

### Genetically decaffeinated coffee

Coffee is a highly popular drink and has been central to many cultures, spreading across the globe – from Indo-China to Europe, from the Arabs and Turks to the South Americans. The earliest written records of coffee date back to Yemen in the early 15th century, but its origin appear to be much earlier in Abyssinia (Ethiopia). The legend is that a shepard from Kaffe (Abyssinia) noticed that his goats became unusually excited on eating the leaves and fruit of a certain bush. This had the same effect on him. On learning about this, the Abbott at the local monastery tried to make a drink out the plant, but it was so bitter that he threw the entire pot into the fire. When the fruit started to burn, the beans inside produced a pleasant aroma. A drink was produced from roasted beans and coffee was born. Coffee was brought to the Malabar Coast of India by Arab traders and was distributed in the Old World largely through Arab and Dutch trade routes (<http://www.ico.org/acoff/cofstor.htm>).

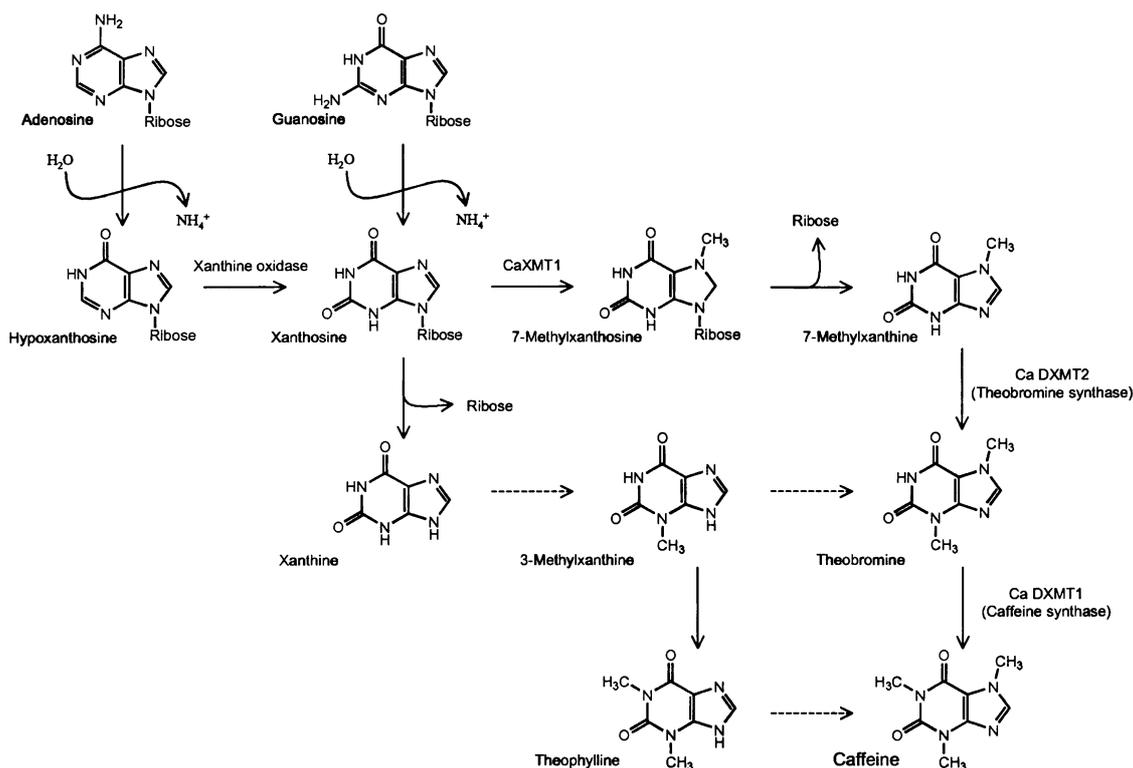
The rich flavour and aroma of coffee comes together with the nerve jangling effects of caffeine. It is present in coffee beans at a concentration of 2–3%, as also in tea leaves at around 5%. Caffeine is a secondary metabolite that is the product of nucleic acid catabolism and must be made by the plant for some specific function. It is speculated that caffeine may guard the plant against insect or pest attack, or prevent the germination of other seeds in the vicinity. The effect of caffeine on the human body is however better understood. These include short-term reactions such as palpitations, gastrointestinal disturbances, anxiety, tremor, increased blood pressure and insomnia. The adverse side effects of caffeine account for a substantial market for decaffeinated products. Decaffeinated coffee accounts for about 20% of all coffee sales in the USA, with the demand going up to 55% for those over 60 years of age.

