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Caixia Gao and a member of her team inspect CRISPR-modified tomato plants in a greenhouse at their growing facility in Beijing.

BASE EDIT YOUR WAY TO BETTER CROPS

Plant scientists are turning to genome-editing techniques to precisely tailor the productivity and consumer appeal of important crops. **By Michael Eisenstein**

Is there anything better than a perfectly sweet summer strawberry? Alas, many commercial berries look better than they taste. But molecular biologist Caixia Gao and her colleagues at the Institute of Genetics and Developmental Biology in Beijing have devised a way to tune the sweetness of strawberries using a few simple genetic tweaks[1]. "We could increase the total sugar content from 20 to 41 milligrams per gram," she says. "And there are so many different levels, you could choose what you like."

Gao's is one of a growing number of research groups turning to strategies known as base editing and prime editing to improve the yield, robustness and consumer appeal of commercial cereals, fruit and vegetables. The methods are adaptations of the widely used CRISPR–Cas9 system, which can be used to introduce specific changes at defined places in the DNA. They allow scientists to tweak the amino-acid sequence of a protein of interest, for instance, or alter sequences that control how strongly a gene is expressed.

Biomedical researchers have pounced on these technologies as tools for studying, and potentially repairing, mutations associated with diverse genetic disorders. Sweeter strawberries might seem like small potatoes in comparison, but the same capabilities are being harnessed to generate crops with greater disease resistance, higher nutritional content or more fruit per plant.

Crucially, these editing systems could one day offer an appealing alternative to adding in genes from other species to generate genetically modified organisms (GMOs), which remain the subject of public scepticism and close regulatory scrutiny. "GMOs have genes from other sources, but for gene-edited plants you can have these plants free from any foreign genes – just some small changes in the plant's

own genes," says Jian-Kang Zhu, director of the Shanghai Center for Plant Stress Biology in China. The first wave of base-edited fruit, vegetables and grains could reach consumers in the next few years, but much work remains before Zhu and other plant scientists can routinely produce bespoke crops to meet the needs of a hungry planet.

Covering the bases

In CRISPR editing, an enzyme known as Cas9 is directed to a particular site in the genome, where it binds to and snips both strands of the DNA. The targeting is achieved by a guide RNA, which seeks out a matching sequence in the DNA.

After Cas9 cuts the DNA, the cell moves to repair the damage through a mechanism known as non-homologous end-joining. This repair process often results in the insertion or deletion of random base pairs at the cut site, thereby disrupting the function of the target gene. "It's efficient, but not precise," says Yiping Qi, a plant scientist at the University of Maryland in College Park. "So that can lead to gene knockout easily, but not necessarily a lot of outcomes you wish to achieve." This lack of predictability is a particular problem if the goal is to optimize the function of a gene rather than simply stopping it in its tracks.

In 2016, chemical biologist David Liu's team at Harvard University in Cambridge, Massachusetts, developed a solution[2]. The researchers fused a modified version of Cas9 to an enzyme called cytidine deaminase, which chemically modifies a cytidine base in such a way that a C–G base pair is transformed into T–A – a process called cytosine base editing.

Liu's team developed an adenine base editor to convert A–T base pairs into C–G the following year[3], and dozens of other cytosine and adenine base editors have been devised since. This technology has translated remarkably well from mammalian cells into plant cells, with only modest modifications required to optimize the efficiency and specificity of the techniques. The efficiency varies wildly depending on the gene target and plant species, but can reach as high as 100%.

In 2019, researchers in France used a cytosine base editor to create a single-nucleotide change in a gene called *eIF4E1*, which encodes a protein that assists in translating RNA into proteins[4]. "That protein is also used by some viruses for their own replication cycles," says Fabien Nogué, a plant geneticist at the French National Research Institute for Agriculture, Food and Environment (INRAE) in Versailles, who was involved in the work. A single edit was sufficient to render the thale cress *Arabidopsis thaliana* essentially immune to the clover yellow vein virus, a common plant pathogen.

That year, Gao's team used base editing to introduce point mutations at two sites in the wheat genome to confer resistance to a variety of herbicides[5].

