New greens

beat the browning blues

Stall-holders at fruit and vegetable markets will often cut open apples or pears for customers to sample the fruits' freshness. But minutes after exposure to the air, the flesh turns brown, spoiling the fruit's appeal.

The browning reaction results from the oxidation of phenolic compounds in the fruit under the action of an enzyme called polyphenol oxidase (PPO), which is ubiquitous in plant tissues. By oxidising phenolic compounds, PPO contributes to the astringent flavours and ruby hues of red wines, but the enzyme is unwelcome in white grapes. It can discolour white wine and imparts a brown colour to dried fruits.

By 'switching off' the action of the enzyme polyphenol oxidase, scentists can prevent browning in some fruits and vegetables.

Dr Simon Robinson, a senior member of Dr Nigel Scott's research team at the Division of Horticulture in Adelaide, cloned the PPO gene from grapevines in 1992.

Cloning PPO genes from other commercially-important crop species is now almost a routine procedure. Using DNA probes derived from highly-conserved DNA sequences in the grapevine gene, Robinson has located and cloned the corresponding gene from potatoes, apples, beans, sugarcane and, most recently, lettuce.

Robinson and his colleagues used the genetic code of the PPO gene to construct an anti-sense PPO gene, a gene whose DNA code countermands the PPO gene's instructions to make the enzyme. Robinson supplied the gene to Dr Peter Waterhouse at CSIRO's Division of Plant Industry in Canberra. His team had already introduced a virus-resistance gene into Atlantic potatoes (see page 8).

The Plant Industry team produced about 100 transgenic lines of Atlantic, some of which exhibit virtually no PPO activity. Concentrations of the enzyme are as low as 1% to 10% of original levels. Atlantic is the Australian industry's main crisping cultivar. It is planned to introduce the gene into chipping potatoes. The chip industry rejects 20%-30% of tubers each year because of browning or bruising.

Other food industries could also benefit from the anti-PPO gene.

'In the short term, we see the main applications being in annual fruit and vegetable crops,' Scott says. 'Browning is a problem in lettuce, and both beans and peas are usually blanched to prevent browning. Processors tread a fine line; if blanching is overdone, peas and beans lose their flavour.'

In a project funded by the Sugarcane Research and Development Corporation, Robinson has recently cloned the PPO gene from sugarcane. Sugar processors use heat to denature the enzyme, which contributes to the brown colour of raw sugar. Silencing the gene in sugarcane would save the industry large amounts of energy and reduce greenhouse gas emissions.

Wood contains phenolic compounds that can turn timber brown as it cures. The anti-PPO gene could prevent discolouration of cabinet-making timbers and reduce the need to for paper-mak-ing, which generates organofluorine effluents.

The CSIRO team would like to introduce its patented anti-PPO gene into two fruits that are notorious for browning: apples and pears. First, it must persuade Australia's orchardists that the advantage they would gain in the lucrative international fresh-fruit market would justify the time and expense of replacing mature apple and pear trees. The industry's decision may be hastened by the interest that overseas orchardists and winemakers are showing in the new technology.

The Australian pineapple industry could also benefit from the anti-PPO gene. Malaysian pineapple growers are interested in curing black heart, which makes fruit unsaleable and unsuitable for processing.

Scott says the French winemaker Moet et Chandon is helping CSIRO to introduce the anti-PPO gene into Chardonnay vines to test the effects on clarifying grape juice. With the division's focus on Australia's wine and dried fruits industries, CSIRO sees mutual benefit in such an arrangement.

'We have a gene they want, and they have a system for transforming vines, which we don't have yet,' Scott says.

Another grape variety for transformation is the Sultana, or Thompson Seedless. Exported Australian dried sultanas bring premium prices because of their golden colour; the lighter the fruit, the lower the PPO activity. Scott says the anti-PPO gene could add value to the industry by upgrading the crop.

The Division of Horticulture is also studying several other genes which affect grape berry quality as part of its role in the Adelaide-based Cooperative Research Centre for Viticulture. The CRC is a joint venture involving CSIRO, Adelaide University, Wagga's Charles Sturt University, the Australian Wine Research Institute and the state agriculture departments of South Australia, Victoria and NSW.

Seeking seedlessness

In a project funded by the Horticultural Research and Development Corporation (HRDC), Dr Anna Koltunow of the Division of Horticulture is studying the molecular genetics of seedlessness. She is looking for genes that could eliminate the seeds in mandarins and other citrus.

'Mandarins are a high-value crop with excellent export potential in Asia and Europe', Koltunow says. 'Many mandarin cultivars grow well in Australia, but all have seeds. The only seedless variety is Silverhill Satsuma, which doesn't do well under Australian conditions. We need an Australian-owned variety that we can export.'

Koltunow's team is targeting the cultivar Murcott, which has almost perfect credentials for export. It has the right shape, size and flavour, but has about 20 large seeds per fruit.

Like the Satsuma mandarin, the navel orange is seedless. In studying the biology of normal seed development, Koltunow has found that seedless citrus cultivars such as the navel typically have defects in their pollen or ovules, and set fruit without being pollinated. This trait is called parthenocarpy.

