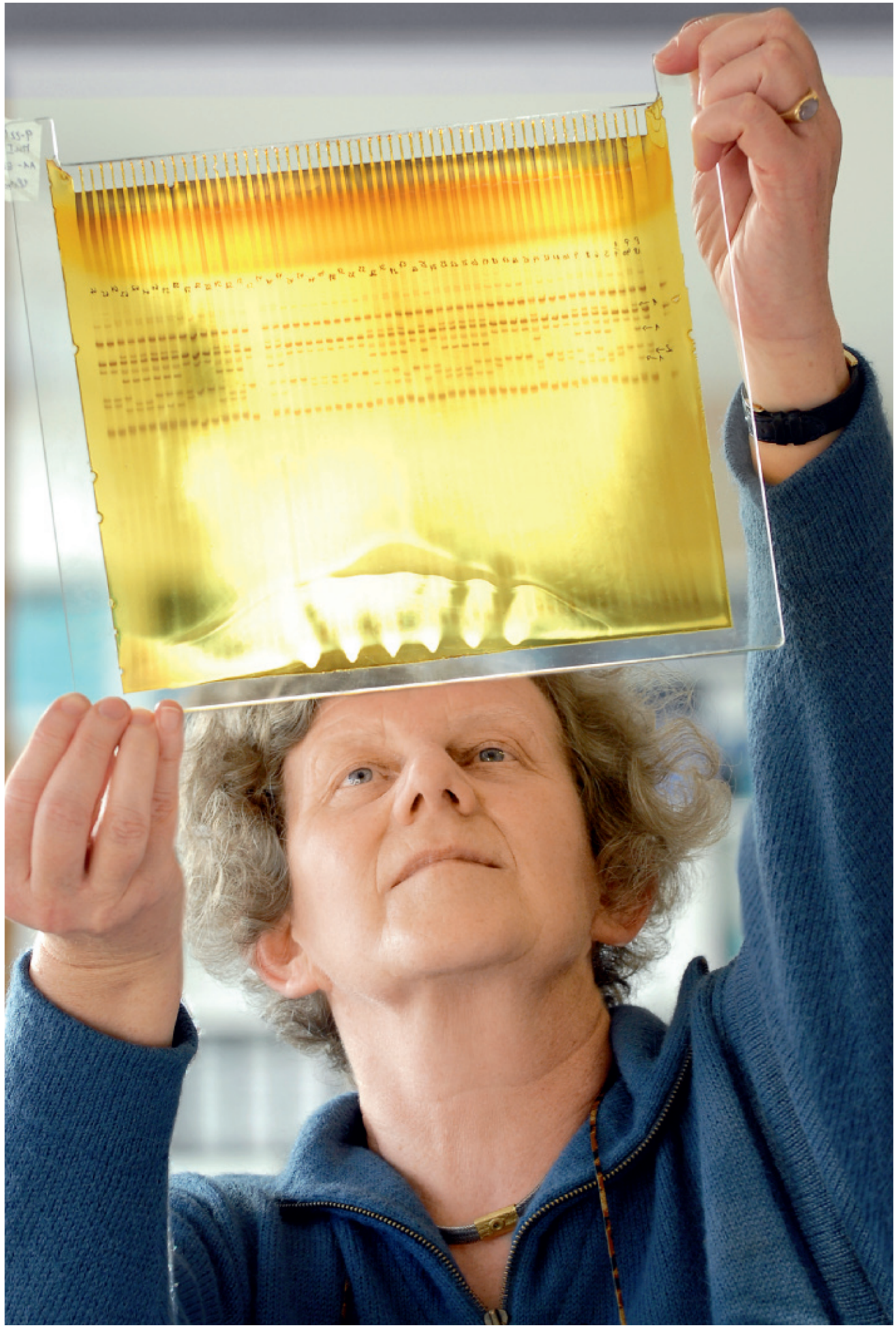
Although most of these mutations have no effect, some can trigger negative consequences while others promote positive characteristics. It is the search for the latter that is keeping people busy in Cologne. However, in view of the one billion base pairs and approximately 30,000 genes that exist in the potato genome, this is a very laborious search.

