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operation mango

The CSIRO mango research team (left to right). Ping Lu, Anna Padovan, Richard Brettell, Sam Blaikie, Alonso González, Graeme Passmore, Alan Niscioli, Jon Schatz and Jay Patterson.

Wendy Pyper meets a team of mango breeders determined to coax more fruit from our delectable but erratic Kensington Pride.

In the 1880s, the northern Queensland town of Bowen was the centre of a thriving horse trade with the British Army in India. Ships anchoring at the port often brought exotic spices and fruits, including the succulent mango.

According to historical notes, ships' masters working in the remount trade made strategic gifts of mango fruit to prominent locals. Bowen harbour and customs officer, GF Sandrock, was one such recipient, and the seeds of his mangoes were planted by a farmer, William Lott. Some years later, Lott's son Harry planted seeds from the best trees at his own property, 'Kensington'.

The 'Kensington Pride' mango proved popular on the Sydney markets, and has since become the main cultivar supplying Australia's $100 million mango industry.

Kensington Pride is popular in Australia for its flavour and texture, but the variety suffers from a range of production problems including erratic flowering and fruiting and a short post-harvest shelf life. The former causes significant fluctuations in annual production, while the latter reduces the fruit's export potential.

Most of Australia's mangoes are grown in Queensland, but the Northern Territory is capturing a larger slice of the market, with about 800 000 mango trees planted and an annual production value of some $25 million a year.

According to Queensland Department of Primary Industry (QDPI) research horticulturist Dr Ian Bally, the biggest problem with Kensington Pride is its poor productivity across much of this growing region.

'The fruit also breaks down quickly and can't be exported to Asian or European ports unless it's via air. This limits the amount that can be exported,' he says.

The Australian National Mango Breeding Program was initiated in 1994 to help tackle these problems.

The program is a joint venture between CSIRO Plant Industry, QDPI, the Western Australian Department of Agriculture and the Northern Territory Department of Primary Industries and Fisheries (NT DPIF).

It aims to develop new mango cultivars that retain many of the favourable Kensington Pride attributes, and possess reliable production characteristics such as a longer shelf-life and less susceptibility to sap burn and post-harvest diseases and disorders.

Since its inception, the program has produced about 1850 hybrid trees from 33 parental combinations. Bally says about 60% of the male parents are Kensington Pride, crossed with female cultivars from Florida, India and South-East Asia. The other parental combinations include different cultivars from Indonesia and Thailand.

Hands on

Unlike other mango-breeding programs around the world, the Australian program uses a hand pollination technique to produce hybrids, rather than the more random, open-air pollination.

While this method is labour intensive, it ensures that the parentage of each hybrid is known.

The crosses were made by each participant in the program in Western Australia, Northern Territory and north Queensland. From the hybrids produced, two trial sites were established, at Southedge near Mareeba (managed by QDPI) and at Coastal Plains near Darwin (managed by NT DPIF).

A target of 50 hybrids per parental combination was achieved in most cases, and the resulting trees and their fruit were evaluated using a range of measurements and taste tests.

'We evaluate the fruit for about 20–30 characteristics including internal and external colour, flavour, skin thickness, size and weight, flesh texture, shelf life and seed recovery,' Bally says.

'We also look at tree performance, and characteristics that benefit the grower in terms of mango production, such as vigour, canopy shape and yield.'

So far, 800 hybrids have been evaluated and nine have been placed on an 'A-list'.

One embryo, one **cross**

MANGOES belong to the same family as the Jamaica plum, pistachio and cashew nut, and are thought to have come from two geographic regions: India and South-East Asia.

Cultivars from India (including Alphonso, Padiri and Bangalora) reproduce via a 'monoembryonic' or single embryo seed. When female flowers are fertilised, a seed containing a single zygotic (sexual cross) embryo develops. Most plants follow this sexual reproductive mode.

Cultivars originating in South-East Asia, however, (including Kensington Pride and Nam Dok Mai), produce 'polyembryonic' seeds.

'When a flower from a polyembryonic tree is fertilised, multiple embryos are produced in a single seed,' QDPI research horticulturist, Dr Ian Bally, says.

'One of the embryos in the polyembryonic seed is the zygotic embryo. The others are asexual, nucellar embryos with the same genotype as the parent tree.

'Nucellar embryos are formed from adventitious budding of the nucellar tissue around the embryo sack. As these buds develop the cells differentiate and become viable embryos.

'If you plant a polyembryonic seed, between two and 15 seedlings can come up and you won't know which is the true zygotic seedling without doing all sorts of tests. So you can't guarantee you'll have a cross.'