Researchers can even conduct 'directed evolution' experiments, with randomized mutations introduced to various genes to identify new variants that improve a particular plant trait. In 2020, for instance, Gao and her colleagues used a combined cytosine and adenine base-editing system to engineer variants of a rice gene that conferred resistance to a class of herbicides known as acetyl-CoA carboxylase inhibitors[6]. "The targeted domain was 400 amino acids, and we designed 200 guide RNAs that fully cover this domain," says Gao. "Then we screened the mutants by spraying herbicide to see which new variants survive." The effort revealed mutations that confer resistance without adversely affecting the health of the plant.

A prime opportunity

Despite its power, base editing has limited potential. Only 4 of the 12 possible base-pair changes can be achieved reliably. Researchers have developed a few cytosine-to-guanine base editors, but an evaluation done last year by Qi's team found these to be generally inadequate[7]. Qi describes them as "not efficient" and says that "there's still a knowledge gap about how to make it better".

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Base editing is also not suitable for making extensive changes in a gene, such as long insertions or deletions. These kinds of modification can be achieved with conventional CRISPR–Cas9 by exploiting a process known as homology-directed repair. Here, the cell incorporates a strand of donor DNA containing the desired sequence change at the Cas9 cut site. But the process remains inefficient in plants. "Maybe in the best possible cases, you might get efficiencies of 5%," says Holger Puchta, a plant biochemist at the Karlsruhe Institute of Technology in Germany.

As an alternative, in 2019, the Liu group described yet another CRISPR-based strategy, known as prime editing[8]. Like base editing, prime editing uses a modified Cas9 protein that makes a single-stranded cut. But this time, the Cas9 is coupled to a reverse-transcriptase enzyme, rather than a nucleotide-modifying one. Prime editing also uses a specially designed prime-editing guide RNA (pegRNA), which not only targets the editing machinery to a specific site in the genome, but also contains a template sequence and a primer-binding sequence. The template encodes the desired genome-sequence change. And after the DNA is cut, the primer-binding sequence hybridizes with the DNA at the cut site, providing a foothold for the reverse transcriptase to convert the RNA template into DNA, and thereby write the encoded sequence into the genome. This process can change any nucleotide, as well as insert or delete sequences dozens of bases long.

The resulting versatility opens the door to sophisticated, and powerful, edits. Single-base edits can do only so much to stave off plant pathogens, Nogué notes. "The simpler your modification, the easier it will be for the virus to escape it," he says. His team, together with collaborators at INRAE in Avignon, has examined naturally occurring determinants of resistance to potyviruses, which can seriously damage plants, and identified a set of five amino-acid variants in the *eIF4E1* gene that collectively protect pea plants against infection[4]. Nogué is now using prime editing to transfer this protection to potatoes. "With multiple amino-acid changes, we think that we will bring durable resistance," he says.

The original prime editor was relatively inefficient – typically on the order of what can be achieved with homology-directed repair. But some genome sequences seem to be more amenable than others, and a well-designed prime-editing experiment can have double the efficiency[9]. "I think there is still room to improve," says Gao, whose team has already devised multiple strategies for upgrading the performance of prime editing, including sophisticated pegRNA designs and variants of the Cas9-based editing complex that have enhanced functions.

For their part, Nogué and his group have found success with prime editing in well-characterized model plant species. Certain improvements "make the technology as efficient as base editing in our hands", he says. "If what we observe in the model plants is true for crops, then I think that this tool will be very, very useful."

Reaping what you sow

Applying base or prime editing is reasonably straightforward for some well-studied crops. Zhu's group has worked extensively with both techniques in rice, and other major crops such as wheat, maize (corn), tomatoes and potatoes have also proved amenable to editing. Qi notes that several web-based tools are available to help researchers choose the editing system that's right for them, including PlantPegDesigner, an app designed by Gao's group[10].

But important parts of the process remain a struggle. The first is transformation, the process by which researchers introduce the editing machinery into plant cells. One of the most common transformation strategies uses the soil bacterium *Agrobacterium tumefaciens*

to infect and subsequently deliver DNA plasmids that encode the Cas9 protein and associated RNAs into plant cells. However, this DNA subsequently also integrates into the plant genome — an undesirable outcome given the field's focus on avoiding permanent introduction of foreign DNA. There's also the risk of unwanted modifications arising from long-term expression of the genome-editing machinery.

