



Production Requirements of the Transgenic Papayas 'UH Rainbow' and 'UH SunUp'

Papaya ringspot virus (PRV) has had a very significant impact on papaya production in Hawaii. Oahu was the major production site in the 1950s, but the spread of PRV caused the papaya industry to move to the Puna area of the island of Hawaii in the 1960s. Since 1992, the introduction and subsequent spread of PRV in commercial orchards in Puna has resulted in a significant decrease in the state's papaya production.

"Transforming" the papaya for virus resistance

Fortunately for Hawaii's papaya industry, help was on the way. In 1987, researchers at the University of Hawaii (UH) and Cornell University in New York had begun trying to create a "transgenic" papaya with virus resistance. They used "genetic engineering" or "genetic transformation" techniques to insert part of the virus into the nucleus of a papaya cell. At Cornell, Dr. Dennis Gonsalves isolated the virus' coat protein, and he and Dr. Jerry Slightom of The Upjohn Company modified it for use in plants. In 1989, quantities of the gene were "shot" into cultured papaya tissue using a "gene gun" developed by Dr. John Sanford at Cornell. Dr. Maureen Fitch, then a graduate student at UH, developed a tissue-culture system needed to grow out the genetically engineered plants from the bombarded plant materials. In 1991, Gonsalves observed the first transformed plant that appeared to have PRV resistance.

At UH, Dr. Richard Manshardt used traditional plant breeding techniques and inbred the resistant plant, producing a true-breeding, red-fleshed cultivar that was named 'UH SunUp' (also referred to as 'SunUp'). Because the papaya industry wanted a yellow-fleshed fruit, Manshardt crossed 'SunUp' with 'Kapoho' to produce a yellow-fleshed F₁ hybrid that was named 'UH Rainbow' (also called 'Rainbow').

Commercializing the transformed papaya

Hawaii's Papaya Administrative Committee (PAC) assumed the task of obtaining license agreements with owners of the patented genetic engineering technology, while Gonsalves and Manshardt prepared and submitted technical documents required by the U.S. Department of Agriculture (USDA), the Environmental Protection Agency (EPA), and the Food and Drug Administration (FDA). In September 1997 the federal regulatory agencies completed their review and approved the transgenic papaya for production and sale. PAC successfully negotiated use licenses with Monsanto Company, Asgrow Seed Company, Cambia Biosystems L.L.C., and the Massachusetts Institute of Technology.

Licenses

The licenses negotiated with the four companies include limitations-of-use and compliance provisions. There are five provisions, common to all of the licenses, which growers must follow to be in compliance with the contracts, as follows:

- Transgenic papaya can only be planted in the state of Hawaii.
- Only PAC can sell seeds of 'Rainbow' and 'SunUp'.
- Fruits can only be sold in countries that have accepted genetically engineered papayas as safe for commercialization.
- Producers must attend an educational session, which covers the requirements of the licenses and PRV resistance management.
- Producers are required to sign an agreement with the PAC to purchase seed.

Characteristics of papaya hybrids

Hybrids such as 'UH Rainbow' have some characteristics that are very different from the inbred papaya lines such as 'Kapoho' that Hawaii growers are used to. It is important that growers understand these differences.

The first difference is that the sex-segregation ratio of hybrids is 1:1 (one hermaphrodite to one female plant) rather than the more favorable 2:1 ratio with inbred lines. Consequently, growers will have to leave *five* seedlings at each hole (rather than three) to ensure a 97 percent chance of having one hermaphrodite per hole at thinning time at flowering.

The second difference is that plants grown from seeds of F₁ hybrids do not breed true, and growers *must* purchase new F₁ seeds for each planting. If growers use seeds taken from the hybrid fruits they have grown, the resulting plants will have mixed characteristics. Some of them will be yellow-fleshed and some will be red-fleshed. More important, some of the plants will be resistant to PRV, while others will be susceptible to the virus.

