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Summary

Papaya is cultivated primarily in tropical and sub-tropical regions. It is an important part of the diet in many developing countries. It is the source of the enzyme papain that is used in the pharmaceutical and food industries. For several decades the global production of papaya has been threatened by ringspot disease caused by the papaya ringspot virus (PRSV).

PRSV was identified for the first time in 1945 in the American state of Hawaii. Since then there have been outbreaks of ringspot disease in other areas of the world where papaya is cultivated. Hawaii is responsible for just 0.1% of global papaya production. Even so, it will always be associated with the fruit thanks to pioneering work in the development of a PRSV resistant strain of papaya.

In an attempt to escape the PRSV, papaya cultivation was moved from the island Oahu to the Puna region. However, in the early 1990’s PRSV was also reported there. Between 1992 and 1997 papaya production dropped more than 30% and local papaya cultivation was considered doomed. Researchers from Cornell University (USA) and the University of Hawaii developed a genetically altered variety of papaya that is resistant to PRSV. A remarkably efficient development and approval procedure allowed the GMO papayas to be introduced as soon as 1998. In less than four years’ time papaya production was back to pre-PRSV levels. Hawaiian papaya cultivation had been saved. In the meantime, Hawaii has started exporting its biotech papayas to Canada and Japan.

Due to the specificity of the virus and the virus resistance mechanism Hawaiian GMO papayas are resistant to the Hawaiian PRSV in particular. However, the developments on Hawaii have inspired and stimulated other papaya cultivating countries to use the same methods to develop virus resistant papayas for their local markets. Resistant papaya varieties have now been developed in Brazil, Taiwan, Jamaica, Indonesia, Malaysia, Thailand, Venezuela, Australia and the Philippines.

Since 2007 China has also been growing its own domestically developed PRSV resistant GMO papayas. The cultivated area devoted to GMO papayas was 3550 ha in 2007 and had risen to 6275 ha by 2012; more than 60% of the total cultivated land area used for papayas in China.
In 2011 nearly 12 million tonnes of papaya were grown on just over 420,000 ha globally, with a total market value of 200 million US dollars. At the global level papaya is the third most important traded fruit.

Papaya is grown in approximately 60 countries, especially developing countries, with India as the leading producer, followed by Brazil, Indonesia, Nigeria and Mexico.

Despite the fact that Hawaii makes up for just 0.1% of global papaya production and that papaya is only its fifth largest crop, Hawaii is still the world leader in research and development relating to papayas.

The papaya ringspot virus is the cause of the most widespread and most destructive disease affecting papaya cultivation. The ringspot disease can cause serious decreases in quality and yield and can even result in the loss of the entire harvest.

PRSV was discovered in 1992 in commercial orchards on Puna, the most important area for papaya production in Hawaii. The virus spread extremely fast and by 1998 papaya production on Puna had nearly halved.

The public sector anticipated the destructive effect of PRSV. Researchers from the University of Hawaii and Cornell University developed genetically altered papayas with resistance against PRSV.

The virus resistant GMO papaya was approved for cultivation in the United States in 1998. In four years the biotech papaya had stopped the decline of Hawaii’s papaya industry and brought production back up to the level it was prior to the emergence of the PRSV.

In 2009 the virus resistant ‘Rainbow’ papaya was cultivated on approximately 676 hectares in Hawaii, 77% of the total papaya acreage.

In 2003 Canada gave the green light to the import of Hawaiian GMO papayas, followed by Japan in 2011.

China developed its own virus resistant GMO papaya. In 2007 this papaya was cultivated for the first time on 3550 ha. Five years later this area had grown to 6275 ha. In Guang Dong province, the most important papaya growing region in China, the level of adoption is 95%.
Papaya cultivation and the papaya ringspot virus

Nearly 12 million tonnes of papaya are produced per year globally. Production in the United States makes up only 0.1% of the worldwide production. Even so, Hawaii will always be mentioned when papaya cultivation and research are brought up. After all, Hawaii successfully developed and introduced the first genetically altered virus resistant papayas.

Global papaya cultivation under threat

Papaya is cultivated on a large scale in the tropics. Not only are the fruits a significant source of antioxidants, vitamins and minerals, they are also the source of papain. This enzyme is used in various industrial processes (e.g. softening leather) as well as in the production of pharmaceutical products and cosmetics (e.g. treatment of scars).1 In 2011 a total of 11.8 million tonnes of papaya were produced, with a market value of 200 million US dollars.2 Papaya is cultivated in approximately 60 countries, especially in developing countries. With a cultivated area of more than 100,000 ha and a production of 4.2 million tonnes, India is the easily the global leader. In production terms India is followed by Brazil (1.8 million tonnes), Indonesia (0.96 million tonnes), Nigeria (0.71 million tonnes) and Mexico (0.63 million tonnes).2 Papaya is the third most important traded fruit on a global scale.

The global papaya industry is under threat from two significant problems. Post-harvest losses due to fungal infections and/or mechanical damage of the fruit result in economic damage. The inadequate infrastructure in the tropics (lack of refrigerated storage and absence of a road network) is often the underlying cause. However, the biggest problem affecting papaya cultivation is the papaya ringspot virus (PRSV). This plant virus appears worldwide, wherever papaya is cultivated.3 It causes a drastic decrease in yield and in the worst cases can even completely destroy papaya plantations.

The name ‘ringspot’ comes from the ring-shaped spots that the virus causes to develop on the fruit of infected papaya plants (Figure 1).3 PRSV destroys the leaves’ capacity to photosynthesise.4,5 Infected plants develop a whole range of symptoms: the leaves fade and turn yellow and the plants remain small and generally lack vigor. If infection occurs in the first few months following planting, the papaya plants usually do not produce any fruit at later stages. Infection of older plants causes delayed and reduced fruit formation. In addition the fruits are smaller, mottled and have a lower sugar concentration resulting in a drastic reduction in the quality of the papayas.

