The ultimate test of tea's quality is probably its taste.

FUMIYA TANIGUCHI

BIOTECHNOLOGY

Engineering a better beverage

Genetic manipulation and innovative breeding techniques are paving the way for new varieties of tea with benefits.

BY ELIE DOLGIN

When people ask Tony Gebely to recommend a caffeine-free tea, he has a stock reply: "I usually just suggest an herbal tea." But strictly speaking, herbal tea is not a type of tea at all. It's a tisane — an infusion of spices, herbs, flowers or roots — rather than a beverage made from leaves of the plant *Camellia sinensis*, which is the source of true teas, be they black, green, yellow, white, pu-erh or oolong.

Unfortunately, there isn't a simple way to remove caffeine, a stimulant, from the plant. Most decaffeinated teas are made with the help of solvents that extract the molecule from tea leaves. The problem, however, is that the process also affects the taste. "You're not just pulling out caffeine," says Gebely, who founded the American Specialty Tea Alliance, a non-profit trade association in Baroda, Michigan, in 2018. "You're pulling out flavour components, too."

In the quest to produce a caffeine-free tea that retains all the flavour and potential health benefits of normal tea, some researchers are turning to genetic engineering. Others are taking advantage of advances in molecular biology, genomics and horticultural science to supercharge conventional breeding techniques to develop varieties of tea that lack the buzz of caffeine, as well as others with improved flavour, resistance to stress or yields.

MAKE IT A DECAF

Despite numerous attempts, scientists have yet to successfully apply genetic-engineering techniques to create a plant that produces caffeine-free tea. Researchers in India, Japan, China and the United States are all attempting to shut down caffeine production in the tea plant by introducing genetic material that is designed to silence the caffeine-synthesis pathway, using methods that rely on pathogenic bacteria or 'gene guns' to deliver DNA to cells.

Modifying the DNA of tea plants in the laboratory proved to be easy, but getting the resulting cells to produce viable, leaf-producing crops is another matter. "Regeneration from the tea-plant cell to a whole plant is generally very difficult," says Misako Kato, a plant biochemist at Ochanomizu University in Tokyo.

Another barrier to using genetic-engineering methods to produce new varieties of tea is the public's antipathy to genetically modified food. According to Kato, a large Japanese agricultural food company initially licensed patents that her team had filed to cover the genetic manipulation of the caffeine synthesis pathway in tea plants. But without a market for such tea, she says, the company never pursued the strategy commercially.

Japan is not unique in this regard. Worldwide, says Zeno Apostolides, a biochemist who leads the Tea Research Laboratory at the University of Pretoria in South Africa, "Any mention of genetic engineering in tea is a total anathema to tea drinkers." So, instead of manipulating DNA to create tea plants that sprout caffeine-free leaves, Apostolides hopes to find plants in the wild that produce only trace amounts of the stimulant. Together with collaborators at the Tea Research Institute in Kericho, Kenya, Apostolides plans to screen roughly 10,000–20,000 plants from tea plantations found in the highlands to the west of Kenya's Great Rift Valley. "We may find a zero-caffeine mutant in 1–3 years," he says.

Tea breeders in China already have a head start on Apostolides. In 2018, Liang Chen and his colleagues at the Tea Research Institute of the Chinese Academy of Agricultural Sciences in Hangzhou described hongyacha, a variety of *C. sinensis* that is naturally free of caffeine, which they found in the mountainous province of Fujian[1]. The team showed that, in hongyacha, the region of DNA that controls transcription of the gene required for synthesis of both caffeine and its precursor, theobromine, differed from that of normal tea plants. They proposed that this region might have lost some of its function, retaining only the ability to produce theobromine — which could explain why hongyacha is rich in that molecule but contains undetectable quantities of caffeine.

Several decades ago, Hung-ta Chang, a botanist at Sun Yat-sen University in Guangzhou, China, found another wild tea plant with only

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trace amounts of caffeine in Guangdong province, which neighbours Fujian. However, the plant belonged to a different species (*Camellia ptilophylla*), and became known as cocoa tea, owing to its high levels of theobromine, which was initially detected in cocoa beans. According to Xiaorong Lin, a food scientist who studies the compounds in cocoa tea at South China Agricultural University in Guangzhou, "Tea made from this peculiar low-caffeine-containing tea species has been sold for decades in China." But, she notes, "Its raw yield is limited." And because of the beverage's unique aroma, cocoa tea does not really offer a decaffeinated alternative to people who want a stimulant-free version of the tea they know and love.

Hongyacha could fill a huge gap in the market. As it's a true tea, it's packed with the same compounds that give caffeinated tea its flavour. Given hongyacha's potential commercial applications, Chen says that he is planning either to propagate the wild tea or to cross the low-caffeine mutation into an existing variety of tea using established breeding techniques.

A BREED APART

If Chen opts to pursue the breeding route, it could be a long time before caffeine-free tea is found in plantations. Tea plants take 3–5 years to mature and bear fruit with seeds, so a multigenerational breeding programme that uses conventional cultivation techniques to produce selectively bred plants, or cultivars, can span 25 years or more. Fortunately, technology offers researchers some time-saving measures.

