The intention of this column is to throw a *spotlight* on individual organisms — not to blind you with science but to reveal important and fascinating aspects of specific plants, animals and microorganisms.

Papaya

Genetic engineering success?

Key words
- Agriculture
- GM crops
- Papaya
- Virus

The papaya 'tree' is actually a tall herbaceous plant found in tropical and sub-tropical areas of the world. It is a useful crop but unfortunately the plants are susceptible to many diseases.

Papayas are known as tropical superfruits — they are rich in vitamins A, C and K, complex B vitamins, potassium, calcium, iron and dietary fibre. As they ripen, the outer rind changes colour from green to yellow and the flesh changes to yellow, orange or red. The brightly coloured fruit, which also has a sweet scent, attracts animals, which help to disperse seeds away from the parent plant in various ways. Fruit may be dropped, seeds can be cleaned off fur or feathers, and some seeds can survive passage through an animal's gut and be deposited in a heap of fresh fertiliser! Unripe, green fruits are cooked and eaten by people, particularly in stews, as they contain a **proteolytic enzyme** called papain that acts as a meat tenderiser. So as well as being useful as food, papayas can be collected and used commercially to extract the papain (see Box 1).

Box 1 Fruit juice

The enzyme papain is found in the milky juice (latex) that oozes out of unripe papayas when they are wounded. It probably functions as part of the plant's defences against attack by herbivorous animals and insects. To produce papain, mature but unripe (still green) fruit are scored and wrapped in plastic overnight. The latex is collected in the morning and dried to produce raw papain. This is further refined and ground to produce a fine powder, which can then be used commercially

Papain digests proteins so it is well known as a meat tenderiser (for example to increase the digestibility of pet food). However, it is also used in many other industries such as brewing (to stop beer turning cloudy when chilled), leather production (to prepare hides for tanning) and the pharmaceutical industry (for example, as an ingredient in contact lens cleaning solutions).

Papain and unripe papayas have long been used in traditional medicine to treat complaints such as indigestion, parasitic worms, swellings, stings and bites. However, while popular in herbal remedies, there is little evidence of the effectiveness of papain in clinical trials and papaya latex can cause allergic reactions in some people.

Growing papayas

Papaya is native to Central America and has been cultivated from as early as the sixteenth century. It grows up to 9 m tall and the leaves and flowers emerge directly from the stem (see Figure 1). Like other cultivated plants, many varieties are available. The variety can be chosen for its suitability for the particular environment that it is to be grown in, or for the fruit type needed. Papayas exported overseas (for example, to Europe) tend to be the

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Figure 1 A papaya plant with fruit.

smaller 'solo' types (so called because one man can eat it all), whereas at local markets the preference is for larger fruits weighing up to 9 kg (see Figure 2). The skin of the ripe fruit is easily damaged, so export crops are picked early and shipped while still partly green. As well as commercial-scale growing, the papaya is also popular in gardens and for small farms, as it is a relatively compact tree that needs little looking after. It starts producing fruit 9–12 months after sowing and crops heavily.

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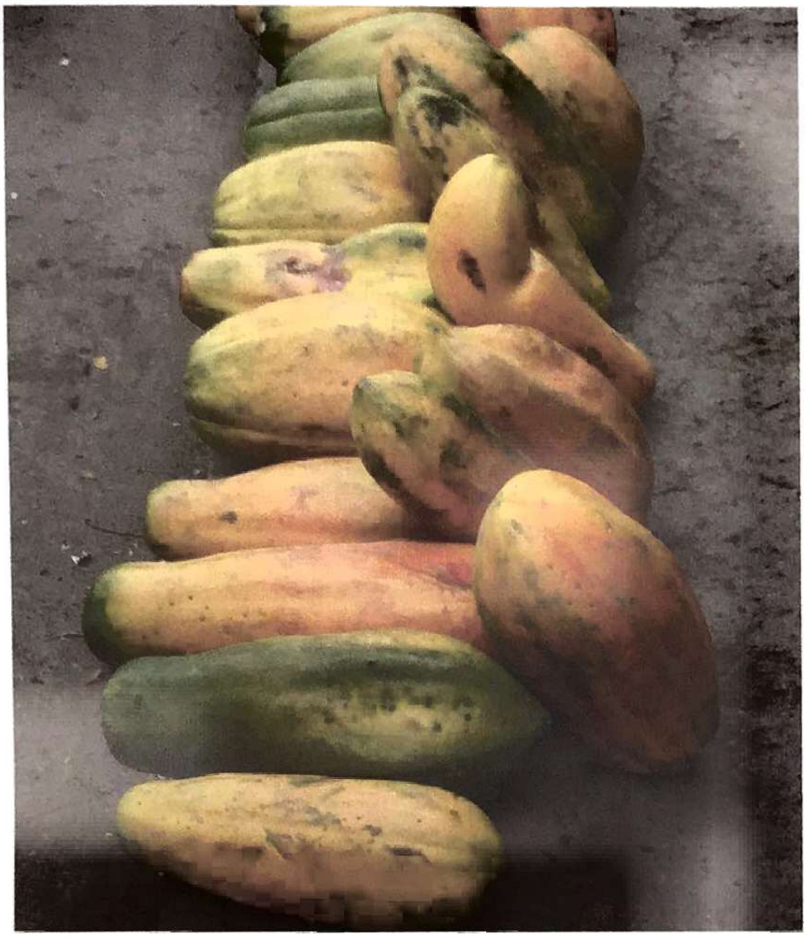

