TURBO-CHARGED SEEDS

TEXT: ANDREAS LORENZ-MEYER

When different varieties of one plant species are crossed with each other, their hybrid offspring are often more robust and grow more quickly than their parents. However, in the next generation this effect disappears. New methods make it possible to preserve the advantageous qualities of these kinds of hybrid plants for the long term and to deliberately design plants with four sets of chromosomes rather than two. The techniques should make it easier to breed particularly high-yielding and resistant crops that could feed a growing global population even in times of climate crisis.

As far back as 1759, over a hundred years before the Austrian Augustinian monk Gregor Johann Mendel published his work on inheritance in peas, scientists were already pondering the question of how plants pass on their traits to their offspring. It was during that year in St. Petersburg that a competition was held by the Russian Academy of Sciences. The task set was to prove that plants also possess sexuality. The winner was Joseph Gottlieb Kölreuter, the son of a pharmacist from Sulz am Neckar. Kölreuter, who later became a professor of natural history in Karlsruhe, had crossed two inbred tobacco plants and, in doing so, noticed that traits specific to each parent were present in the next generation after crossing. He concluded that they were passed on from the parent plants in equal proportions. In addition, the first-generation plants from the cross looked the same – a finding that Mendel also formulated in his principle of uniformity. But the botanist noticed something else too: the offspring thrived better than their parents.

Thus, more than 250 years ago, Kölreuter discovered the "heterosis effect", otherwise known as hybrid vigor. This occurs when first generation hybrids – that stem from a deliberate crossing of two inbred varieties of the same or closely related species – are superior to their parents in terms of vitality and growth. How this phenomenon comes about has not yet been conclusively clarified. Still, modern agriculture has this effect to thank for the cultivation of high-performing hybrid varieties of maize, rapeseed, rice, rye, and many other crops.

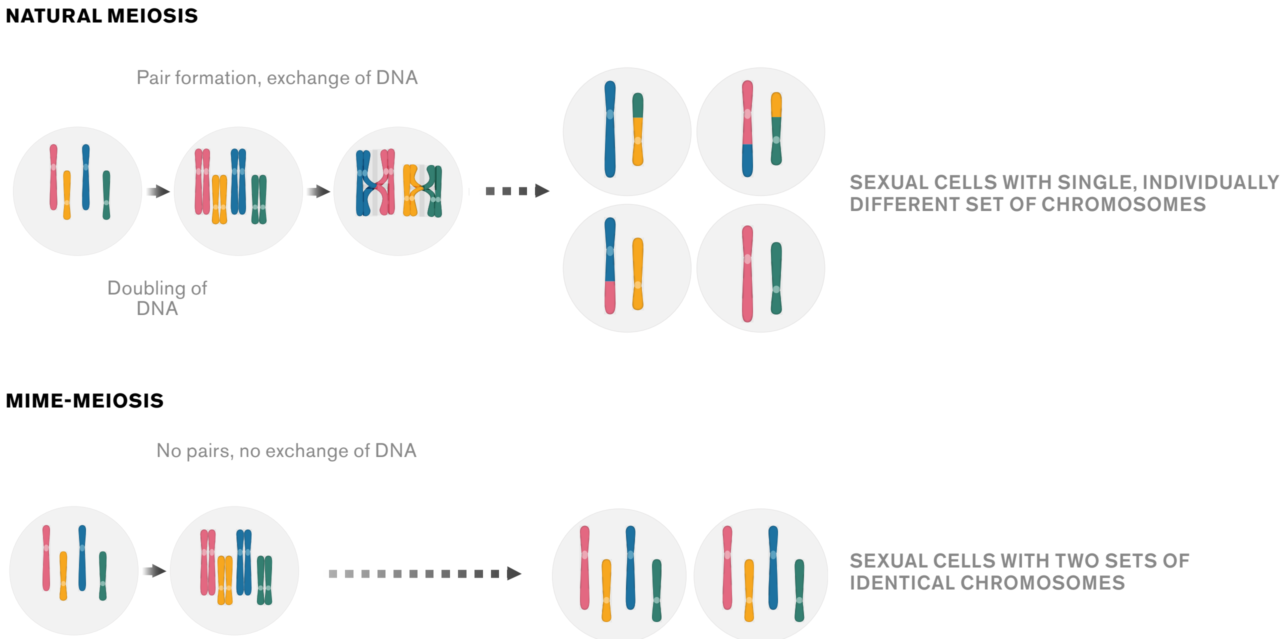
Short-lived effect

Outwardly, hybrid crop varieties grow quicker and are more robust to abiotic and biotic stresses than their inbred relatives. Hybrid maize, for example, produces 30 percent greater yields. But there's a problem: the heterosis effect doesn't last. The yield increase achieved by the cross in the first generation of offspring is lost by the second. The plants also lose their outward uniformity. The reason for this is the processes involved in sexual reproduction: during the meiotic cell division necessary for the formation of germ cells – i.e., egg cells and pollen – the genetic material of the germ cells is mixed up – or recombined – so in the next generation none of the plants are exactly like their parents. In this way, meiosis enhances genetic diversity in plants and animals. If, however, the hybrids could be propagated asexually, i.e., cloned, through seeds they could pass on their complete genetic material and thus their advantageous traits to the next generation. This would massively reduce the costs associated with hybrid seed production and could lead to the development of many more hybrid varieties than are currently available.