Gebhardt would like to generate the basis for marker-assisted selection (MAS) with the help of genetic analysis. MAS requires the development of diagnostic gene markers – artificially generated snippets of DNA that visualize or ‘mark’ specific sections of the genome. These DNA snippets are distributed across the plant’s genome like landmarks. Usually they do not cause any



above | Potatoes reproduce vegetatively through tubers.

below | The principle of marker-assisted breeding: The gene for tuber skin color has two versions (alleles); the tuber has red or white skin depending on the allele present. The numbers 1 and 0 symbolize a DNA marker that is linked to the gene for skin color: 1 stands for red skin color and 0 for white skin color. The diagram shows how the skin color can be predicted with a high degree of certainty with the help of the marker.



left	Christiane Gebhardt, head of a research group in Maarten Koorneef's department at the Max Planck Institute for Plant Breeding Research, examines the bands on a polyacrylamide gel.
right	The DNA isolated from a potato is multiplied using PCR (polymerase chain reaction) and cut into smaller fragments using so-called restriction enzymes.
bottom right	DNA samples from different potato varieties are prepared for gel electrophoresis.

phenotypic effect. What matters is that they are located near so-called quantitative trait loci (QTL) and can thus indicate the existence of certain genes.

PRECISION BREEDING SAVES TIME AND MONEY

With the help of the artificial DNA snippets, the plant genome can be scanned for certain gene variants, the presence or absence of which indicates a characteristic of the living individual examined. The purpose of the analysis of the plant genome is to bring suitable crossing partners together and to select the right offspring. The terms "precision breeding" or "marker-assisted breeding" are also used to describe this approach. The advantages of MAS arise from the fact that, theoretically, it is possible to establish at the seedling stage whether or not a plant has the desired characteristic. Consequently, the time-consuming process of field-based selection is avoided, costs are reduced, and the use of pesticides minimized – in theory, at least.

Much to the delight of the ketchup industry, this process can already be used to breed tomatoes with a higher sugar content, and a rice variety that is less sensitive to flooding has also been bred in this way. However, "more work needs to be done to enable precision breeding in the potato," says Lübeck. Potato genetics is far more complex. While tomatoes and rice have only two sets of chromosomes and one or two variants (alleles) per gene, the potato has four sets of chromosomes with as many as four different alleles per gene. Moreover, only in some cases are the desired characteristics manifested by

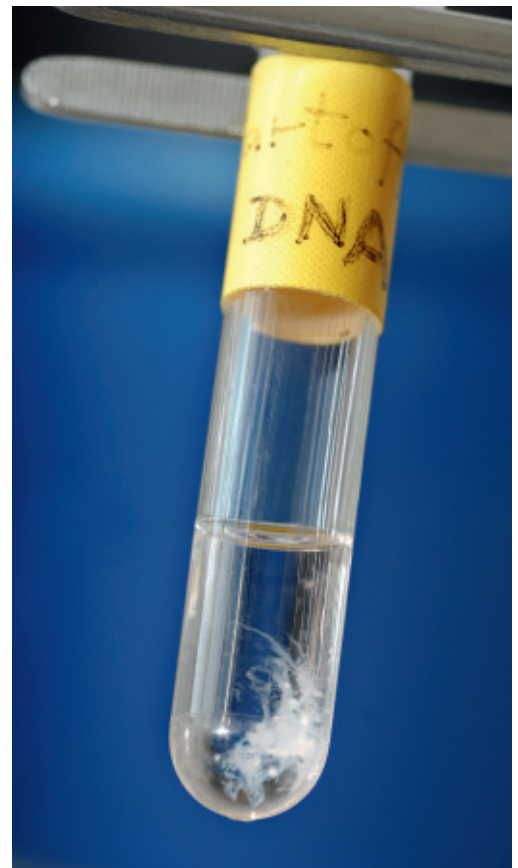
one gene alone. "Over 50 genes can determine a resistance character that we then describe as a quantitative trait," says Gebhardt. It would be fantastic to know the ten most important of these.