For this reason, the Australian National Mango Breeding Program only crosses male Kensington Pride plants with monoembryonic female varieties.

Top left: The Kensington Pride mango has acquired a variety of names including 'Kensington, 'Bowen Pride', 'Bowen Apple', 'Bowen Special' and just plain 'Bowen'.

Many other varieties of mangoes are grown in Australia. They include the R2E2 variety developed by the Queensland Department of Primary Industries, Nam Doc Mai from Thailand (above left), Kent, Irwin (left), Honeygold, Celebration and B74.

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These may have commercial potential and will be vegetatively propagated and grown in larger replicated trials to boost the amount of fruit available for further evaluation. Bally says the program is now negotiating with industry to take an active role in testing and commercialisation of the A-list varieties.

'By involving a commercialisation partner early in the project, we can maximise industry input into the selection process and utilise their resources in coordinating grower testing of potential varieties around the country,' he says.

'It is important to test the performance of these varieties on different soils and under different climatic conditions.

'Then when we do release a variety we can give growers some sort of assurance on its performance in different parts of Australia.'

Bally expects that trial plots on selected growers' properties will consist of about 10 trees of each A-list variety. This will provide enough fruit for export trials and other market tests as well.

In the future, the project team would like to tackle the difficult long-term goal of introducing some resistance to anthracnose, the major post-harvest fungal disease of mangoes.

To make hybrids, bags are placed over the inflorescence or 'panicle', just before the parent plants flower, to prevent foreign pollen entering. The next morning, up to 12 flowers will have opened. These are emasculated and pollen from the desired male parent is introduced. A capsule is placed over the flower to prevent the entrance of foreign pollen, and the remaining unopened flowers on the panicle (which can hold up to 500 flowers) are removed. Each panicle can set 50-100 fruit, but trees generally hold only one fruit through to harvest, for every two or three panicles. According to Dr Ian Bally of QDPI, a lot of natural fruit drop and a lot of hand-crossed pollination is lost in this way. 'That's why most mango-breeding programs don't use this technique,' he says. 'But the advantage is that we know which tree was the male parent.'

Furthering flowering

The National Mango Breeding Program will provide a long-term solution to the Kensington Pride shortfalls. But short-term help is also needed for growers whose livelihoods are based on hundreds of established and newly planted Kensington Pride trees. This problem is particularly acute in the Northern Territory, where the climate is less favourable to reliable flowering and fruiting.

According to Dr Richard Brettell, leader of the CSIRO Plant Industry Horticulture group based at the Tropical Ecosystems Research Centre (TERC) at Darwin, high night temperatures during the early dry season are partly to blame.

'You need a sustained cool period, where night temperatures go below 20˚C in order to get good flowering,' he says.

'But in Darwin there's a degree of variability from year to year, and in 2001 there were only five nights in June where the temperature went below 20˚C.

'This is one of the reasons why trees in the Territory are producing about five tonnes per hectare when they should be yielding 20 tonnes.'

When poor yields are coupled with the high costs of harvesting, packing and transport, mangoes cost growers about $13 a tray (7.5 kg). But when fruit floods the market, growers may receive only $10–$15 a tray.

To try and improve flowering, Plant Industry scientist Dr Sam Blaikie, in collaboration with NT DPIF and local growers, has conducted a three-year trial of two flower inductive treatments.

The first treatment used a commercially available growth retardant called morphactin, which arrests shoot growth.

'Trees grow large and unmanageable very quickly in this climate, and if they're producing leaves they can't produce flowers,' Blaikie says.

'But if we can arrest vegetative growth before the cooler temperatures come in, the chances of flowering will increase.'

Experiments with morphactin or 'mango flowering treatment' (MFT) on 12 properties in the Darwin / Katherine region, increased yields by 150%, on average. MFT treated trees also flowered and fruited earlier than controls, a positive effect that would allow growers to capture the more lucrative start-of-season market.

The second trial tested a commercially available soil drench known as 'Cultar®', which is based on paclobutrazol (PBZ). PBZ inhibits the synthesis of the plant hormone gibberellin, associated with vegetative growth, but it has a reputation for producing unreliable results.

The trial revealed that when the weather was conducive to flowering, PBZ, like MFT, boosted the yield response of trees by an average of 1.5 times, compared with controls. Flowering and fruiting also occurred earlier.

In the absence of a prolonged cool period, however, flowering was reduced in both controls and PBZ-treated trees.

'This suggests that the response to PBZ treatment depends on the weather,' Blaikie says.