Figure 1. The papaya ringspot virus causes ring shaped spots on infected papayas.

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2 FAOSTAT. Available via http://faostat.fao.org/site/567/default.aspx#ancor
4 Photosynthesis is the biochemical process used by plants to convert energy from sunlight into sugars.
Papaya (Carica papaya L.) grows best in warm, humid, tropical regions and originated in the tropical regions stretching from South Mexico to the Andes in South America.10 According to their botanical characteristics papayas are perennial herbaceous plants, not trees.11 They grow to a height of six to nine meters and have a single stem (see photo). Papaya plants can remain productive for up to ten years but in most commercial plantations the plants are replaced every three years.3 Depending on the papaya variety and its surrounding conditions fruit formation can start between 6 and 12 months after planting. The fruits then need 5 to 9 months to fully develop.11 It is therefore possible to harvest papayas within a year and to replant them every year. In order to save on labor and new plant material this annual cultivation cycle is only used when disease and pests make multiannual cultivation impossible.

Viruses are made up of genetic material (DNA or RNA) encased in a protective protein coat. They are only capable of multiplying using infected cells, and need the infected cell’s molecular machinery in order to replicate their genetic material and the viral coat proteins that make up the protein coat.

There is a great variety of different kinds of virus. Some infect animals, others infect plants or bacteria. Plant viruses are either rod-shaped (like the papaya ringspot virus) or spherical. They show great variation in size but the largest plant viruses are still extremely small (one thousandth of a millimeter).

During a typical infection process of an animal or bacterial cell the virus attaches itself to the cell in order to inject its genetic material or to fuse with the host cell. Plant cells on the other hand have sturdy cell walls that plant viruses cannot penetrate without help. For this reason plant viruses are generally transferred when damage has been caused by other organisms, such as aphids. The first step in infection is the uncoating of the virus.13 A few dozen coat proteins are removed from one side, partly exposing the genetic material. The plant’s ribosomes recognize this genetic material. Ribosomes are specialized deciphering machines that can translate the message that is hidden in the virus’ genetic material into the production of proteins. While the ribosomes are translating the virus’ genetic material, the virus continues uncoating.13 In this way new coat proteins and other proteins necessary for the virus’ multiplication are made. The virus effectively puts the infected cells to work making new virus particles. Once the virus’ genetic material has multiplied sufficiently and enough coat protein has been synthesized, the genetic material is recoated by the coat proteins. The newly formed virus particles then infect new cells and if the infection is not stopped this can lead to the eventual death of the host organism.

PRSV is a virus that is spread primarily by aphids.6,7 The aphids take in virus particles while extracting sap from infected plants. When these aphids move on to healthy plants and extract sap the virus particles are transferred to the healthy plants. To prevent the virus from spreading, papaya plantations are treated with aphicides (insecticides that target aphids) and affected plants are destroyed immediately.8,9

10 http://www.fruit-crops.com/papaya-carica-papaya/
12 RNA or ribonucleic acid is made up of a long chain of nucleotides, just like DNA. In higher organisms its main function is to transfer the information stored in DNA to the production of proteins. In the absence of DNA, such as in plant viruses, RNA can also serve as a carrier of genetic information. It can appear in both single and double stranded forms.
The papaya industry in Hawaii

Papaya production in the United States is relatively low key when compared to the larger producers. All the plantations are located in the state of Hawaii. According to national statistics the area used for cultivation of papaya in Hawaii in 2011 was just over 800 ha, of which 526 ha of papaya plants were harvested. So with a yield of 24.7 tonnes per ha Hawaii produced nearly 13,000 tonnes of papaya in 2011. These figures indicate that the United States has just a 0.1% share of global papaya production. Despite its small scale the papaya is still very important to farmers in Hawaii where it is the fifth largest local crop. In 2011 Hawaii had 172 papaya growers, which gives an average of 4.7 ha per company.

PRSV was discovered on Hawaii around 1945 on the island of Oahu, at that time the most important papaya producing region in Hawaii. Five years later the production on Oahu was transferred to the Puna region of the main island Hawaii, where there was as yet no commercial papaya production, but also no known cases of PRSV. The area used for papaya cultivation grew steadily from 26 ha in 1960 to 910 ha in 1990. This was in strong contrast to the infected island Oahu, where the papaya acreage decreased from 200 ha in 1940 to just 20 ha in 1990.

PRSV belongs to the Potyviridae family, the largest and most economically important plant virus family. Approximately 30% of all plant viruses belong to this family. There are two types of PRSV. The P type (papaya infecting) causes ringspot disease in papaya, while the W type (watermelon infecting) is especially responsible for causing damage within the cucumber family. The papaya ringspot virus that was first described in 1949 after it caused so much damage in Hawaii was given the name PRSV-HA and is a typical type P virus. Potyviruses are RNA viruses (see text box ‘Plant viruses’, page 7). The genomic RNA consists of one strand of 10,326 nucleotides that codes for one large polypeptide made up of 3340 amino acids. The polypeptide is then cleaved into 11 functional proteins.

The RNA is enclosed in a protein coat that is made up of approximately 2000 identical copies of a coat protein. PRSV viruses are extremely small rod-shaped structures with a diameter of approximately 12 nm and a length of 760-800 nm. The molecular characterization of PRSV has progressed so much in the last few decades that it no longer holds many secrets for plant virologists. The RNA sequences and thus the genetic information of the PRSV viruses that appear in Hawaii and Taiwan have been fully sequenced.