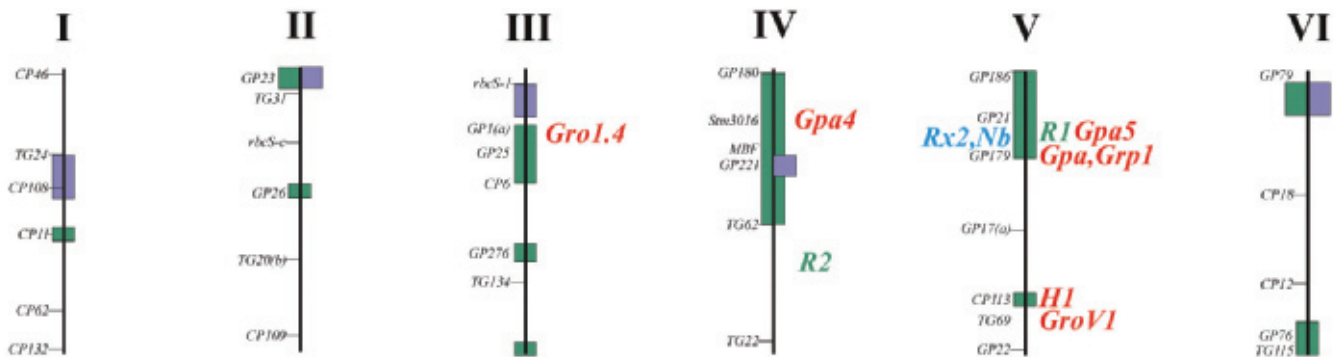
The molecular biologist points to a map of Germany that extends across the wall in the hall of the institute. "We are constructing new maps," says Gebhardt. The Earth has already been fully surveyed, and next up for surveying are genomes. Schematic diagrams of the twelve potato chromosomes, long rods on which green or blue bars are indicated, hang in her office: these are QTLs that have already been identified. They indicate DNA regions that contain genes for which, in previous studies, it was possible to demonstrate a quantifiable influence on the expression of a phenotypic characteristic.

Thin arrows and combinations of letters and numbers in various colors surround the schematic chromosomes. For example, GPA5 is shown in red, R1 in green and GP179 in black. "The black labels indicate DNA markers without any particular biological significance," explains Gebhardt. However, they are still part of the basic framework of the genetic map – like the lines of latitude and longitude on a world map.

GENETIC MAPS FOR AN EXPEDITION IN THE GENOME

It is possible to create a genetic map for each potato chromosome. The markers are lined up in a row like pearls on a string, and the traits must be localized between them. For example, gene locations that play a role in resistance to nematodes are shown in red, and those involved in resistance to fungi, in green.





above: The scientists have already identified various areas on the twelve potato chromosomes that, for example, play a role in resistance to certain pathogens.

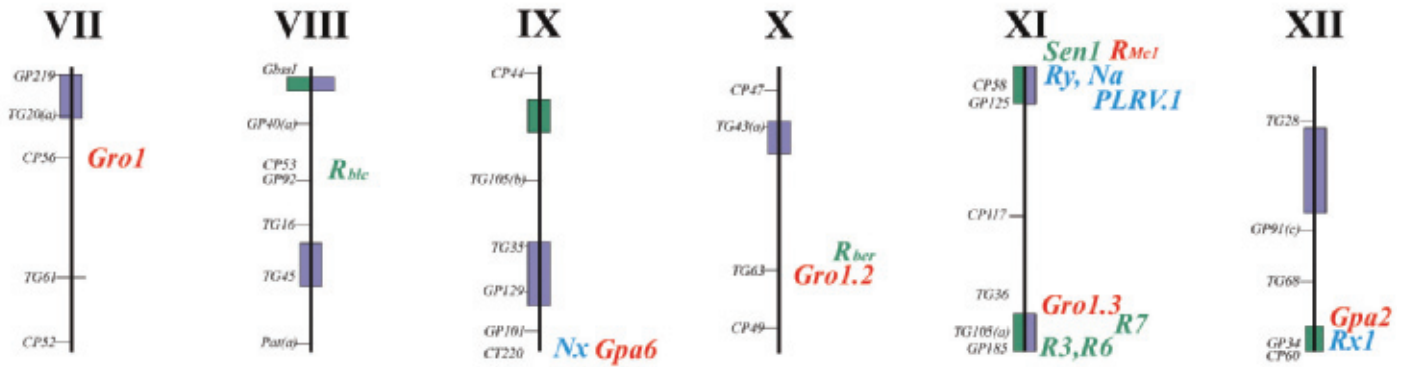
left: Researchers check on the computer the varying distances that DNA fragments have migrated during gel electrophoresis.