'If the weather is with you, then PBZ boosts performance compared with

Right: The temperature in Darwin typically hovers at about a 35°C maximum for most of the year, with a minimum of 20–25°C. For good flowering of Kensington Pride, however, a sustained cool period, with night temperatures below 20°C, is needed in the early part of the dry season (May).

Below right: A characteristic profile of carbon assimilation in field-grown trees of Kensington Pride in the Northern Territory. Carbon assimilation rates are low during the dry months of June to October, just when the demand for carbon is the highest. To produce fruit then, the trees must rely on their carbon reserves for initial fruit development. 'Flushing' is associated with growth in mangoes and other species. In mango, a group of leaves – about 20 leaves for Kensington Pride in the NT – constitutes a single growth event, which is called a flush.

Bottom right: CO_2 assimilation is negatively correlated to Vapour Pressure Deficit, or VPD (note the reverse scale in the figure). During times of high VPD (June–October), when the air is relatively dry, leaf stomata remain almost fully closed to conserve water. This restricts the amount of CO_2 that can be assimilated by photosynthesis. Management practices can do little to overcome this problem at the leaf level.

controls. But when the weather is not conducive to flowering, you cannot rely on PBZ.'

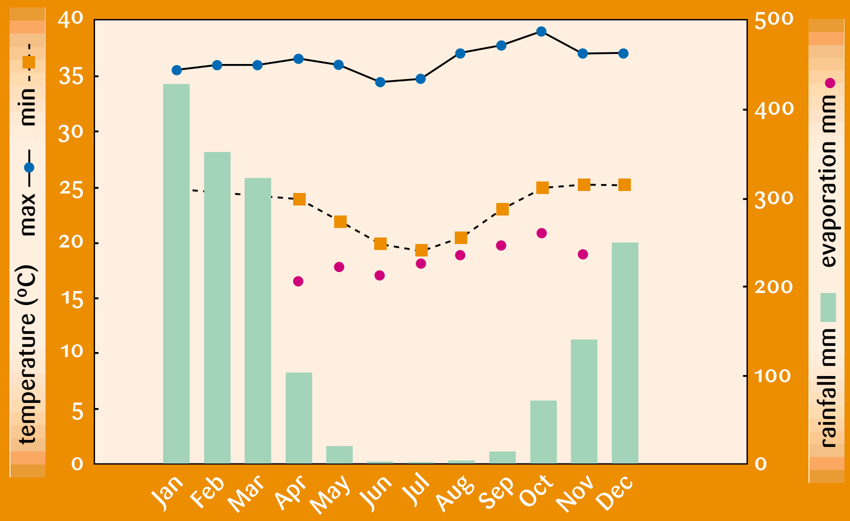
The most likely recommendation to come out of the trial will be for growers to use MFT to boost flowering. However the treatment used will also depend on the size of the tree.

'On some properties we achieved an enormous yield response to MFT or PBZ and on others we had a zero or negative response compared to the controls,' Blaikie says.

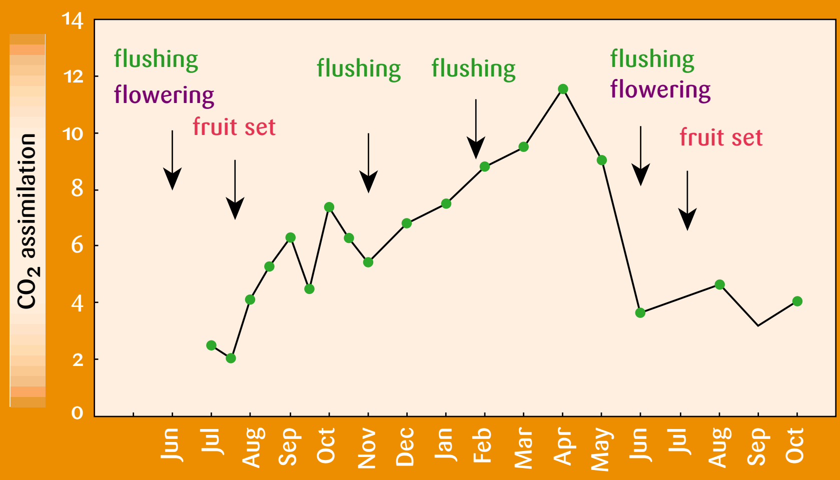
'We've linked this variation to the size of the tree. Large trees of 90–120 cm in circumference are well suited to MFT, while middle-sized trees (60–90 cm) can be treated with either PBZ or MFT. However small trees (less than 60 cm) are best treated with PBZ.'