20 1 nanometer (nm) is one millionth of a millimeter.
To everyone’s surprise Puna remained safe from PRSV for 30 years. Geographical shifting of cultivation in an attempt to escape virus infections is effective in the short term. However, viruses move with the crop. Just one PRSV infected aphid or papaya is all it takes, and sooner or later the new cultivated area will become infected. It only becomes more and more difficult to continue shifting cultivation to other places. In 1992 PRSV was discovered in the papaya plantations in Puna. At that time 95% of Hawaiian papayas were being grown in Puna. The government immediately initiated a program of eradication requiring plants from infected plantations to be destroyed. Nevertheless the virus continued to spread and in 1994 the Hawaiian state government admitted that the situation was out of control. In less than three years one third of all the plantations in Puna had become infected. The first drastic decline in production was witnessed in 1995 with a drop of 25% compared to 1994. Production dropped a further 10% in the following year (Figure 2). Over the course of four years (1994-1998) yield dropped from 25.5 tonnes per ha to 16.8 tonnes per ha, a decrease of nearly 35%. In 1998 the American government decided to allow the cultivation of the genetically altered papaya varieties ‘Rainbow’ and ‘Sunup’ on Hawaii (for more information about the development of the GMO papaya see chapter 2, page 13). This gave Hawaii’s 250 papaya growers a way to protect their crops against PRSV. The PRSV resistant GMO papayas showed results immediately. In four years ‘Rainbow’ and ‘Sunup’ not only stopped the dramatic decline in papaya production but levels of production returned to where they were prior to the PRSV outbreak (Figure 2).

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**Figure 2**

*Infection of Hawaiian papaya plantations with PRSV in 1992 led to a significant decline in production. The GMO papaya returned production to normal levels, since 2002 production has been declining due to a persistent decrease in land area used for papaya cultivation, distribution problems and unfavorable climate conditions. Source: National Agriculture Statistics Service (NASS).*
The introduction of the virus resistant papayas was a widespread success and in 1999 the ‘Rainbow’ and ‘Sunup’ GMO varieties were cultivated on 607 ha, approximately 50% of the land used for papayas in Hawaii. In 2000 ‘Rainbow’ alone made up 54% of the harvested land area and 40% of the total area cultivated for papaya in Hawaii.

The proportion of GMO papaya declined at first, only to start rising again in 2002 (Figure 3). In 2009 (latest available data) the GMO papaya ‘Rainbow’ had a share of 76% of the total harvested land. For the non-GMO papaya variety ‘Kapoho’ this share declined from 49% in 2002 to 9% in 2009.

**Figure 3**

The proportion of GMO papaya ‘Rainbow’ expressed in hectares increases in terms of both total area (above) as harvested area (below). The proportion of the GMO papaya ‘Sunup’ is included with non-GMO varieties in ‘Other’. ‘Kapoho’ and ‘Sunrise’ are non-GMO papayas.

*Source: for 2000-2008 Ref. 25 (/xpap01.pdf /xpap08.pdf); for 2009 Ref. 26.*
Despite the GMO papaya’s success in protecting papaya plantations from PRSV, production has declined since 2002. According to Stephen Ferreira at the Department of Plant and Environmental Protection Sciences in Honolulu (Hawaii) there are several reasons for this. During the PRSV crisis in Hawaii other countries took over Hawaii’s exports. Once production had recovered Hawaii had problems approaching its previous markets. In order to continue exporting to Canada and Japan, Hawaii had to continue producing the non-GMO papaya ‘Kapoho’. Cultivation of the virus resistant papayas lowered the risk of disease, but it still became increasingly more challenging to cultivate non-GMO papayas. The large decrease in production in 2002 can therefore also be attributed to the large harvested land area of the PRSV sensitive ‘Kapoho’ (Figure 3B, page 10).

In January 2003 Canada approved the import of GMO papaya. The effect on the harvested area of GMO and non-GMO papaya is clearly visible (Figure 3B: page 10). Since 2004 more GMO ‘Rainbow’ has been harvested than non-GMO ‘Kapoho’. The total area cultivated for ‘Kapoho’ has also steadily declined since 2004 (Figure 3A, page 10).

The second drop in production from 2004 to 2006 (Figure 2, page 9) was primarily caused by unfavorable weather conditions; too little rain in 2004 and 2005 and too much rain in 2006. Both GMO and non-GMO papaya productivity was significantly lower in 2004 (Figure 3B, page 10).

Despite the absence of sharp production drops since 2007, Hawaiian papaya production is still in decline (Figure 2, page 9). Over the past few years Hawaiian papaya sales in the US and Canada have been suffering from competition from Brazil. In addition the distribution potential of GMO papaya and the global reluctance to eat food derived from genetically modified plants remains a serious problem. It was necessary to wait until 2011 for Japan to approve the import of the ‘Rainbow’ GMO papaya after carrying out a food safety analysis. Any initial optimism was quickly eroded. The traditional wholesalers and distributors were still hesitant to risk their reputation by selling GMO papaya. An alternative distribution chain was established, but after three shipments this export was also stopped. The Hawaiian papaya industry is currently making a considerable effort to ensure the smooth running of exports to Japan but it is highly probable that it will take several years before GMO papaya is fully accepted.

27 Personal e-mail correspondence with Dr. Stephen Ferreira
Papaya is known for being available in a large number of varieties. These varieties differ in size, taste and color of pulp, and are also adapted to local climate and soil conditions. Each country grows its own selection of papayas. Hawaiian papayas are usually pear-shaped, with yellow or red pulp and weigh approximately 450 g each. This contrasts with the Mexican papayas that are much larger (up to 40 cm) and can easily weigh more than 5 kg each.

