Make it a decaf

The enduring quest for a coffee bean without the buzz.

BY BRENDAN BORRELL

Paulo Mazzafera punched a pea-sized disc out of a waxy green coffee leaf, then placed the disc in a small vial with a mixture of chloroform and methanol to dissolve it. Later, he loaded the extract, along with 95 other samples, into a high-performance liquid chromatography machine, which separates out each chemical component. When the plant physiologist returned to his lab at the University of Campinas in Brazil

IMAGE SOURCE/CORBIS

the next morning, he sat down at his laptop to examine the results. Scrolling from one chromatogram to the next, he scrutinized the peak representing caffeine. In one plant, it was missing.

Mazzafera ran the sample twice more and then, just before noon, called his collaborator Bernadete Silvarolla, based at the agricultural station nearby, to share the news. “Are you sure?” she asked. He was. In fact, he was thrilled. After screening thousands of plants over the course of two decades, his project to find a naturally caffeine-free coffee finally seemed to be bearing fruit. That was in late 2003.

Coffee contains some 2,000 chemical compounds that give the drink its enticing aroma and flavour, including caffeine, a stimulant and natural pesticide. Removing the caffeine while leaving all the others intact poses a significant challenge. Brewers have generally turned to chemistry: Ludwig Roselius of Bremen, Germany, patented the first commercial decaffeination process in 1905. But his coffee, marketed as Kaffee HAG, used benzene in the extraction process, and the chemical was later replaced by less toxic solvents. Today, companies may instead douse raw green coffee beans in high-pressure liquid carbon dioxide or soak them in hot water for several hours to remove the caffeine before roasting. Aficionados say that all these methods destroy the taste, but the decaf market is still worth US\$2 billion a year.

Researchers have long sought a better bean, harvested directly from the plant caffeine-free. This would preserve coffee’s complex flavour and give growers a high-end slice of the decaf market. But developing such a bean through conventional breeding or even genetic modification has proved more difficult than anyone

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anticipated (see ‘The ups and downs of decaffeination’). Coffee plants take years to begin producing beans, and can be fickle when they do. Moreover, to make them profitable to farm, the plants need to be productive, ripen synchronously and be of a size and shape that can be harvested easily by hand or by machines. The loss of any of these traits can render a plant worthless. The caffeine-free quest has produced a string of high-profile papers, but not a drop of marketable coffee.

Basic research often proves hard to translate into industrial agriculture, says Rod Sharp, a retired plant cell biologist who tried to produce caffeine-free coffee in the 1980s while at DNA Plant Technology in Cinnaminson, New Jersey. “Those in the research lab are always a bit naive. We jump up and down when there is a breakthrough, but turning that into a commercial operation is another challenge altogether.” However, Sharp remains confident that the plant will one day yield its secrets. “It will happen,” he says. “It’s just a long incubation period.” This is certainly true for Mazzafera’s plant: more than eight years after its discovery, his colleagues are still trying to turn it into a crop.

Coffee is worth a total of \$15 billion to \$20 billion per year to exporting countries, which include Brazil, Colombia and Vietnam. A relatively recent innovation, compared with tea or wine, it dates to about the fifteenth century when — according to at least one account — a Yemeni mystic described a revitalizing beverage prepared in Ethiopia by roasting and boiling berries. Two coffee species dominate the market today: *Coffea arabica*, the better-tasting bean, which grows in cooler climates, and *Coffea canephora*, commonly known as ‘robusta’, which is used mainly in instant coffee and lower-quality blends. Caffeine, which is present at 1.2% in commercial *C. arabica* and 2–3% in *C. canephora*, has helped to make both species part of a worldwide addiction.

But the stimulant beloved by many is avoided by others who are unusually sensitive to its effects, abstain for religious reasons or simply don’t want to be kept awake. Gabriel Bertrand of the Pasteur Institute in Paris discovered a caffeine-free species of coffee on Grande Comore island near Madagascar in 1901. In fact, many of the hundred or so species of *Coffea* are either caffeine-free or contain low levels of the stimulant. Several naturally occurring intermediate-caffeine coffees (with 0.6–1% caffeine) have already reached the market, including one produced by the Italian coffee-maker Illy. Unfortunately, most plants with the lowest caffeine levels produce few beans, or contain high concentrations of bitter compounds. Nevertheless, the existence of such natural variation suggests that a diligent breeder could create a commercially viable caffeine-free strain.