The subsequent grading of the plants is based on a scale of one to nine. Quantitative characters must be recorded in several intermediate stages – phenotypic characters are rarely as purebred or monogenic as the flower color in Mendel’s pea plants. The lowest grade is 1, which indicates that the corresponding plant is highly vulnerable to *Phytophthora*.

The freeze-dried leaves travel 532 kilometers through Germany and are processed in the laboratory in Cologne. Sections of the isolated DNA are multiplied using the PCR method (polymerase chain reaction), a kind of biochemical photocopying process. Restriction enzymes are then used to cut the DNA at specific short nucleotide sequences – or not, if an SNP, a single base substitution, is present in this recognition sequence. This causes certain DNA fragments in this individual to become longer. In the gel electrophoresis that follows, longer fragments – driven by the electric field – migrate more slowly than the shorter ones. Considering a marker for resistance, for example, a DNA fragment that is unfavorable for resistance moves faster than a favorable one.

In order to be able to establish a link between such genetic maps and plants in the field, the Cologne-based research group receives sample material from plant breeding companies, such as SaKa Pflanzenzucht GbR in Windeby.

An experimental population comprising an average of 200 individuals is planted there in the field in spring. The researchers cut a fresh leaf from each of the numbered plants for DNA isolation. The plants are then further monitored to see whether infection with diseases such as *Phytophthora* arises. Otherwise, artificial infection is provided to enable an assessment of the phenotypic effects of the pathogen on each individual plant over a few weeks.



Whether a marker is favorable or not can be established only through comparison with the breeders' documentation on the corresponding test plants. The breeders examine the quality of the test plants and their tubers. Such phenotypic tests must be carried out frequently, in different places at different times, and over several years. This is because the effect of genes can be defined only in reaction to a specific environment.

FINANCIAL INPUT FOR MORE COOPERATION

The evaluation of the genotype and phenotype is accompanied by statistical calculations that allow a link to be established between phenotypic characters and DNA sections. Thousands of bands are thus tested by association analysis to determine whether they correlate with certain characters. In successful cases, the plants whose laboratory analyses withstand the phenotypic and statistical comparisons will be propagated. It is then a matter of waiting to see whether the expectations are fulfilled.

"Breeding is a really slow process," says Lübeck. A breeding cycle for just one potato variety takes around ten years to complete. MAS could accelerate this process. Whereas few diagnostic markers were previously known that could be used to identify monogenic characters, the German genome research program GABI (Genome Analysis in the Biological System of Plants) has been providing strong support in this field over the past eight years. The projects

funded by the Federal Ministry of Education and Research (BMBF) facilitate cooperation between research centers, such as the Cologne-based Max Planck Institute, and plant breeding companies, and have thus resulted in the identification of the first diagnostic markers for polygenic characters of the potato.

"One of the biggest successes for the economically relevant breeding programs thus far is a molecular marker that diagnoses resistance to the nematode *Globodera pallida*," says Lübeck. The cysts of this threadworm can survive in the soil for up to 20 years. If potatoes are planted in an infected area, the larvae penetrate the roots, rob them of their nutrients, and cause the plant to starve.

INITIAL SUCCESSES IN THE FIGHT AGAINST PREDATORS

Other successes can also be reported in the fight against the potato's numerous enemies: "We have now discovered a first gene for field resistance to foliage and tuber late blight," explains Gebhardt. The gene is named *StAOS2* (*Solanum tuberosum allene oxide synthase 2*). The scientist speculates that the potato genome will be fully decoded in approximately five years' time. This will be a huge help in the search for other genes. Jens Lübeck assumes that other markers for resistance genes will also be found soon. He is optimistic when he looks at the genetic map in Christiane Gebhardt's office. "Potatoes à la genetic carte" could thus be a real prospect in the not-too-distant future. ◀

GLOSSARY

Phenotype/phenotypic expression

Externally recognizable characteristic of an organism that is influenced by genes and environment.

Genotype

The individual set of genes an organism possesses.

Marker

Generally: a biochemical tool for identifying certain characters; here, specifically: a DNA fragment that corresponds to a known and precisely localized sequence in the genome and thus helps in the search for genes.

MAS

Abbreviation for marker-assisted selection: the search for breeding-relevant characters with the help of gene markers.

Quantitative trait locus mapping

A method for finding the locations of genes that influence a character.