Resistance management

Whenever new resistant varieties are planted widely, disease-causing organisms may change, acquiring the ability to overcome or "break down" the resistance. In papaya, this could occur in two ways:

- A new PRV strain could be brought into Hawaii; both 'Rainbow' and 'SunUp' are highly resistant to the PRV strains found in Hawaii, but they are highly susceptible to strains from Asia.
- New PRV strains could develop from strains now present in Hawaii; this could occur if virus-infected plants are allowed to grow in or near fields of 'Rainbow'.

'SunUp' is totally resistant to the virus, but 'Rainbow', in greenhouse studies, has shown susceptibility to PRV until about seven weeks of age. This is referred to as "young plant susceptibility." The plants become fully resistant to PRV after three months of age. These observations suggest that in areas of high virus pressure, such as Puna, growers are likely to find infected seedlings. It has also been observed that side-shoots on the main trunk may show infection. If PRV symptoms are observed in a seedling field, all infected plants un-

der three months of age should be removed from the field immediately. If PRV symptoms are observed in plants older than three months, the symptomatic plants should be removed from the field and samples should be taken to the local Cooperative Extension Service office for testing to determine if a new strain has developed.

It is important that virus pressure on the new 'Rainbow' and 'SunUp' cultivars be kept at a minimum to maintain their resistance to PRV. This means that growers must cut down *all* infected plants in and around fields of transgenic papaya plants. In addition, all abandoned papaya orchards must be rogued completely of papaya plants to reduce virus pressure.

Review of virus symptoms

Frequent surveys of young plantings will ensure that infected trees are identified at the early-infection stage.

- Leaves become yellowed and show a "mosaic" pattern of yellow to yellow-green discoloration.
- Leaf shape can become distorted with an upward cupping, and "shoe-string" symptoms (thinning of the leaf between the lobes) can be seen as the distortion becomes severe.
- The leaves may also show "green islands" (patches of green tissue).
- Water-soaked streaks may be present on the leaf petioles.
- Ringspot on the fruit is usually evident only after the infection has been present for an extended period.

Culture of 'UH Rainbow'

The culture and production of 'Rainbow' will be a little different than growers are used to with the cultivar 'Kapoho'. 'Rainbow' seem to be sensitive to calcium deficiency and requires application of 1500 pounds of calcium carbonate and 500 pounds of dolomite per acre. Since 'Rainbow' has 'Sunset' parentage, it requires applications of 400 pounds of a fertilizer such as 14-14-14 at intervals of four to five weeks.

'Rainbow' seems to be a little more sensitive to phytophthora than 'Kapoho' and will require timely application of fungicides. 'Rainbow' fruits seem to be more tolerant of anthracnose than 'Kapoho' fruits. The transgenic cultivars have the same susceptibility to mites and leafhoppers as 'Kapoho'. The fruits seem to ripen faster than 'Kapoho' but slower than 'Sunrise' fruits.

Marketing concerns

Growers must ensure that transgenic and non-transgenic papaya fruits are kept segregated at harvest so that transgenic fruits are sent only to accepting markets. The necessary practice of segregating transgenic varieties by blocks at planting should help to support this control.

It is imperative that growers do not plant seed from their own fruits of the F₁ hybrid 'Rainbow' plants. If this is done, the field will include a mixture of plants bearing yellow-fleshed or red-fleshed fruits. This would create a serious problem for the farmer and marketers because there is no way to determine if the fruit flesh color is red or yellow without cutting each fruit. Also, some of the plants in the field will not be PRV-resistant, which can contribute to breakdown of resistance in the transgenic plants. The related marketing concern is that transgenic fruits from a mixed field may be moved by mistake into markets that do not permit them.

Fruit quality

The fruits of both 'Rainbow' and 'SunUp' are larger than 'Kapoho' by about 50 percent. 'Rainbow' has a yellow-fleshed fruit which is slightly sweeter and juicier than 'Kapoho' but has a slightly less intense aroma. 'SunUp' has a pink-fleshed fruit and has fruit characteristics similar to 'Sunset'. 'Rainbow' has shown good tolerance to the heat treatment that is required for fruit fly disinfection.