Raphaël Mercier, Head of the Department of Chromosome Biology at the Max Planck Institute for Plant Breeding Research, and Charles Underwood, Research Group Leader in Mercier's department, are aiming to make that possible. The two scientists present their progress in the production of hybrid seeds in the Institute's greenhouses, in which, in addition to the thale cress – an inconspicuous wild herb on which the scientists have gained fundamental insights – barley, potatoes, and tomatoes grow. To do so, they must ensure two preconditions are met: first, the entire genetic material of the mother plant must be preserved in the female gamete, which is only possible if the meiotic cell division, during which the genes are mixed up, does not take place as usual and a clonal egg cell is produced. Next, the new plant must develop from the clonal egg cell without fertilization by a male gamete, because, without meiosis, the number of chromosomes is not halved. So, if a pollen cell were to fertilize this kind of egg cell, it would then have too many chromosomes. "We need to overcome two obstacles: meiosis and fertilization. Only in this way can we produce seeds that are

Shoot of a tomato plant in a culture medium. Thanks to the growth factors it contains, just a small piece of tissue can develop into a complete plant several meters high.

PHOTO: FRANK VINKEN FOR MPG



GRAPHIC: GCO BASED ON DATA FROM CHARLES UNDERWOOD / BIORENDER.COM

Formation of gametes in plants: in the meiotic cell, pairs of chromosomes of the same type form and exchange DNA segments with each other. The pairs then arrange themselves in one plane, and the previously duplicated genetic material separates in an orthogonal direction. This results in four cells with a single, individually different set of chromosomes. In the MiMe process, sexual cells are formed without meiosis, and there is no exchange of DNA segments between chromosomes. This produces two cells that each have two sets of chromosomes identical to that of the initial meiotic cell.

genetically identical to one another and to the mother plant. With this kind of clonal hybrid seed, the hybrid state can thus be extended almost indefinitely," explains Mercier.

Mercier began his investigations in 2009 at the INRA Jean-Pierre Bourgin Institute Versailles-Saclay in France. "It is still my goal to find out which genes are involved in the meiotic cell division and development of egg and pollen cells. On a fundamental level, I want to know how these processes work." In the thale cress, he identified three genes that control important processes for meiosis and differentiate meiosis from mitosis, a process in which a cell divides into two identical daughter cells. When Mercier deactivated these three genes simultaneously, meiosis reverted to mitosis, and the plants formed clonal germ cells. The genetic material of the egg cells and the number of chromosomes were therefore identical to those of the mother plant. Through this experimentation, Mercier discovered a process that bypassed meiosis.

Rice from cloned seeds

In 2016, Mercier and Emmanuel Guiderdoni from the French Agricultural Research Centre for International Development (Cirad) applied the process, dubbed MiMe (mitosis instead of meiosis), to rice and thus for the first time to a crop. Alongside maize and wheat, rice is one of the most important cereal crops worldwide and is a staple food for 90 percent of the world's population. The three genes have been conserved during evolution and control meiosis in both thale cress and rice. It emerged that, without these genes, in the rice too an egg cell formed that was genetically identical to the mother plant.

In 2019 Mercier and Venkatesan Sundaresan from the University of California Davis, tackled the second obstacle: through activation of the BBM1 gene in the egg cell, which is otherwise only active in pollen and the embryo, the development of the embryo could be triggered without fertilization. BBM1 is a transcription factor that triggers embryogenesis. It becomes active in the cell as a result of the fertilization of the egg cell by a pollen cell. The feasibility of clonal reproduction for a crop plant through seeds was thus proven. But the process is not yet ready to be put into practice. "Compared to sexually reproducing rice, these plants still produce 30 percent fewer seeds. This is a problem, of course, because that means a 30 percent lower yield for crops from which we harvest the seeds. However, I am confident that this problem can be solved in the near future."

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Another crop with which the researchers have now tried out the MiMe technique is the tomato, the world's number one vegetable crop. Among other varieties, the scientists used date and vine tomatoes in their research – hybrid varieties that are also available in the supermarket. Charles Underwood and his team have not only developed a MiMe technique for this, but also utilized it in a different way. First, they established MiMe in different hybrid tomato plants to generate clonal sex cells. The fertilization of a clonal egg from one plant by a clonal sperm from another led to plants containing the complete genetic information of both parents. This approach – termed "polyploid genome design" – allowed Underwood and his team to design plants with a four-fold set of chromosomes instead of a two-fold one. The polyploidy observed in these tomato plants is similar to that observed in many other cultivated crops like wheat, rapeseed, bananas, and potatoes. The difference here is the polyploidy was induced by the MiMe process. "The result is a kind of super-hybrid," says Underwood.