The most commonly cultivated varieties in Hawaii are as follows:

- Kapoho (Solo): a pear-shaped papaya with a high sugar content and a greenish yellow skin that gets yellower during ripening. The pulp is deep yellow and tastes like pear and melon. Kapoho is the standard export product but it is a variety that is PRSV sensitive.

- Sunrise/Sunup: the Sunrise variety is known locally as ‘strawberry papaya’. When ripe the fruits have yellow skin and intensely red-orange pulp. The Sunup variety is resistant to PRSV and is the genetically altered version of Sunrise.

- Rainbow: a hybrid made by crossing the yellow pulped Kapoho Solo with the red pulped Sunup. Rainbow is genetically altered to be resistant to PRSV. It is the most popular variety amongst consumers and is also the most widely cultivated on the main island Hawaii and on the islands Oahu and Kauai. The fruits strongly resemble the Kapoho papayas with golden yellow pulp.

- Kamiya/Laie Gold: Kamiya is rounder and larger compared to the aforementioned varieties. It is cultivated exclusively on Oahu for local consumption and, just like Sunrise, has orange colored pulp. The Laie gold variety is resistant to PRSV and is the genetically altered version of Kamiya.

2. Genetic engineering as protector of the harvest

Up until 1992 it was possible to protect the papaya producing region Puna from the destructive papaya ringspot virus. Scientists at the University of Hawaii and Cornell University (USA) saw and anticipated the danger to Puna. In 1984 they started the development of a genetically altered papaya. This GMO papaya was made resistant to PRSV through the activation of the plant’s immune system.

Building up to a success story

In order to be prepared for PRSV infections in Puna, Cornell University (research group led by Dennis Gonsalves) and the University of Hawaii (research group led by Richard Manshardt) initiated a research program in 1979. Within this program they wanted to explore the possibilities of cross protection. Cross protection is a method involving the deliberate infection of plants with weak viruses. These viruses do not usually cause any disease but do activate the plant’s immune system whereby later infection by viruses related to the mild virus can be better dealt with. Cross protection against PRSV showed limited success. This method relies on the existence of a mild virus that can effectively offer protection against PRSV. It was possible to create a low level of resistance in some papaya varieties, primarily resulting in a delayed progression of the disease, but in many cases this resistance was too weak or the mild virus still caused certain disease symptoms. Additionally, this method requires extra work from the farmer and very close monitoring, making it too impractical to be viable.

Using the weapon as the defense

In the early 1980’s researchers at Washington University discovered that resistance to plant viruses could be attained by getting the plant itself to produce one of the virus’ coat proteins (see text box ‘Plant viruses’, page 7). This phenomenon was named coat-protein-mediated resistance. At first the underlying mechanism could only be guessed at, but eventually it became clear that resistance was a result of ‘post-transcriptional gene silencing’. Such complex concepts require further explanation, which is provided in the text box ‘RNA and gene-silencing’, page 32. The papaya researchers based their work on this new technology and decided to produce the PRSV HA 5-1 virus’ coat protein in papaya. PRSV HA 5-1 was the mild variant of the stronger PRSV HA virus that was used in the cross protection experiments (see previous paragraph). The HA 5-1 gene was mechanically inserted into the papaya variety ‘Sunrise’ (see text box ‘Agrobacterium versus the gene gun’, page 17). In order to distinguish it from the non-GMO ‘Sunrise’ the genetically altered variety was given the name ‘Sunup’. Two years later the genetically modified ‘Sunup’ papayas could be tested in the lab. The test showed that the transgenic papayas were completely protected, not just against the mild PRSV HA 5-1 virus but also against the stronger PRSV HA virus that is present in Hawaii. Protection against PRS viruses from outside Hawaii was much less predictable and turned out to be dependent on the age at which the plant became infected, the similarity to the PRSV HA 5-1 virus and the quantity of viral RNA (See text box ‘RNA and gene-silencing’, page 32) produced in the plant. The ‘Rainbow’ variety (see page 14) that produces less viral RNA than ‘Sunup’ was only protected against some PRS viruses from outside Hawaii at a later age, whilst ‘Sunup’ was resistant at an earlier age and against a wider range of PRS viruses. However, despite the large quantity of HA 5-1 RNA in ‘Sunup’ plants they still remained sensitive to viruses that differed too much from PRSV HA 5-1 (for more information see text box ‘RNA and gene-silencing’, page 32).
In 1992, the year when the first PRSV infections were noticed in Puna, the GMO papayas were tested in the field for the first time. For this purpose the heavily infested island Oahu was chosen. The Sunup GMO papayas retained full resistance to PRSV during a two year long field test, while the non-GMO papayas Sunrise and Kapoho began showing symptoms after just 77 days.\(^3\)

In 1997, when the entire Puna region was infected with PRSV, the authorities were presented with an important dilemma. Either all papaya plantations in Puna would have to be cleared and a new area on Hawaii would have to be found and used to rebuild papaya production from scratch, or the genetically modified papaya would have to be given a chance.

### A new characteristic and a new variety

Virus resistance was first introduced into the red pulped ‘Sunrise’ variety. However, at that time the main papaya variety in the Puna region was the yellow ‘Kapoho’ papaya. In order to make a virus resistant yellow pulped papaya available to growers, the GMO ‘Sunup’ papaya was crossed with the non-GMO ‘Kapoho’ variety. Not only was the resultant hybrid virus resistant but a new variety was also born, namely ‘Rainbow’. For an overview of the different papaya varieties see the text box ‘Papaya varieties in Hawaii’ on page 12.

