Genetic Evolution

New Breeding Techniques, or NBTs for short, could be the future of apple breeding: a silver bullet against new pathogens and the climate crisis. Our *dossier* explains what NBTs are all about and why they are the subject of tough negotiations in the European Commission.

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AUDIO STORY

The research community sees *New Breeding Techniques* as a promising third option alongside time-consuming conventional breeding, which can take decades, and the sometimes controversial classic genetic engineering methods that involve inserting foreign genes into plants.

“NBTs bring about changes that happen in nature – only faster and targeted better.”

Dr. Thomas Letschka, *head of the Breeding Genomics working group at the Laimburg Research Centre*

Trial and error is the principle of evolution. Whatever threats await animals and plants, nature usually finds a way. But this takes time – a lot of time. Through the mixing of parent species and natural mutations, offspring are produced that are all slightly different from one another. Those with an advantage in the face of current threats will prevail over the others. This also applies in agriculture, but only to a limited extent: after all, what use is an apple that is resistant to scab but has a mealy texture and not much flavor? It is still rejected – by humans.

In apple growing, humans have always gently influenced fruit development. The apples that succeed are the juicy, flavorsome ones that appeal to buyers. “So consumer taste has – quite naturally – become an important selection characteristic. And that’s a good thing: after all, the entire apple industry is based on it,” says Dr. Thomas Letschka, head of the Institute of Agricultural Chemistry and Food Quality and head of the Breeding Genomics working group at the Laimburg Research Centre near Bolzano. But conventional breeding is a complex, protracted process. For example, a Gala apple may be painstakingly crossed with a variety that is resistant to the bacterium *Erwinia amylovora*, which causes the dreaded fire blight. Only a fraction of the offspring will then have a degree of resilience against the disease. But the ones that do may have lost their great Gala flavor. And that only comes to light after five or six years of cultivation. Back to the drawing board!

Apple breeders who start at a young age have often retired by the time a new and acceptable variety has been created. Breeding is not a matter of years but often of many decades. That’s because the way the genes mix when two apple varieties are crossed is purely random.

The 1980s saw the introduction of technical methods that made breeding a little more targeted. But only a little, and that’s the problem. These conventional genetic engineering methods – the best known of which is transgenesis, often used nowadays as a synonym for all conventional genetic engineering methods – involve the delivery of foreign genes into a plant. The plant then becomes a transgenic plant: a genetically modified organism, or GMO for short.

Exactly where in the plant’s genome the new genes are inserted, however, is again largely random. Sometimes the gene comes from a completely different species. A gene from a frog could be inserted into a tomato to make it less mushy, for example. But even if we succeed in improving the tomato, we don’t know what else in it changes because the insertion is too imprecise. “Consumers are uncomfortable with this, of course. So it’s

Glossary

Transgenesis:
Conventional genetic engineering method. Foreign genes are transferred from one organism to another, e.g., to a plant. Today, the term is often used as a synonym for all conventional genetic engineering methods. Transgenically modified plant species authorized for cultivation so far include soy, maize, cotton, and rapeseed. This method is often rejected by consumers, especially in Europe, where transgenic plants – genetically modified organisms, or GMOs for short – must be labeled as such.

New Breeding Techniques (NBTs):
New methods of genetic engineering. The best known is CRISPR/Cas, which involves intervening in specific parts of DNA in exactly the same way as spontaneous mutations that occur in nature. This distinguishes NBTs from conventional genetic engineering methods (transgenesis). The EU is therefore currently leaning toward not equating NBTs with conventional GMO methods.

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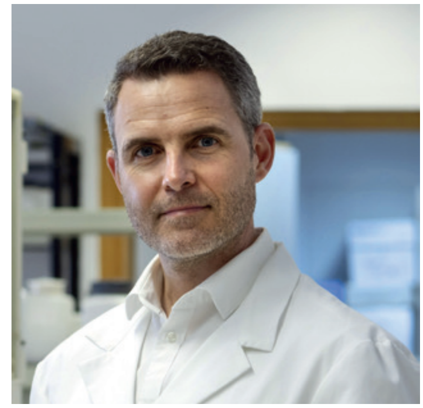

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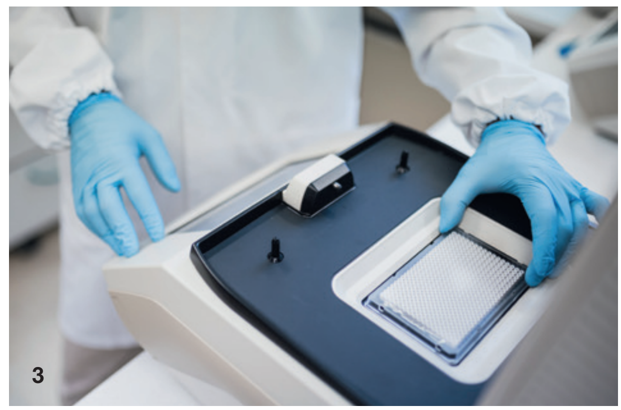

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no surprise that these crossings are often referred to as 'Frankenfoods'," Letschka notes. On top of that, the authorization process is highly complex; which is one of the reasons why you still don't find apples on sale in Europe that have been modified using conventional genetic methods.