Food safety concerns

What is genetic engineering?

Genetic engineering is a molecular technology that allows the transfer of a genetic trait, such as virus disease resistance, from one kind of organism to another. In the case of the genetically engineered papayas, a gene that prevents infection by papaya ringspot virus was found in the virus itself. It was transferred to a papaya, creating a PRV-resistant variety.

Why haven't I seen other genetically engineered fruits and vegetables in the market?

Genetic engineering is a new technology, and 'SunUp' and 'Rainbow' papayas are the first genetically engineered fruits to be commercialized in the United States.

Is genetic engineering necessary?

In some cases, yes. Some problems, such as overcoming PRV, cannot be solved without it. In papayas, there are no naturally occurring genes for effective resistance to PRV, so plant breeders have nothing to work with to improve the crop. Without the genetically engineered form of PRV resistance, the papaya industry in Hawaii would be destroyed by the virus, resulting in lost livelihoods for growers and much higher prices for consumers.

Is genetic engineering risky?

Genetic engineering is a powerful tool for manipulation of biological organisms, and as such it has great potential for beneficial use. However, as in any situation, human fallibility raises concerns about the proper application of the new technology. We need to be assured that genetically engineered products serve the public good, not just the agenda of the producer. There is a societal need for strict monitoring of the objectives and methods of genetic engineering.

How can I be assured that genetically engineered foods are safe?

Development of genetically engineered crops and foods is monitored by three federal regulatory agencies: the U.S. Department of Agriculture, the Environmental Protection Agency, and the Food and Drug Administration. These agencies have examined the development of the 'SunUp' and 'Rainbow' papaya cultivars and approved them as safe.

Can I be infected with the PRV virus by eating genetically engineered papayas?

Definitely not! Plant viruses like PRV cannot infect humans. Furthermore, 'Rainbow' and 'SunUp' fruits contain only a tiny part of the virus's genetic information, not the complete virus. There is no danger in eating genetically engineered fruits.

Does genetic engineering make 'UH SunUp' and 'UH Rainbow' better than other papayas?

Yes! 'SunUp' and 'Rainbow' are high-quality solo-type papayas, very similar (respectively) to 'Sunrise' and 'Kapoho', which are popular standard varieties developed in Hawaii and grown around the world. Following

the recent devastation of much of Hawaii's papaya acreage by papaya ringspot virus, genetic engineering has provided the two new transformed papaya varieties that are resistant to the virus. This has enabled local growers to continue producing delicious, nutritious papayas and offering them to consumers at affordable prices.

Summary

This publication is designed to accompany the video that must be viewed as part of the licensing process for anyone wishing to purchase seed of transgenic papaya cultivars. In addition, a sublicense agreement with the Papaya Administrative Committee must be signed before seed can be purchased. The video covers PRV resistance management and the requirements of the sublicense agreement.

It is very important that everyone—both commercial producers and home gardeners—growing 'Rainbow' and 'SunUp' adhere to the conditions in the sublicense that they sign with PAC. The new transgenic cultivars

will allow papaya production in PRV-affected areas where it formerly was not possible. People growing them should know the symptoms of PRV and the basic principles of PRV management. By carefully observing their plants and following the conditions of the sublicense agreement, papaya growers can help to ensure that the virus resistance of 'Rainbow' and 'SunUp' is not compromised and lost.

For answers to questions about the sublicense, call the Papaya Administrative Committee at (808) 969-1160. For questions regarding papaya cultivation or PRV resistance management, call your local Cooperative Extension Service office.

Melvin S. Nishina¹, Stephen J. Ferreira², Richard M. Manshardt³, Catherine G. Cavaletto³, Emerson Llantero⁴, Loren Mochida⁴, and Delan Perry⁴

¹Cooperative Extension Service—Hilo, ²CTAHR Department of Plant Pathology, ³CTAHR Department of Horticulture, ⁴Papaya Administrative Committee