During a two year field test in the Puna region the non-GMO papaya ‘Sunrise’ was severely compromised. The yield per harvesting of the non-GMO papaya dropped from 419 kg/ha to 56 kg/ha in one and a half years’ time. On an annual basis production levels of 5.6 tonnes per ha were achieved. In contrast, the GMO papaya ‘Rainbow’ remained disease free for the entire test, produced more than 2000 kg per ha per harvesting and achieved 112 tonnes per ha per year.\(^2\) In addition, the growers were positively surprised by the characteristics added to the new hybrid. Compared to the ‘Kapoho’ variety ‘Rainbow’ could be harvested earlier and gave a higher yield.\(^2\) The success of GMO papayas in Hawaii is not only attributable to virus resistance but also partly to the new papaya variety.

In 1998 Hawaiian papaya growers were given permission to plant the ‘Rainbow’ and ‘Sunup’ varieties. The growers were open to the new ‘Rainbow’ varieties and planted the transgenic papayas on a large scale. The previously abandoned farmland could be recultivated and the papaya industry in Puna was saved.
RNA AND GENE-SILENCING

The genetic information of all organisms is contained in nucleotide sequences (see text box ‘Plant viruses’, page 7). With the exception of viruses, DNA can be seen as the universal carrier of genetic information. In higher organisms (plants, animals) DNA consists of two strands (see Figure 4). Every cell in a given organism contains the same DNA. However, not every cell needs the same information. For example, a liver cell needs to make different proteins than an eye cell. In order for specific information to be used in the production of a protein, two steps are necessary (Figure 4). First, a piece of code (gene) that is necessary for protein production is transcribed into RNA. This single strand moves from the nucleus to the cytoplasm of the cell, where the code is translated. For this reason this RNA is often referred to as ‘messenger RNA’. The translation of the messenger RNA coincides with the production of the protein for which the original piece of DNA encodes.

RNA plays a crucial role in this process. If the RNA is broken down before it can be translated then the message contained in the DNA will never lead to the production of a protein. This phenomenon is called ‘gene silencing’, literally shutting down a gene’s function by deactivating the corresponding RNA. The process is naturally used by plants to defend against viruses. The immune system of a plant is based on the presence of double stranded RNA. As previously mentioned a plant’s messenger RNA consists of a single strand. When viral RNA multiplies in the plant cell, double stranded RNA molecules are formed. These are recognized as foreign structures by the plant cell and to defend itself it breaks down the double stranded RNA. This process activates a mechanism that destroys all similar RNA molecules, even if they are single stranded. This RNA silencing process can be considered a kind of immune system that works at the genetic level. Not all plants are equally efficient with regards to carrying out this system and viruses also make attempts to defend themselves against such attacks. That is why papaya is naturally susceptible to PRSV.

**Figure 4**

Information in double stranded DNA is first transcribed to single stranded RNA before being translated into a protein.

Both cross protection of papaya (see page 13) and coat-protein-mediated resistance are reliant on this RNA-dependent silencing mechanism. Both cross protection in papaya and coat-protein-mediated resistance are reliant on this RNA-dependent silencing mechanism. In cross protection the initial infection with the mild virus activates the silencing mechanism. When a subsequent infection occurs the plant is then able to break down viral RNA that is similar to the first virus and in doing so stops the infection. In GMO papayas the silencing process is given some help. The mRNA for the viral coat protein is made by the plant in large quantities. When a certain RNA molecule is present in large quantities plants react in the same way as when they encounter double stranded RNA, recognizing it as foreign material and breaking it down. This reaction was observed for the first time during research into Petunia flower color. In order to make a purple color more intense American and Dutch researchers activated the expression of extra pigment genes, To their surprise the resulting plants had less intensely colored flowers and in some cases even had white petals. The plants with white flowers had so much pigment mRNA that the RNA was broken down before pigment could be produced. The GMO papaya’s virus resistance is based on the same principle. The plant makes the mRNA that codes for the viral coat protein in large quantities causing the silencing mechanism in the plant to be activated which results in the RNA for the viral coat protein being broken down.

RNA AND GENE SILENCING: CONTINUED

**Figure 5**

Diagram showing how the function of the messenger RNA can be shut down so that no protein is produced.

Original RNA → Translation → Functional Protein

Overproduced RNA molecules are broken down → RNA fragments can bind to the original RNA strand → Binding induces RNA silencing → No Protein
During lab experiments the researchers who developed the GMO papaya noticed that the more mRNA for the coat protein was produced, the more resistance the plants showed to the virus.31 *Carica papaya* is a diploid plant, meaning that each gene is present in two forms (alleles). If the two forms are identical then one is dealing with a homozygous characteristic. This can best be compared to a person’s blood group. Humans are also diploid organisms. Humans with the O blood group are homozygous for that characteristic: both forms of the blood group gene are O (OO). By contrast the blood group AB is determined by the A allele and the B allele. The ‘Sunup’ papaya is homozygous for the virus resistance characteristic. In other words, it has two identical alleles. But the ‘Rainbow’ papaya is a cross between the GMO papaya ‘Sunup’ and the non-GMO papaya ‘Kapoho’. As a result ‘Rainbow’ has only received virus resistance from one parent (‘Sunup’). As a result ‘Rainbow’ produces less mRNA for the coat protein than ‘Sunup’ and exhibits a lower resistance against PRSV.31

As mentioned earlier the silencing process is based on double stranded RNA. Therefore two RNA molecules must bind to each other before the process is activated (Figure 5, page 16). Greater similarity between the molecules allows for easier and stronger binding and a more efficient RNA degradation process. In Hawaii the coat protein from PRSV HA 5-1 was used. The GMO papayas are protected against other PRS viruses as long as the coat protein’s genetic code does not differ too much from that of PRSV HA 5-1. For example, the GMO papaya ‘Sunup’ was also resistant to PRS viruses from Jamaica and Brazil. However, protection against the Thai PRSV was much less effective because the Thai PRSV’s DNA coding has less in common with PRSV HA 5-1 compared to the Jamaican and Brazilian viruses.31