Figure 2 Ripe papayas on sale at a local market.

Ringspot disease

Unfortunately, papaya plants are subject to attack by many insect pests, as well as fungal and viral diseases. The biggest threat to cultivation comes from the papaya ringspot virus (PRSV). PRSV is a flexible, rod-shaped virus consisting of a single-strand of RNA protected by a coating of proteins. It is transmitted by the **stylet** of aphids as they probe the plant looking for suitable feeding sites. There are two different types of PRSV — P and W, but only type P can infect the papaya family. Additionally, there are many different **strains** of the virus, which are found in different locations across the world. Scientists studying the genetic sequences of PRSV coat protein have found that the greatest diversity occurs in India, perhaps suggesting that this is where the disease originated.

The virus is named for the ringspots that develop on the fruit (see Figure 3), but diseased trees show several other symptoms, including oily streaks on the **petioles** and stem, **chlorosis** and distortion of leaves, stunted growth and flower drop. The effects of this disease on an orchard

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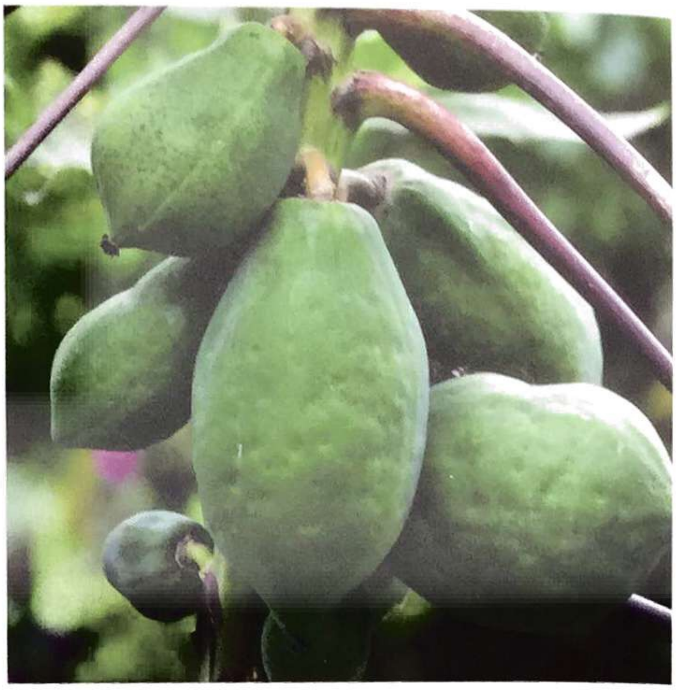

Figure 3 An unripe papaya showing spots characteristic of ringspot disease.

are devastating — fruit quality and yield are poor and eventually the plant dies. To deal with an outbreak, farmers must uproot and burn the infected plants, removing all possible hosts and quarantining orchards. Breeders have tried to develop resistant plants using conventional cross-breeding techniques, crossing the commercial plants with wild relatives that show resistance to PRSV. These attempts have met with limited success. Deliberately inoculating young plants with a mild strain of the virus (known as cross-protection) can reduce the severity of the disease or delay the development of more serious symptoms. However, none of these methods can prevent the spread of PRSV or give complete protection to the plants.

A GM solution?

Although the success of cross-protection was limited, it inspired scientist Dennis Gonsalves and his collaborators to attempt to develop papayas with 'pathogen-derived resistance' (PDR). These are **transgenic** plants that have been modified to include genes from the **pathogen**, which results in protection from the disease. Normally a pathogen such as a virus would use the processes taking place in a plant cell to reproduce itself, but PDR interferes with this hijack of the plant cell. PDR was first used against plant viral disease in the 1980s with tomato, and in 1989, Gonsalves and his team began trying to transform papaya using a method called particle bombardment. DNA including the gene for the PRSV coat protein coupled to an antibiotic resistance marker gene was coated onto tungsten particles

Further reading

Gonsalves, D. (2004). 'Transgenic papaya in Hawaii and beyond', *AgBioForum*, Vol. 7, Nos 1 and 2, pp. 36–40: **http://tinyurl.com/2cbkv3**

Terms explained

Biosafety Safety procedures dealing with biological research and its effects on people and the environment.