COFFEE AND DREAMS

Mazzafera began his attempts to do just that in 1983 at the Agronomical Institute of Campinas (IAC), a century-old agricultural station in the rolling hills northwest of São Paulo, Brazil. He set out to study the genetics and physiology of caffeine biosynthesis under Alcides Carvalho, a pioneering plant breeder who established the IAC’s collection of coffee plants, which now number 70,000 and represent more than 1,000 wild strains, breeding lines, hybrids, mutants and cultivated varieties from around the world.

At first, Mazzafera used an old-school spectrophotometer to measure caffeine content one sample at a time. In 1987, he got a job at the University of Campinas and installed a high-performance liquid chromatography machine in his lab, allowing him to process samples more efficiently. By this point, scientists had sketched out the basic four-step pathway by which *C. arabica* synthesizes caffeine. Mazzafera studied caffeine production and breakdown in great detail in seven species, hoping to find one with defects in a pathway that rendered the plant low in caffeine. At the same time, he and Carvalho, who died in 1993, were cross-breeding commercial coffee cultivars with wild non-*arabica* species low in caffeine. But it proved impossible to eliminate caffeine while maintaining the desirable attributes of *C. arabica*. “We

were just wasting time,” says Mazzafera.

In 2000, Mazzafera teamed up with Silvarolla, a coffee breeder at the IAC. They shifted their focus to a group of *C. arabica* plants originally collected during a 1964 United Nations expedition to Eritrea and Ethiopia. Seed samples — 620 in total — were divided up and grown in several countries, including Costa Rica. Later, 308 of these lineages were collected in Costa Rica and sent to Brazil. Mazzafera believed it would be much easier to produce marketable coffee by starting with the Ethiopian *C. arabica* plants than by hybridizing with other species.

It was from this collection that Mazzafera discovered the promising strain in 2003, as well as two more like it. After confirming that, like the leaves, the beans were caffeine-free, he worked out that the plants were defective in the final step of the chemical pathway that turns theobromine — a mild stimulant and diuretic — into caffeine¹. The Brazilian government offered the research group a \$1.2-million grant along with an order to keep the location of the precious plants under wraps. Mazzafera felt certain that commercial growers would be planting the new variety in five years. That is, so long as others didn’t get there first.

BEAN MODIFICATION

The advent of genetic engineering led many scientists to try to make decaf by splicing the right genes into the right beans. But coffee has proved resistant to this kind of tinkering. In 1992, geneticist John Stiles of the University of Hawaii in Honolulu wanted to use ‘antisense’ technology, whereby a gene inserted into the plant reduces production of a target protein. This was the technology used to produce the Flavr Savr tomato, the first genetically modified organism to be approved for human consumption. Stiles’s goal was to target a protein in the caffeine-producing pathway.

Problems cropped up almost immediately. Creating any genetically modified plant involves culturing a blob of plant cells on nutrient-rich agar, inserting the desired genetic material into those cells, and then convincing the tissue to sprout into a plant. For *C. arabica*, that process is mysteriously inefficient.

Over the next seven years, Stiles and two postdocs, Kabi Neupane and Stefan Moisyadi, struggled to overcome the biological roadblocks.

They produced plants that seemed to have low levels of caffeine, and Stiles made some enthusiastic claims. In August 1999, for example, he told *The Wall Street Journal* that he would be beginning field trials that month in Hawaii before expanding to Mexico, with commercial prospects after three years.

The plants, however, did not cooperate. As they grew, their caffeine levels rose. Moisyadi and Neupane moved on to academic careers and, in 2000, Stiles left the university, setting up a private lab in Waialua. In 2008, after disagreements with the local community and the state legislature over the right to field test his transgenic coffee, the company folded, and Stiles now says that he was never 100% certain that he had created caffeine-free coffee. Many coffee researchers also doubt that he had. “We were always doing this on a shoestring,” Stiles says. “We were never Monsanto.”

Neither was Shinjiro Ogita, a postdoc in Hiroshi Sano’s lab at the Nara Institute of Science and Technology in Japan. In 2001, he began a research programme targeting an enzyme in the caffeine pathway that had recently been identified in tea. His group used an efficient gene-silencing technology called RNA interference, and worked with robusta coffee in the hope that cell culture would be easier. It wasn’t. Few cells took up the engineered DNA. Ogita was able to recover just enough to produce 35 transgenic seedlings.

He tested some leaves and found they had as much as 70% less caffeine than his control plants. “It was kind of unbelievable,” he says. He remembers popping a bottle of Dom Perignon champagne the day

Aficionados say that chemical extraction destroys the taste, but the decaf market is still worth US\$2 billion a year.

The ups and downs of decaffeination

For more than a century, brewers and growers have looked for ways to create tasty, caffeine-free coffee.

Chemistry

▲ Several well-established chemical methods strip beans of their caffeine, which can be sold for use in drugs and cosmetics.