**AGROBACTERIUM VERSUS THE GENE GUN**

Nowadays there are various methods available to genetically modify plants. The best known method is through using the soil bacteria *Agrobacterium tumefaciens*’ natural DNA transfer mechanism. This bacterium infects certain host plants and inserts a part of its bacterial DNA into the plant’s cells during the infection process. The bacterial DNA is stably incorporated into the plant DNA and carries the information necessary for the synthesis of nopines, with which *Agrobacterium* can feed itself. It is one of evolution’s most intriguing tricks: a bacterium that forces a plant to produce its food by inserting the necessary information (‘the menu’) into the plant’s DNA. During the 1970’s molecular biologists realized the possibility presented by this infection process, to use *Agrobacterium* as a DNA delivery service. When a piece of bacterial DNA (that would normally be inserted into the plant) is replaced with information that we want to insert, then *Agrobacterium* will transfer our desired information to the plant.
In addition to this biological method there are also mechanical methods for inserting DNA into plants. The most important is the ‘particle acceleration’ method, also known as ‘particle bombardment’ or the ‘gene gun’. For this method tiny gold particles are coated with the DNA that is to be inserted into the plant. The gold particles are then ‘fired’ into the plant tissue at high pressure. In some cases the DNA penetrates into the nucleus where it may be spontaneously incorporated into the plant’s DNA. This method leaves more to chance, is less efficient and in many cases only part of the desired DNA is inserted into the plant DNA. However, this still remains the most effective way to modify plants that cannot be infected by Agrobacterium. In the late 1980’s Hawaiian papayas were genetically modified using this method. Since then methods have been developed to genetically modify papaya using Agrobacterium. For example, the GMO papayas cultivated in China (see page 25) were modified using Agrobacterium.

How do you get from a genetically modified cell to a whole plant? Unlike humans and other animals, plants have the unique ability to make a new plant from just one plant cell. So when one plant cell has been genetically modified (with Agrobacterium or the ‘gene gun’) it is then possible to grow a full, genetically modified plant from it. Sometimes this happens spontaneously, but in most cases the process must be directed by adding plant hormones that stimulate the development of shoots and roots.

The power of biotechnology

The development of the virus resistant GMO papayas shows that in certain cases using biotechnology can improve plants more efficiently than traditional plant breeding. By using traditional crossing techniques breeders are limited to the genetic information that is available within a certain plant species. In the case of PRSV, the genetic information required for resistance is not present in papaya (Carica papaya). This resistance is known to be present in the Vasconcellea papaya. The Vasconcellea papaya belongs to the same family as the Carica papaya, but a different genus. Attempts have been made since 1958 to transfer the PRSV resistance in Vasconcellea to Carica using intergeneric crossing (between genera). Such hybridizations are exceedingly inefficient and not only do they require the development of special procedure and laboratory techniques, they also rely on a great deal of luck. Over the course of more than 50 years only a small number of infertile offspring have been generated. Filipino and Australian researchers have recently reported the first fertile, classically bred Carica papaya plant that is resistant to PRSV. Despite the very significant contribution that classical breeding has made, and will continue to make in the future, the classically bred plant was far too late to save the damaged Hawaiian papaya plantations.
3. A public sector development

The development of a virus resistant GMO papaya was an important step in the fight against PRSV. However, getting the transgenic papaya to the grower was at least as important. This final step involved obtaining permits and the approval for both cultivation and consumption. Showing unprecedented efficiency and foresight the Hawaiian papaya organization managed to get the GMO papayas to growers just three years after the first field tests.

Patent transferred to the local papaya organization

When a particular innovation, technology or product is developed the developer can obtain intellectual property rights. To do this the developer must apply for a patent from the authorities, in which he/she must explain what has been discovered in great detail. When a patent has been granted, the inventor has the right to deny other parties from making, using, importing or selling the product or offering for sale. This protection is only valid in the countries where the patent is granted and typically lasts for 20 years. It is primarily intended as a means to allow the inventor to earn back his or her investment costs.

Depending on the country where the patent is filed, it can be taken out on a product, technology, methodology, the use of molecules, ... When someone wants to use the protected technology for commercial purposes or needs a protected product during a development process, an agreement (license) can be drawn up whereby the product, the technology or part of it, may be used in exchange for (financial) compensation. However, patents are not valid when the protected product is used for research purposes”. So the research groups that developed the Hawaiian GMO papaya were allowed to freely use patented technology and/or genetic components. However, this free use was no longer applicable once the GMO papaya was to be distributed commercially. Despite the fact that the researchers granted the intellectual property rights for the virus resistant papaya to the Papaya Administrative Committee (PAC), the PAC could not commercially introduce the virus resistant GMO papaya because certain components and technologies that were used during the papaya’s development were protected by third parties.

PAC wanted to be able to offer the transgenic papayas to the local growers for free. To achieve this, permits had to be obtained for the protected parts of the GMO papaya production process. More specifically, the areas in question were the use of technology to genetically modify plant cells by bombardment (see text box ‘Agrobacterium vs. the gene gun’, page 18) and the discovered ability to make plants virus resistant by having them express virus genes. Furthermore, certain genetic components such as the PRSV coat protein that was used, the selection marker (antibiotic resistance gene NPTII) and the GUS reporter gene were protected, as well as certain sequences (5’ UTR) for the efficient expression of plant genes. In order to avoid certain financial concessions in the form of royalties depending on the volume of distributed plant material, PAC wanted to obtain these licenses with a one-off payment.