Chlorosis Leaf yellowing caused by loss of the green pigment chlorophyll.

Gene escape Transfer of genes from a transgenic organism to a wild relative.

Pathogen A disease-causing agent.

Petiole The stalk attaching a leaf to a plant.

Proteolytic enzyme An enzyme that digests the bonds between individual amino acids in a protein.

Strain A genetic variant of a microorganism.

Stylet The piercing and sucking mouthpart of an aphid.

Transgenic Containing a gene transferred from another organism.

and fired at the plant tissue. This bombardment technique does not manage to insert the DNA into all the treated cells, so it is first necessary to separate out those cells that have the new DNA from those that have not taken it up. Those cells that have incorporated the foreign DNA into their genome can be selected out using their antibiotic resistant properties — the antibiotic will kill only those cells without the foreign DNA. Once a population of transgenic cells is obtained, tissue culture methods are used to grow them into plantlets.

By 1990 the team had succeeded in developing the first transgenic papaya, called 'SunUp'. In Hawaii there are no wild plant relatives of the commercial papaya crop so **gene escape** into the natural environment was not thought likely and field trials of the transgenic variety were soon approved. Following the success of this trial, 'SunUp' was then crossed with a papaya variety that was popular with both growers and consumers in Hawaii to create 'Rainbow', which became commercially available in 1998. Both varieties have excellent resistance to Hawaiian strains of PRSV, showing no disease symptoms and cropping heavily even when neighbouring non-GM plants were dying. This was very timely, because in 1992 the Hawaiian papaya industry had suffered a serious outbreak of PRSV disease. Consequently, the Hawaiian papaya growers quickly adopted the new transgenic varieties.

The future for GM papaya

The development of transgenic papaya varieties rescued the Hawaiian papaya industry. Over 10 years of cultivation later, the GM papayas remain the most successful attempt so far to combat PRSV. However, the very success of these transgenic plants could threaten their future. Whenever crops are grown as monocultures — i.e. the same crop is grown over large areas of land, year after year — there are inherent risks. Pests and diseases evolve all the time and can eventually overcome the plant defences. In addition, all the papaya plants are genetically similar to one another and this means that if a new strain of PRSV evolves that is able to infect one plant, then it is highly likely to be able to infect the others.

Future research may, therefore, be needed to improve genetic diversity of the GM papayas in the fields. Greater variation in the genetic background of the crops in a field means it is less likely that they will all succumb to a disease. Additionally, the plant resistance in 'SunUp' is based on the expression of the coat protein gene from a Hawaiian strain of PRSV. If crop developers want to grow GM papayas elsewhere, they would have to consider not only the preferred local fruit variety but also the local PRSV strains. Plant biotechnology institutes in many countries, including Thailand, Venezuela and Jamaica, have been keen to capitalise on the knowledge and technology developed in Hawaii. Several region-specific transgenic papaya cultivars have been developed, but currently Hawaii remains the one location where GM papayas are grown.

Problems

The tide of public opinion has turned against GM crops since the introduction of the Hawaiian papayas and no new cultivars have been released for cultivation. In each case, problems have arisen, including concerns over ineffective **biosafety**, bioethical concerns and difficulties with legislating for these plants. To address these problems, researchers have done further studies looking into subjects such as the public perceptions of the crop, the safety of the fruit for human consumption and the impact of the crop in its environment. As a direct result of these studies, Japan has ruled that Hawaiian transgenic papayas pose no threat to human health and has lifted the import ban on them.

Researchers have now sequenced the transgenic 'SunUp' papaya genome. It was published in the journal *Nature* in 2008, making it the fifth plant and the first transgenic fruit crop ever sequenced. Greater understanding of the genetics of papaya gives scientists an opportunity to make further progress by adding other pathogen-resistance genes. This might help not only against PRSV, but also against other viruses such as papaya mosaic virus and against fungal diseases such as mildew and blight. However, whether new papayas are developed and are a commercial success anywhere outside the USA depends on the willingness of producers and consumers to grow and buy transgenic produce.

Advocates for transgenic crops argue that GM technologies are essential tools to avoid future problems with food availability, resulting from increasing world population pressures and environmental change. In the near future, however, public suspicion of GM foods is likely to continue to limit their commercialisation worldwide. Even in Hawaii, producers of organic or non-GM papayas are critical of their continued use. One thing is certain: this debate will continue for many years to come, and the success and failures of existing crops such as 'SunUp' and 'Rainbow' papaya will contribute to the case-by-case discussions for each new application of GM biotechnology.

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