Mahasen Ranatunga, who leads plant breeding at the Tea Research Institute of Sri Lanka in Talawakelle, estimates that his team has trimmed the length of their process by around six years by culturing plant embryos in the lab — enabling the mass propagation of genetically identical young plantlets — instead of depositing seeds in soil and taking cuttings from the resulting plants. The researchers then transfer the plantlets outside for further experiments in the field.

TEA RESEARCH INSTITUTE OF SRI LANKA

A tea plantlet grown in the lab from a cultured plant embryo.

In the past, Ranatunga and his team would wait for tea plants to mature fully before measuring characteristics related to their yield, quality and stress responses. Now, they run chemical analyses, conduct morphological evaluations and look for genetic markers in immature plants to get an earlier read on which plants to move forward with in their breeding effort. "We can identify putative quality cultivars at very early stages," he says. With optimization, Ranatunga thinks that he can shave off several more years from the breeding cycle.

Although Ranatunga says that his "number one priority is increasing yield", he is also hoping to breed tea plants that have enhanced resistance to certain pests and diseases. To do that, he plans to alter levels of plant metabolites known as catechins. In 2017, Ranatunga and his colleagues showed that high levels of one such molecule, epicatechin, and low levels of another, epigallocatechin gallate, helped to protect the leaves of green-tea plants from blister blight, a disease caused by the fungus *Exobasidium vexans*[2]. His team is now looking for genetic and chemical determinants of tea plants' resistance to the shot-hole borer beetle (*Xyleborus fornicatus*), the live-wood tea termite (*Glyptotermes dilatatus*) and canker-causing fungi such as *Botryosphaeria dothidea*.

In the past, researchers who wanted to engage in such marker-assisted selection were hampered by a lack of genetic information. But in the past two years, several genomic resources for tea have become available.

In 2018, Apostolides and his colleagues described dozens of sites in the tea-plant genome that are linked to tea's caffeine level, antioxidant content and taste, as well as the tea plant's tolerance to drought[3]. Meanwhile, two teams of researchers — one led by Lizhi Gao at the Chinese Academy of Sciences' Kunming Institute of Botany, and the other led by Xiaochun Wan at Anhui Agricultural University in Hefei, China — published genome sequences of the two main varieties of tea plant[4,5].

Not only do these genome maps offer a window on the domestication of tea (see page S2), they also reveal many of the genes that are involved in synthesizing the compounds that give tea its distinct flavour. Knowing the location of such genes and closely linked DNA sequences should further assist marker-assisted breeding efforts, says Jeffrey Bennetzen, a plant geneticist at the University of Georgia in Athens, who also leads a team of tea researchers at Anhui Agricultural University and was involved in the university's project to sequence the tea-plant genome. "It will be useful for a number of things," he says, "including for crop improvement."

That has already started to happen in Japan, where Fumiya Taniguchi and his colleagues at the Institute of Fruit Tree and Tea Science in Makurazaki have determined the location of a gene that confers resistance to a sap-sucking insect known as white peach scale (*Pseudaulacaspis pentagona*). Taniguchi's team used this information to lay down an important milestone: the first use of DNA marker-assisted selection to create a variety of tea. They described the tea, known as nanmei, in 2018, and reported that it could also withstand attack by various species of fungi[6].

Between 2007 and 2011, the team grew nanmei at more than a dozen research stations throughout Japan. These field trials showed that nanmei had a greater yield than yabukita, the most widely planted variety of tea in Japan, as well as an improved flavour profile, as gauged by professional tea tasters. "We are now making concerted efforts to promote nanmei to tea farmers and tea sellers," Taniguchi says.

TOOLS OF THE TRADE

Elsewhere, researchers are taking a more systematic approach to growing tea. In California, where farmers are hoping to jump-start tea production as a new industry, a team led by Jacquelyn Gervay-Hague, a chemical biologist at the University of California, Davis, is planting 30 varieties of tea at 7 sites across coastal, flat agricultural and mountainous regions of the state. Gervay-Hague's goal is to better understand the links between growth conditions and tea-plant quality. Her team is also feeding the plants chemically labelled nutrients to track their metabolic processes and interactions with soil microorganisms. "We expect to find that certain varieties are going to do better in certain areas, and we'll have a chemical basis for understanding that," says Gervay-Hague.

Amita Bhattacharya, a plant biotechnologist at the Institute of Himalayan Bioresource Technology in Palampur, India, also sees a need for more tools to streamline the process of genetic modification in tea plants — even when the plants are not destined for human consumption. She and her colleagues have optimized gene-gun technologies to alter genes that modify traits, including drought tolerance, that define plants' interactions with the climate and growing environment. They aim to reveal characteristics that might be amenable to manipulation through more conventional breeding techniques.

"We want to understand tea completely," says Bhattacharya. As a bonus, she says, should government regulations and consumer tastes change, enabling the mass cultivation of genetically modified tea, "We want to have all the technologies in place and ready to go."

That has meant developing the gene-editing technique CRISPR–Cas9 for use in tea plants. Bhattacharya has only started to work with this state-of-the-art technology, but researchers at Hunan Agricultural University in Changsha, China, have already described such a platform for deleting genes in tea plants with needle-like precision[7]. Its target? The gene that encodes the enzyme caffeine synthase. ■

Elie Dolgin *is a science journalist in Somerville, Massachusetts.*

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