Thanks to the limited level of papaya cultivation in the US, the crisis affecting the Hawaiian papaya industry and the fact that none of the patent holders were involved in an independent development of a PRSV resistant papaya, there was a certain degree of ‘goodwill’ amongst the patent holders and, with some help from the USDA, PAC eventually managed to obtain all the licenses under reasonable terms and conditions. PAC had anticipated this and during the negotiating phase they had already asked the Hawaii Agricultural Research Center to start the propagation of ‘Rainbow’ seeds. One month after the last license agreement

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42 The PAC is an organization that was assembled by the USDA to help the Hawaiian papaya industry to market the papaya.
* Patent law in the United States is generally more strict then in Europa, as such there might be some restrictions for the use of patented technology even for research purposes.
was signed the research center had already produced enough seed to plant 400 ha of transgenic papaya plantations.\(^3\) In May 1998 the seeds were distributed amongst Hawaiian papaya growers free of charge. However, a number of limitations were imposed onto the free distribution of the GMO papayas. For this reason the cultivation of the Hawaiian GMO papaya was only permitted in Hawaii. The harvested papayas were approved for sale outside of Hawaii, which was essential for export activities.\(^3\) Growers were also required by PAC to follow a training course about cultivating the GMO papaya.

**Safe for the environment and approved for consumption**

Before a genetically modified plant is brought onto the market in the United States it must first be approved by the relevant authorities. The GMO papaya needed approval from the APHIS (Animal and Plant Health Inspection Service), the EPA (Environmental Protection Agency) and the FDA (Food and Drug Administration).

APHIS was primarily concerned with the effects the produced coat protein could have on other plant viruses and the presence of small RNA fragments as a result of the RNA silencing mechanism (see text box ‘RNA and gene silencing’, page 15). Theoretically, an infecting virus X could use the PRSV coat proteins resulting in virus X acquiring the ability to be transferred in a different way. Alternatively the infecting virus X could use the gene or part of the gene responsible for PRSV coat protein production resulting in the creation of a new virus. Both concerns could be refuted thanks based on the fact that the PRSV coat proteins are naturally present in all plants infected with PRSV and so no new situation was being created. A third concern was that the new information could be spread to wild papaya varieties, giving them a selective advantage and causing them to become invasive plants. However, there are no wild papaya varieties in Hawaii and even in areas where there are no PRSV infections papaya is not considered invasive. Approval from APHIS was obtained in November 1996.\(^3\)

According to the EPA’s definition the coat protein would have to be considered a pesticide due to the resistance to plant viruses that it provides. In theory this meant that the coat protein would need to be subjected to the same risk analyses as a pesticide. However, as mentioned earlier the coat protein is present in all PRSV infected papayas and, despite their spots, these are still consumed without causing any health problems.\(^3\) In addition, the coat protein’s concentration is much lower in the GMO papaya than it is in infected non-GMO papayas. The applicants also argued that there was no evidence suggesting that coat proteins from any plant virus could produce allergic reactions or have any other negative effects on human health. The EPA gave its approval in August 1997.

The FDA was particularly interested in how much the GMO papayas differed from non-GMO papayas with regards to nutritional value and gave positive advice to commercialize the biotech papaya. By initiating this approval procedure before the plant was ready to put on the market, Hawaii saved time again and in September 1997 all the necessary procedures had been completed successfully.
4. Economic aspects of the Hawaiian papaya

The Hawaiian papaya industry is responsible for just 0.1% of global papaya production. Even so, in 2012 papaya exports generated 23 million US dollars. Canada and Japan have been the biggest customers for decades.

Canada and Japan share the papayas

The largest share of Hawaiian exports consists of the virus resistant ‘Rainbow’ and the non-genetically modified ‘Kapoho’ and ‘Sunrise’ papayas. The United States mainly exports fresh papaya to Canada, Japan and Hong Kong. In the first half of the 1990’s Japan was the biggest importer of Hawaiian papayas; more than 60% of exports went to Japan and approximately 30% went to Canada. In 1996 exports to Japan peaked at 15 million US dollars (Figure 7). This changed dramatically after the introduction of the GMO papaya in 1998. The share of exports to Japan plummeted, while for Canada the opposite happened (Figure 7). Between 2010 and 2012 Canada was the biggest buyer of Hawaiian papayas with a share of 83%, followed by Japan (6.3%), Hong Kong (4.2%) and other countries like China and Mexico.

**FIGURE 7**

The Hawaiian papaya’s export figures in millions of US dollars. Import by Japan decreases drastically after the introduction of the GMO papaya. In 2003 Canada opened its markets to GMO papayas resulting in extra import.

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When GMO papayas were introduced, Hawaii lost access to the Japanese market. Former top importer Japan refused to import the GMO papaya as a result of a high level of caution regarding genetically modified food. In addition, there were concerns about the possible mixing together of GMO and non-GMO papayas. Between 1996 and 2010 exports to Japan decreased at an average rate of 1 million US dollars per year. This decline in exports was offset by Canada. In 2003 Canada opened its markets to the biotech papaya and as a result export figures rose from 14.2 million US dollars in 2003 to 23.5 million in 2012 (Figure 7, page 21). While the import of Hawaiian papayas between 2000 and 2003 was worth an average of 6.5 million US dollars, by 2012 this had increased to nearly 20 million US dollars.
The GMO papaya in Japan

According to official Japanese import figures the annual import value of papaya between 2005 and 2008 was approximately 11 million US dollars (from all parts of the world). Despite a decrease in papaya import over the past few years, the yearly import since 2009 has still been worth more than 6.5 million US dollars.\(^{46}\) Approximately 1 million US dollars’ worth originated in Hawaii (Figure 7, page 21).