Researchers can achieve foreign-DNA-free transformation by delivering the materials required for editing into protoplasts — cultured plant cells that have been enzymatically stripped of their outer cell wall. Such cells are much easier for researchers to transform transiently, with either DNA or RNA reagents encoding the editing machinery. "You are making cells with only a cell membrane, just like a human cell," says Qi, who notes that this process also offers a robust method for rapidly testing and optimizing base- or prime-editing experiments. Another possibility is to use a 'gene gun' to fire tiny projectiles laden with protein and RNA into embryonic plant cells. In both scenarios, the editing machinery will be active in the cell only temporarily, before being degraded, in contrast to the long-term expression that happens when DNA integrates into the host genome.

Whatever the transformation method, researchers must then use the edited cells to regenerate an entire plant. But for many plant species, biologists simply do not have the knowledge or expertise to isolate, cultivate and transiently transform the appropriate cells. "In nature, there are more than 370,000 higher plant species," says Zhu. "But we can only make transformation successful in a few dozen of these." Some emerging technological solutions could help; for instance, overexpression of genes that encode growth-regulating factors can greatly enhance the efficiency of regenerating gene-edited plants[11]. "It might well be that we will see many more plants that are very hard to transform being transformed and edited because of this," Puchta says.

Researchers are also stymied by a fundamental lack of understanding about the underlying biology of many key traits related to plant growth, resilience and quality. "Without knowledge of the genomes and a very deep knowledge of the mechanism that is behind a particular trait, these types of tools are completely useless," says Nogué.

The falling cost and increasing efficiency of genomic analysis technologies should be a boon here, and efforts are under way to begin applying some of the techniques routinely used in clinical genetics to agricultural sectors. For example, Pairwise, a biotechnology firm based in Durham, North Carolina, which was co-founded by Liu and has licensed his base-editing technology, is collaborating with government and academic scientists in the United States and Canada to identify the genetic bases of more than 50 traits in at least 300 unique species and varieties of berry, says chief technology officer Ryan Rapp. "We went from having almost nothing to over 600 sequenced genomes through this collaboration."

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CRISPR-modified wheat is planted in greenhouse at a growing facility in Beijing.

Evolving beyond GMOs

Even with just existing tools, however, the field is moving quickly. Rapp says that Pairwise's first gene-edited product, a leafy green vegetable with enhanced flavour, is expected to reach the US market next year. This was edited with standard CRISPR, but other, base-edited crops are in the works. One of those is a stoneless cherry, but that is still being tested and will take a while to reach the market because cherry trees take longer to cultivate than do crops grown in rows.

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Such products — alongside similar projects such as Gao's sweeter strawberries — could be just what is needed to help build trust with the public and with regulatory agencies alike. Some regulators, including the US Department of Agriculture and the Chinese Ministry of Agriculture, have afforded more latitude to CRISPR-edited plants than to transgenic GMOs, as long as the organisms do not incorporate foreign DNA. Other jurisdictions are more reluctant. But unless the public is persuaded, the technology could be dead in the water. "I think the entire community has seen that if you want people to accept genome-edited crops or food, you better make it more appealing to the public," says Qi.

Success could open the door to some truly transformative applications, including future-proofing global agriculture against the impacts of climate change. Puchta notes that attempts to bolster drought-resistance or salt-tolerance by transplanting individual genes have made only limited headway. But he sees considerable potential in moving in the other direction: domesticating rugged wild crops by tweaking their edibility and agronomic performance.

Gao has already shown that the idea can work. In 2018, her team and its collaborators used conventional CRISPR–Cas9 to domesticate a wild South American tomato by manipulating five genes linked to traits such as fruit size, yield and nutrient content[12]. "Through natural domestication, this process takes 8,000 years from start to finish," she says. "Now it's one-and-a-half years."

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Correction

This Technology feature article erroneously implied that Pairwise's leafy green vegetable was being base-edited to improve the nutritional content. In fact, the firm used standard CRISPR and targeted flavour. Also, the stoneless cherry is not yet in field testing.

Corrected 9 May 2022