▼ Extraction processes can be very expensive. Coffee aficionados say that the flavour is destroyed.

Hybridization

▲ Caffeine-free species of *Coffea* might be crossed with better-tasting *Coffea arabica*.

▼ Maintaining all desired traits is complex. One potential low-caffeine (~0.37%) strain is a hybrid of three species.

Breeding

▲ Maintaining the desired traits of *Coffea arabica* might be easier if it were bred with a caffeine-free strain of the same species.

▼ Caffeine-free strains are hard to find. Some undesirable traits may still be difficult to 'breed out'.

Genetic engineering

▲ Can specifically target caffeine-biosynthesis genes.

▼ Coffee species are notoriously difficult to engineer. Public acceptance of genetically modified foods is low.

Mutagenization

▲ A well-accepted method for creating variation in crop traits.

▼ Resultant specimens need to be screened, which can be a long and arduous process. Non-targeted traits may be affected.

his paper was accepted by *Nature*². He has since tailored the methods to *C. arabica*, but the plants have still not produced beans. Now at Toyama Prefectural University, Ogita tends about 40 transgenic plants. Each year, he says, the female part of the flowers, called the pistil, matures and deteriorates a week before the pollen is ready.

Even if Ogita can overcome these breeding issues, it is hard to imagine that transgenic caffeine-free coffee will land on supermarket shelves any time soon. One basic hurdle, says biochemist Alan Crozier at the University of Glasgow, UK, is that side routes in the caffeine pathway result in some production of caffeine, so transgenic coffee may never be caffeine-free. Gaining public acceptance is likely to be another challenge. Monsanto and other agricultural giants have generally commercialized technologies with the grower in mind — for example, pest resistance or herbicide tolerance — rather than focus on consumer-oriented traits such as low caffeine or high antioxidants. So finding financial backing may be tricky.

BREED APPEAL

A general aversion to genetically modified food has made the naturally caffeine-free Ethiopian strains that much more appealing. But breeding the trait into a commercially viable cultivar has taken longer than Mazzafera or Silvarolla anticipated. During the flowering season, Silvarolla spends all day in the field, clipping the pollen-producing stamens off the flowers and bagging the pistils so that she can hand-pollinate them later. She produces 800 new plants every year.

The beans that are produced taste good, according to tasting panels put together by the IAC, but the plants tend to be bushy and don't flower uniformly. The team is now working on breeding plants that have the low-caffeine trait but not the low-productivity one. "At first, we thought it would be a piece of cake," says Miriam Maluf, a geneticist at the IAC working on the project. But the researchers may be facing the biggest challenge of all in removing caffeine from living plants: it is there for a reason. Caffeine is a natural insecticide, which explains why wild coffee plants that lack caffeine tend to contain other bitter compounds — to deter pests.

And the researchers have to contend with these issues largely without Mazzafera's help. Soon after the 2004 discovery and subsequent publicity, the IAC took greater control of the programme, and

Mazzafera, based at the university, has only a limited role. "It's disappointing," he says.

Nevertheless, despite so many years without success, the quest for caffeine-free coffee shows little sign of fading. Plant geneticist Benoit Bertrand of the French Centre for Agricultural Research and Development in Montpellier has been searching the centre's collection for caffeine-free plants. And Chifumi Nagai of the Hawaiian Agriculture Research Center in Waipahu has been working in Madagascar with the Japanese coffee manufacturer UCC Ueshima to develop a three-species hybrid that tastes good, has a moderate yield and contains just 0.37% caffeine³. Its success is uncertain; Madagascar faces significant logistical challenges even growing and harvesting normal *C. arabica*.

Even Mazzafera, now 51, hasn't given up. On a cloudy November day, he walks out past two mesh shade houses behind the biology department in Campinas and reveals several hundred hip-high coffee plants. Some have rows of green coffee berries clustered on their branches. Many, he says, are nearly caffeine-free.

Miffed at his inability to continue his work with the Ethiopian varieties, he came up with a new plan in 2006. He took the seeds of a productive *C. arabica* variety, soaked them in chemicals that cause mutations, and then screened the caffeine levels of 28,000 seedlings. "It was a shot in the dark," he says. He ended up with 7 plants that have only 2% of normal caffeine levels⁴. He has already trademarked their name: Decaffito.

Challenges remain — the strain is susceptible to cross pollination, which can reinstate caffeine production in the beans — but he is determined to breed a commercially viable strain. He has even been talking to one company about investing in his research. But knowing the hurdles ahead, he is willing to settle for less. "If I had a farm," he says, "I would grow this coffee for myself." ■

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The biggest challenge of all may be that caffeine is there for a reason.

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