Prior to the introduction of the GMO papaya Hawaii supplied 97% of Japan’s imports. Exports were worth 12.8 million US dollars. The Philippines, that up until this point had barely supplied any of Japan’s imports, could supply papayas that were guaranteed non-GMO and gradually took over from Hawaii as main supplier (see Figure 8).\(^{46}\)

In 1999 the Hawaiian papaya industry got started on the procedures necessary to enable the export of biotech papayas to Japan. Following evaluation of all the food safety and environmental factors, the import of the Rainbow papaya was approved in Japan on 1 December 2011.\(^{47,48}\) The biotech papaya’s introduction was linked with an education program and at the instigation of the Hawaiian papaya industry all individual papayas were labeled with the Japanese text ‘idenishi kumikae’ or ‘genetically modified’.\(^{47}\)

Despite the fact that the export of the American papaya to Japan is at a historical low, the Hawaiian GMO papaya growers now have a chance to regain a share of the Japanese market. However, achieving this plan will rely on the readiness of the Japanese public to consume genetically modified papaya (see page 11).

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5. The biotech papaya outside Hawaii

Despite its minimal impact on global papaya production, Hawaii turned out to be a pioneer with regards to PRSV resistance. Countries such as China, Thailand, Taiwan, the Philippines, Australia and Jamaica followed Hawaii’s example and developed their own PRSV resistant papayas.

The Hawaiian technology spreads

For several decades PRSV has been rampaging through papaya producing regions. In India, the primary papaya producing country, PRSV was discovered in 1958 after which it spread from West India to other regions. PRSV was responsible for harvest losses of 85 to 90%.[49,50] In 1975 PRSV was detected in Taiwan. Three years later 44% of the land used for papaya cultivation had been rendered useless and by 1980 the ring-spot disease had spread across the entire island.[51] In 1982 PRSV was detected in the Philippines, in 1990 in Thailand and then 2 years later in Vietnam. In most virus infested regions the only way to continue papaya cultivation is to stop production and resume it somewhere else.

However, Taiwan is a small island and there are limited opportunities for relocation of the papaya industry.[5] The destructive effects of PRSV forced farmers to grow their papaya as an annual crop. After the first harvest all plants are destroyed and the fields must be replanted. This results in higher production costs and a smaller harvest.[3]

In 2003 researchers at the ‘National Chung Hsing University’ and the Taiwanese agricultural research institute published the development of a transgenic papaya resistant to Taiwanese PRSV.[52] The Taiwanese GMO papaya is not just resistant to PRSV viruses from Taiwan but also to PRSV from Hawaii, Thailand and Mexico.[52] However, the Taiwanese GMO papaya is not yet being cultivated commercially. The same applies to the virus resistant GMO papayas from Brazil, Indonesia, Malaysia, Australia, the Philippines, Thailand and Vietnam.[53] In most of the countries the plants have been tested in the field successfully but are awaiting approval for commercialisation.[5]

During the early 1990’s the Jamaican papaya industry was shaken up by PRSV infections. There it also had disastrous consequences for the incomes of papaya growers and on distribution. Estimates suggest that the land area planted with

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papaya in Jamaica declined by more than 50% due to the PRSV infections, from 400 ha in mid-1990 to 180 ha in 2008.54 The annual production levels fell from an average of 15,300 tonnes between 1993 and 1998 to 5,800 tonnes in 2011.2 Cornell University sprang into action and a GMO papaya was developed based on coat proteins from the Jamaican variant of PRSV.55 The GMO papaya was ready for field testing in 1998 but because the necessary legal framework for commercialization was absent no further steps have been made to this day.55 In Venezuela the situation is similar. The University of Los Andes (Venezuela) got in contact with Cornell University as early as 1992. The collaboration yielded a GMO papaya resistant to Venezuelan PRS viruses56 but Venezuelan growers still have to manage without a virus resistant papaya.

**China takes things into its own hands**

In China the papaya ringspot disease was first reported in Guangdong province in 1959. From there it spread itself to other papaya producing provinces.8 Motivated by the success in Hawaii, researchers from South China Agricultural University initiated a similar research project. Instead of expressing the PRSV coat protein the Chinese researchers chose to work on the viral replicase gene. The GMO papaya ‘Huanong 1’ was approved for cultivation and commercialization in 2006 by the Chinese Biosafety Committee after numerous field tests.57 The Huanong 1 plants are only available from a Chinese seed company that sells them as small plants.1 Much like in Hawaii the GMO papaya also helped papaya production to recover in China. In addition, it has been responsible for rising levels of production. In 2009 the four papaya producing provinces produced more than before the PRSV infections broke out. The increased levels of production were accompanied by a significant decline in the import of foreign papayas. By 2009 import levels had decreased to just one sixth of what they were in 2006 (Figure 9).57 In Guangdong province, the most important papaya growing region in China, the adoption level in 2012 was 95%.58 4275 of the 4500 hectares were planted with PRSV resistant GMO papayas. On the island Hainan an extra 2000 ha of biotech papaya were planted, 40% of the 5000 ha of papaya cultivated land on Hainan.58

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**Figure 9**

_After the introduction of the virus resistant GMO papaya in 2007 imports decline dramatically._57

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Conclusion

Genetically modified crops are often associated with multinationals and large scale cultivation. The story of the GMO papaya demonstrates that this does not necessarily always have to be the case. The Hawaiian GMO papaya was developed by the public sector and the intellectual property rights were transferred to the local papaya industry.

The GMO papaya has been cultivated since 1998 to deal with the papaya ringspot virus, a pathogen that does not just cause serious losses in yield but can also make commercial papaya cultivation impossible. Thanks to the use of GMO papayas cultivation has been able to continue on Hawaii. This success story inspired many papaya producing countries to look for a similar solution to deal with local papaya viruses. Until now virus resistant GMO papayas are only being cultivated in Hawaii and China but more countries are ready to make use of biotech papayas in the fight against the papaya ringspot virus.