'What's important to realise is that there is no panacea for poor and erratic flowering in mango. No treatment will be right for every situation and we're starting to understand the nuances of that.'

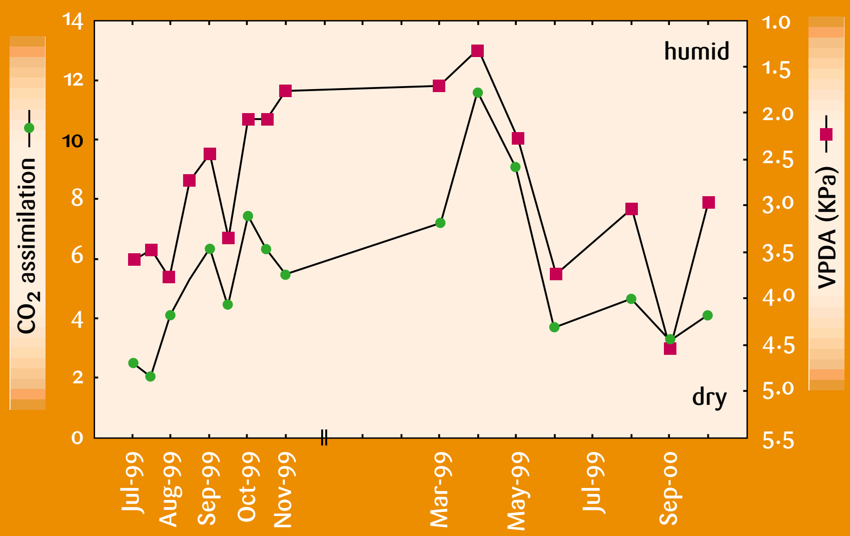
Darwin NT Australia



Phenophysiological profile of mango
(Kensington Pride) Northern Territory Australia



Seasonal effect of VPDA on photosynthesis of mango
(Kensington Pride)



Seeking mango **flowering** genes

THE CSIRO Plant Industry Horticulture group is investigating almost every facet of mango biology, including the molecular aspects of mango flowering.

For the past 18 months, plant molecular biologist Dr Anna Padovan has been using genes known to regulate flowering in *Arabidopsis* – the 'green rat' of the plant world – to identify flowering gene counterparts in mango. So far she's found three: 'Leafy', 'Terminal Flower1' or TFL1 and 'Apetala1'.

According to Padovan, the *Arabidopsis* Leafy gene 'tells' the shoot tip to become a flower. Apetala1 performs a similar role by promoting floral formation and being involved in the development of floral organs. TFL1 works in an opposite manner, by maintaining vegetative growth.

'Now that we've found counterparts to the *Arabidopsis* genes in mango, we want to find out if they have the same role in controlling flowering,' Padovan says. 'Just because they're in mango doesn't mean they have the same function, So we're trying to measure the levels of expression of the genes before flowering and leading up to flowering.'

To do this, Padovan collects 10 buds per tree at weekly intervals, beginning in April, and examines gene expression by measuring the messenger ribonucleic acid (mRNA) made from each gene.

MRNA is the template for the protein product of genes, and the amount of mRNA in a sample provides a measure of how strongly a gene is expressed.

'We hope to see increased levels of Leafy and Apetala1 leading up to flowering, and a drop in TFL1, if these mango genes are really involved in mango flowering,' Padovan says. 'But how soon before flowering we can detect the gene products remains to be seen.'

Padovan says that in the future the expression of these three genes could be compared among mango cultivars. As flowering is often poor in Kensington Pride, Leafy expression, for example, may be weaker in Kensington Pride than in better flowering cultivars.

'This would be another piece of evidence that Leafy is important in mango flowering,' she says.

The mango genes will also be inserted into the genome of *Arabidopsis* to see if they switch flowering on. This would provide more direct proof that they regulate flowering.

It is hoped the work will eventually allow scientists to predict when flowering in mango will occur and to what extent. This information could then be developed into simpler detection tools to help advise growers.

'Tree management strategies would be far simpler if growers knew with surety when flowering was due to commence,' Padovan says.

New light on photosynthesis

Unfortunately, increased flowering does not necessarily lead to increased fruit production, and Blaikie, together with CSIRO's Dr Alonso González and Dr Ping Lu, have found that low rates of photosynthesis during the dry season could be a contributing factor.

'During the wet season, maximum rates of photosynthesis are achievable,' González says. But during the dry season, photosynthesis drops by up to 80%.'