'We have asked an indirect question: what happens if we cut off the anthers (the pollen-bearing organs of the flower) of seeded mandarins, or exclude bees to prevent the flowers being pollinated?' Koltunow says. 'We have found that some varieties, like Kara mandarin, have an inherent ability to undergo parthenocarpic development if they are prevented from setting seed.'

While working at the University of California in Los Angeles, Koltunow identified a gene which functions during pollen development in the anther. A Belgian company, Plant Genetic Systems, has taken the gene's anther-specific DNA promoter and attached it to a gene encoding an RNAse enzyme, which breaks messenger RNAs from genes to form a male sterility gene. This male-sterility gene has been shown to function exclusively in the anthers of a range of crop species, where it prevents pollen forming.

The CSIRO team is now attempting to identify genes that are expressed early in the development of citrus seeds, whose promoters could be attached to RNAse genes and introduced into mandarins and other citrus fruits. But even if such genes can be cloned, there is another obstacle: the plant-infecting microbe *Agrobacterium tumefaciens* is normally used to ferry new genes into plant cells. *Agrobacterium* does not infect mandarins.

Agrobacterium does not infect plant cereals either, but in recent years genetic engineers have produced transgenic lines of rice, wheat, maize and barley by using a 'gene gun', a device that fires microscopic pellets of tungsten or gold, coated with the DNA of the chosen gene, through the tough cellulose outer wall of the plant cell. Occasionally, a pellet penetrates the cell nucleus and the gene integrates with the plant's DNA.

A post-doctoral researcher at Koltunow's laboratory, Dr James Bond, built a gene gun which has been used to introduce the Belgian male-sterility gene into the embryo-forming cells of Kara mandarin. Plants are now being regenerated from these embryos, but until they flower it will not be known if they are male-sterile.

A potential problem is that abolishing pollen does not necessarily ensure seedless fruit. Some cultivars, like Murcott, lack the alternative, parthenocarpic development pathway, and the entire fruit aborts. So Koltunow's team is experimenting with a different approach, focusing on seeds rather than pollen. They have identified a seed-specific DNA promoter, and spliced it into an RNAse gene.

The gene is being tested initially in the tiny crucifer, *Arabidopsis*, and tobacco (*Nicotiana*), the 'green rodents' of plant biologists. When introduced to tobacco, the synthetic gene prevented the formation of the nutrient-laden endosperm tissues that sustain the embryo's growth after it germinates and also stopped embryo development. Both tissues died.

It seems that with citrus, nothing is easy. Koltunow says genes can only be shot into embryogenic citrus callus by the gun, and then plants are regenerated from embryos which form from that callus. Here lies the Catch-22. The gene is turning on at a low level and killing the embryo as it grows during the regeneration process from callus. Koltunow's team is modifying the promoter to see if they can make it work selectively in developing seeds and not turn on in the semi-seed like embryos that form in the tissue culture process.

If it works – and preliminary experiments with tobacco have yielded promising results – Australian researchers should be able to remove the seeds from any citrus fruit, including lemons. Juice-processors and consumers would benefit.

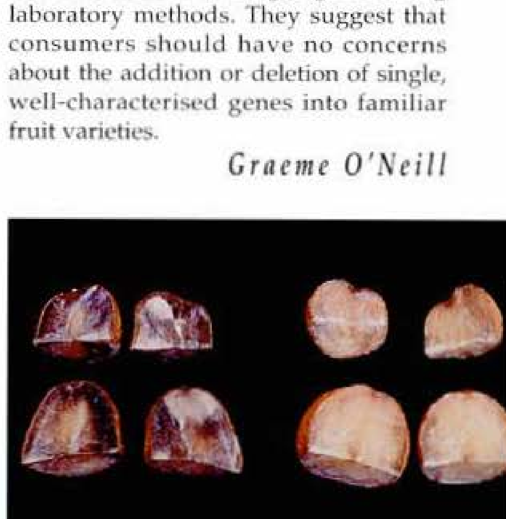
The transformation and regeneration techniques could be used to introduce other genes, such as genes for disease resistance, into citrus plants. Beyond citrus, seedlessness is a highly desirable trait in table grapes, and even large-seeded fruits like avocadoes, mangoes and lychees might be candidates.

Koltunow says that despite the problems encountered in transforming citrus, the citrus industry, through the HRDC, has been supportive and wants research to continue. Research is expensive, but the potential payoff is high.

Will consumers accept transgenic citrus and grape varieties?

Koltunow and Scott say people are already familiar with naturally-occurring seedless fruits, and have no concerns about the grapefruit and tangelo, both the result of conventional hybridisation between relatives in the citrus family. Here, the mixing of thousands of anonymous genes produced no adverse effect, but for some reason consumers worry about the introduction of a single gene using laboratory methods. They suggest that consumers should have no concerns about the addition or deletion of single, well-characterised genes into familiar fruit varieties.

Graeme O'Neill



Browning in Atlantic potatoes with (right) and without the anti-PPO gene. The chip industry rejects between 20% and 30% of tubers each year because of browning or bruising.