So, back to traditional time-consuming breeding, then? In fact, there are other options on the horizon. A third approach – New Breeding Techniques, or NBTs for short – is seen as promising by researchers and many breeders. "There is a clear dividing line between these new techniques and conventional genetic engineering methods," explains Letschka. "Basically, the aim is to quickly bring about very precise changes in the genome that take decades to achieve naturally through traditional breeding." NBTs therefore still involve intervening in the apple genome, but instead of introducing foreign genes, you are only changing as much as nature itself would. "And we can control this very precisely, which is one of the main differences between these methods and conventional genetic engineering. We can make minimal changes to a very specific gene at the exact spot where the best effect is achieved," says Letschka.

The most common method used for NBTs is CRISPR/Cas. CRISPR/Cas is an enzyme produced by a bacterium and is often referred to as genetic scissors, because it can cut very precisely – down to DNA building block level – and can therefore make highly selective changes. The potential of genetic

1 The most common method in NBTs uses so-called *CRISPR/Cas genetic scissors*: an enzyme produced by a bacterium that can cut DNA building blocks with great precision.

2 "With NBTs, we can make minimal, highly targeted changes to specific genes," explains *Thomas Letschka*. An intervention in the genome that doesn't involve inserting foreign genes.

3 Discussions are currently underway in the *European Commission* as to whether NBT-bred apples should be classified as conventionally bred apples, as the risks are said to be just as low.

1 The EU is discussing *compulsory labeling* for NBT-modified plants – like with classic genetic engineering.

2+3 It is impossible to establish retrospectively whether plants have been modified using CRISPR/Cas or by natural mutation. This makes *tracing and checking* difficult.

4 In Zurich, *Giovanni Broggini* is researching a specific gene that makes Gala apples more resistant to fire blight.

“We are attempting to turn off genes that make apples susceptible to disease.”

Dr. Giovanni Broggini, *researcher in molecular plant breeding at ETH Zurich*

scissors was first described in the journal *Science* in 2012, earning their discoverers Emmanuelle Charpentier and Jennifer A. Doudna the Nobel Prize in Chemistry in 2020. In medicine, scientists are currently researching new therapies using CRISPR/Cas. And in agriculture, the hope is that this will significantly speed up breeding, revolutionizing it in the process.

In Switzerland, chemist Dr. Giovanni Broggini is running greenhouse trials to improve the disease resistance of well-known apple varieties. The researcher in molecular plant breeding in the Department of Environmental System Science at ETH Zurich wants to introduce a special gene into Gala apples to improve their resistance to fire blight. “And in other experiments we don’t introduce a resistance gene at all, but instead switch off specific genes – the ones that make an apple susceptible to certain diseases or pathogens. When we switch them off, the apple is less susceptible,” Broggini says.

Is that still natural? An apple modified with the CRISPR/Cas genetic scissors still goes through a laboratory process. To cut the apple genome using CRISPR/Cas, DNA from the bacterium that produces the genetic scissors must be introduced. This DNA ensures that CRISPR/Cas is produced in the apple plant and can then function. Afterwards, the elements containing the genes for CRISPR/Cas are cut out again. But these intermediate steps could be skipped in the future: Broggini is already investigating how to produce CRISPR/Cas artificially in the lab and introduce it directly into the plant cell, ensuring that the intervention only targets the specific location in the apple genome.

However, it is not yet possible to determine whether plants have been selectively edited with CRISPR/Cas. “It is impossible to establish retrospectively whether the change was the result of a natural mutation or intervention using CRISPR/Cas,” Letschka declares. “This makes tracing and checking difficult. But it does highlight the fact that we can target the kinds of changes that can also occur naturally through mutations.”

So, is CRISPR/Cas a step forward in apple breeding? Some of the most popular varieties like Gala and Golden Delicious are susceptible to pests. CRISPR/Cas could be used to increase their resistance, as Broggini is currently trialing in a greenhouse in Zurich. However, no-one in Europe has yet applied for authorization for an apple that has been improved using CRISPR/Cas. That is because in the EU, apples bred with NBTs are currently treated in the same way as GMO

Glossary

Genome Editing:
Generic term for all technological methods used to edit the genome without adding foreign genes. These include NBTs such as CRISPR/Cas.