González says this dramatic decline in photosynthesis is due in part to the trees' response to the amount of water in the air, measured as water vapour pressure deficit .

During the dry season in the Northern Territory (May–October), the vapour pressure deficit is high, which means the air is relatively dry.

Under these conditions, leaf stomata are almost closed to conserve water, but this restricts the amount of CO_2 that can be assimilated into sugars by photosynthesis. Even if more water is pumped onto the soil, the rate of photosynthesis remains low.

'Our concern is that from June to October, when mango fruits are developing, the trees' demand for sugars is higher. But if the photosynthetic apparatus is not operating at full capacity, this could reduce the productivity of the trees,' González says.

The team is looking at ways to increase carbon assimilation, and one approach is to manipulate the tree canopy.

In the Northern Territory, mango trees grow large and dense, so most photosynthesis occurs in the upper layer of leaves, where light can penetrate. Opening up the canopy by chopping off branches in strategic places could alter the amount of photosynthesis the tree is capable of, and therefore reduce the amount of carbon and energy-consuming respiration.

To determine the feasibility of this approach, the CSIRO team is measuring the amount of photosynthesis undertaken by leaves that are external, intermediate, or deep in the canopy. These measurements will form part of a mathematical model that simulates the effect of canopy pruning on photosynthesis and respiration.

A second potentially yield-dampening phenomenon that mango trees, and physiologists, must contend with, is photoinhibition.

'Photoinhibition occurs when external stresses such as high light levels cause damage to the photosynthesis machinery,' González says.

'Depending on the level of stress, photoinhibition can be temporary or more permanent, resulting in long-term effects on tree productivity.

'When the leaves are experiencing a high VPD and their stomata are closed, they can't utilise this excess light via photosynthesis and photoinhibition is the consequence.'

A long-term strategy to cope with the excess light is the development of extra pigments such as carotenoids and xanthophylls in the leaves.

These filter or reflect the light, but reduce the ability of the leaves to photosynthesise efficiently under more advantageous light and vapour pressure deficit conditions.

The introduction of new mango cultivars that don't suffer so dramatically when vapour pressure deficit is high, could provide a long-term solution.

Mangoes originated from two gene pools, which differ from each other in the way they reproduce (see story on page 15). Studies have shown that these monoembryonic and polyembryonic gene pools may also operate differently physiologically.

'Our data support the idea that there are genetic differences in mango in response to photoinhibition, and we're now exploring how the two gene pools respond to conditions of high light,' González says.

While photoinhibition and low photosynthetic rates may contribute to the poor performance of Kensington Pride, González says there are many other factors to consider.

'We're trying to dissect all the factors and test them so we can design a management plan for growers that will enable efficient mango production,' he says.

Parts of this project were funded by Horticulture Australia, the Australian Centre for International Research, mango growers in the NT and the Rural Industries Research and Development Corporation. NT DPIF is now known as the Northern Territory Department of Business, Industry and Resource Development.

More about breeding mangoes

More about mango research by the Tropical Ecosystems Research Centre is available on the web at http://www.terc.csiro.au/pi-/mango.asp.

Below: To measure photosynthetic rates of whole trees, a chamber is placed over the small tree to seal it off from the external environment. A fan is used to force air into the chamber and air pumps are used to sample air before and after it has passed through the chamber. Sensors measure the amount of CO_2 taken up by the plant and the amount of water released. The measured changes in CO_2 allows the scientists to calculate the plant's photosynthetic and respiration rates.

Above: For large trees, a bag is used to measure the photosynthetic rate of individual branches.

Abstract: Kensington Pride is the main cultivar supplying Australia's $100 million mango industry, but it suffers from a range of production problems, including erratic flowering and fruiting. CSIRO Plant Industry's Horticulture group in Darwin is involved in projects spanning mango breeding, physiology and molecular biology. A national mango breeding program aims to develop new hybrids that combine desirable Kensington Pride characteristics with improved production characteristics from other cultivars. Nine promising hybrids from more than 1850 generated in the past seven years, will soon be evaluated in commercial trials. In the short-term, growers may use chemical treatments to improve flowering and fruiting. Physiologists are investigating ways of improving photosynthesis and understanding a second yield-dampening phenomenon, photoinhibition. Three genes involved in mango flowering have been identified and research is under way to confirm their role in flowering and measure their expression during and leading up to flowering.

Keywords: mangoes, cultivars, plant breeding, flowering, photosynthesis, plant physiology, photoinhibition, Australian National Mango Breeding Program, Kensington Pride.