The scientist is standing in front of an LED-lit greenhouse filled with tomato plants and points to the plant at the front to the right, which boasts particularly large fruit. "This plant has a quadruple set of chromosomes, so it carries the complete genetic material of both parent plants. As far as we are aware, this was the first time clonal sex cells from two different parents have fused – in any plant or animal – ensuring complete inheritance from both parents." Next to the "super-hybrid" is a plant that admittedly bears considerably smaller fruit, but is nonetheless very robust. "This is the result of crossing a MiMe tomato hybrid with a wild relative of tomato, *Solanum pennellii*. This wild accession comes from a barren location in South America and is particularly resistant to heat, drought, and salty soils. The genes for this stress tolerance are now also present in this hybrid plant," says Underwood. This also explains why the fruit are smaller: large fruit actually don't occur in nature at all, but are the result of thousands of years of artificial selection by humans. The hybrid tomatoes are part of an effort by Underwood and his colleagues to harness MiMe in developing new breeding systems that can fully make use of the stress tolerance of crop wild relatives.

Another candidate for the MiMe method is the potato. Potatoes and tomatoes may look very different, but the plants themselves are closely related. They both belong to the nightshade family and indeed to the same genus. "Many of today's varieties are already quite old – the 'Russet Burbank' variety, for example, has been cultivated in the US for over a century. There is an urgent need to speed up the development of disease-resistant potato varieties that can tolerate the increasingly variable summer climates, because potatoes are still one of our most important crops," explains Underwood.

One problem that arises in the cultivation of potatoes is disease. The pathogen that causes potato blight, for example, damages both the aboveground parts of the plant and the tubers that lay underground. If the pathogen attacks the potato plants during the growth phase, this results in high losses in yields. In Ireland in the mid-19th century, the fungal disease led to a devastating famine. As with the wild tomato, genetic material from wild potato species could make its domestic relatives more resistant. "MiMe could enable breeding varieties that are more resistant to potato blight, but otherwise have the usual characteristics of potatoes. This could help to reduce the need to spray the plants with pesticides." For Raphaël Mercier too, MiMe hybrid po-

SUMMARY

Crossing two varieties can produce hybrid daughter plants with particularly advantageous traits, but these traits are then lost in the following generation.

With the MiMe technique, hybrid plants can form clonal sex cells without meiosis. Clonal egg cells can be used to develop clonal plants without fertilization. The genetic material of these new plants is identical to that of the mother plant, preserving the high performance of hybrid plants for the long term.

The MiMe technique can also be applied in polyploid genome design. This offers a route to increase the genetic diversity within a single plant by, for example, engineering plants with four sets of chromosomes rather than two.

"The process could enable us to breed varieties that are more resistant to potato blight."

RAPHAËL MERCIER

PHOTO: FRANK VINKEN FOR MPG

Yazhong Wang, Charles Underwood, and Raphaël Mercier (left to right) examine a super-hybrid tomato plant that contains the complete genetic information of both parent plants.

tato varieties have huge potential, partly because it is not the seeds or fruits that are harvested, but rather the tubers lying under the ground. "The fact that MiMe hybrid potatoes don't form as many seeds is therefore not as relevant as for rice, since this doesn't have a negative impact on the yield."

There's a barrier to the application of this technique, however – namely the strict EU regulations concerning genetically modified crops. These regulations restrict techniques like MiMe that are based on genome editing, i.e., the targeted alteration or deactivation of genes. "The EU should follow the example of the US and UK and make it easier to cultivate genome-edited plants. Ultimately, we need to make future food production more efficient so that we can feed a growing global population in times of more frequent extreme climatic events. Hybrids that can produce higher yields and be made more robust with genetic scissors can make a contribution here," says Mercier.

Other researchers are therefore also calling for modernized genetic technology legislation in the EU that takes into consideration new techniques and findings, since the existing legislation is now more than 20 years old. A legislative proposal by the European Commission that would facilitate the approval of genome-edited plants was approved by the European Parliament at the beginning of the year. Now the EU member states have to agree on a final draft of the text for the legislation.

It is politicians who will therefore decide whether these kinds of plants will one day grow in the fields of Europe. Ultimately though, it depends on whether consumers would like to see genome-edited produce on their plates. Perhaps that decision might be influenced by the fact that the MiMe technique is not as unnatural as it might seem at first glance. The dandelion, and other plants such as various blackberries and grasses, reproduce in nature entirely without female meiosis or fertilization of the egg cell. Clearly, the yellow carpets of dandelions that appear on our meadows every spring are evidence of just how well this mode of reproduction works.

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