CRISPR/Cas:
An enzyme produced by bacteria that can make cuts at specific points in DNA, also known as “genetic scissors.” It can be used either to switch off individual genes such as a susceptibility gene that makes plants more susceptible to certain diseases, or to insert specific genes such as a gene from a related apple variety to make the apple more resistant to certain fungi without changing it significantly in any other way.

Cisgenesis:
If a plant is cisgenically modified, only genes from plants of the same species are inserted, either using conventional genetic engineering methods or NBTs. They must come from a biologically compatible species, e.g., from a wild apple whose gene is transferred into a variety of the domestic apple.
If an apple receives a gene from a tomato, it is no longer a cisgenic plant.

NBTs: Facts & Figures

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FIELD TRIALS WITH GENETICALLY MODIFIED PLANTS IN THE EU (2023)

742,000,000

BASE PAIRS, THE APPROXIMATE LENGTH OF THE GENOME OF A CULTIVATED APPLE. IT CONTAINS ABOUT 42,000 GENES.

50 %

REDUCTION IN PESTICIDE USE BY 2030 TARGETED BY THE EUROPEAN COMMISSION. NBTs ARE EXPECTED TO INCREASE PLANT RESISTANCE.

300,000

APPLE AND PEAR TREES CLEARED IN SWITZERLAND (2000–2014) DUE TO FIRE BLIGHT. NBTs ARE BEING USED TO RESEARCH RESISTANCE GENES.

17 %

DROP IN REVENUES DUE TO CLIMATE CHANGE BY 2050

45

APPLE PROPERTIES CAN BE CHANGED THROUGH BREEDING

97 %

OF CRISPR/CAS PROJECTS DO NOT INVOLVE FOREIGN DNA

90 %

OF CRISPR/CAS APPLICATIONS ARE KNOCK-OUT PLANTS (GENES ARE SWITCHED OFF)

7.5 million

PEOPLE IN GERMANY ARE ALLERGIC TO APPLES. NBTs COULD HELP REMEDY THIS.

Foods bred with NBTs are already widely authorized outside Europe.

apples. And this puts breeders off, given the enormously complex and costly testing and studies needed to obtain authorization.

But that is about to change. The European Commission is currently deliberating whether apples bred using NBTs should be classified as normal apples bred without genetic intervention. As NBTs can be equated to a natural breeding process, the risks are equally as low, the argument goes. Therefore, additional genetic engineering legislation is being drawn up that would treat foods edited with CRISPR/Cas in almost the same way as conventionally bred foods. They would then face much lower authorization hurdles than GMO foods.

"If this legislation is actually implemented across the EU, this could be a breakthrough for NBTs in Europe," Letschka remarks. The technology is already widely authorized outside Europe.

The fact that the normally strict European Commission, which follows the precautionary principle, is seeking a more lenient approach to NBTs lies in the goals it has set itself. One of these is to halve the use of chemical pesticides through the European Green Deal. NBTs could play a key role in this: a more resistant apple variety needs fewer pesticides.

But there are also critics. "A comprehensive risk assessment must be undertaken before CRISPR/Cas is authorized. And in the current drafts of the EU legislation, this isn't provided for: it's simply skipped. That is irresponsible," warns Jan Plagge, president of the organic grower's organization Bioland.

Another hotly debated topic is compulsory labeling. If plants bred using NBTs had to be labeled, this would push up costs, and could potentially result in consumers rejecting the new method. On the other hand, proponents argue that consumers must have the freedom to choose not to buy apples bred with NBTs. "I expect a decision on the treatment of NBTs and whether labeling will be made compulsory in 2025," says Letschka.

How a simplified authorization procedure for NBTs would change apple breeding is difficult to say. The first marketing authorizations have already been granted in other parts of the world. In Japan, there is a tomato that contains healthy amino acids, a functional food designed with CRISPR/Cas. And in the United States, the first apple modified with CRISPR/Cas to prevent browning after cutting is already on the market under the Arctic® brand (see next page).

But for now, the main issue for the apple industry is to make apples more resistant to pests. Some of these traits can be achieved with individual gene edits. This option would increase the competitiveness of the European apple industry. But whether apples treated with CRISPR/Cas will really catch on is largely down to the consumer. CH

Glossary

Precautionary principle:
This principle sets the direction for legislation on genetic engineering in Europe. It not only looks at the end product but also at the processes that led to the creation of the product.

Principle of substantial equivalence:
Unlike the precautionary principle, this principle primarily focuses on the end product, the processes that led to its creation being inconsequential. This principle assumes that a newly developed food is just as safe as an existing one if it has the same composition. The principle is widely applied in places such as North and South America.