First developed in the early 20th century, decaffeination techniques used organic solvents to extract caffeine from the coffee bean. The organic residues often caused greater harm than caffeine. At present, decaffeination is carried out by supercritical extraction that involves washing the beans with liquid carbon dioxide. The procedure is not only expensive it also removes other compounds that give coffee its rich taste and aroma. There are some species of the *Coffea* plant that have low levels of caffeine in their seeds. However, these either make horribly bitter coffee or are difficult to grow. Plant breeders have also tried to transfer the low caffeine trait to *Coffea arabica* without any success.

This is where molecular biology has stepped in. Recent work by Japanese scientists has led to the isolation and characterization of enzymes responsible for caffeine biosynthesis and the genes coding for these enzymes. A report appearing in the 19 June 2003 issue of *Nature* has now used this information together with the RNA interference (or RNAi) technique to create a coffee bean with reduced caffeine content (Ogita 2003).

The biosynthesis of caffeine starts with hypoxanthosine or xanthosine, themselves the products of hydrolytic deamination of adenosine or guanosine, respectively. Caffeine is synthesized from xanthosine through one ribose removal and three N-methylation steps (see figure 1). Earlier this year, Hiroshi Sano and colleagues at the Nara Institute of Science and Technology reported the cloning and expression of three cDNAs encoding the N-methyltransferases involved in caffeine biosynthesis (Uefuji *et al* 2003). These included CaXMT1, CaMXMT2 (theobromine synthase) and CaDXMT1 (caffeine synthase) that successively add methyl groups to the N-7, N-3 and N-1 positions of the purine ring (see figure 1). A minor biosynthetic pathway also converts xanthine into caffeine through 3-methyl-xanthine and theophylline.

In an attempt to produce genetically decaffeinated coffee beans, the same group constructed transgenic coffee plants in which expression of the theobromine synthase gene was repressed by



**Figure 1.** The biosynthetic pathway for caffeine. Solid and dashed arrows represent the major and minor pathways of caffeine biosynthesis, respectively.

RNAi. This versatile technology involves the use of short (21–23 nt) pieces of complementary RNA. Once inside the cell, these RNAs bind to the target mRNA and mark these for degradation by the cellular machinery. This causes specific inhibition of gene expression and has been used to create functional knockouts in species ranging from the nematode *Caenorhabditis elegans* and the fruitfly *Drosophila melanogaster* to mammals and plants (Dykxhoorn *et al* 2003).

The Japanese researchers designed hairpin constructs with fragments from the 3' untranslated region of the theobromine synthase mRNA and used these for *Agrobacterium*-mediated transformation of somatic embryos from *Coffea canephora*, a robust coffee plant grown in Central and West Africa, and throughout Southeast Asia ([www.ico.org/frameset/coffset.htm](http://www.ico.org/frameset/coffset.htm)). The seedlings that grew out on a selection medium were then tested for their theobromine and caffeine content. Compared to wild type plants, the transgenic plants showed up to 70% reduction in its caffeine content. Though the caffeine content in coffee beans was not tested, it is expected to reflect the reduced levels seen in leaves. The researchers are now applying this RNAi-based technique to *C. arabica*, which accounts for about three-fourths of the global market and is widely grown in Latin America, Central and East Africa, and India (<http://hspinfo.org/toxin.htm>).

Coffee is good, but can this approach be used to improve crops that feed the world's hungry? An attractive target would be the plant *Lathyrus sativus*, commonly known as “grasspea”, or in India as “kesari dal”. The entire plant is used as fodder for livestock and is consumed by humans in many ways. It thrives in poor soil, during drought or waterlogging and contains twice as much protein as wheat (<http://hspinfo.org/toxin.htm>). However, it also contains an amino acid called *b*-N-oxalyl-L-*a*-*b*-diaminopropionic acid (ODAP) that is a mimic of glutamate and a neurotoxin. This mimicry causes excessive triggering of the fast excitatory signals running through the brain and nervous system resulting in exhaustion and death of neurons. When consumed in large quantities, kesari dal causes a spastic disorder called lathyrism. If the biosynthetic pathway for ODAP is targeted analogous to that of caffeine, it may be possible to produce kesari dal with reduced levels of the toxin. Another target

may be the biosynthetic pathway for oxalic acid in tomato and spinach, as this is the main cause of kidney stones.

The initial observations on transcriptional co-suppression were made in plant systems. Subsequent developments in the field of RNA interference made initially in worms and fruitflies and then in higher animals appear to have come full circle. The technology appears poised for developing better crops in a world with shrinking land and water resources.

### References

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