Papaya Ringspot Virus-Resistant (PRVR) Papaya
"Why genetically engineer virus resistance into papaya?"

Fact Sheet

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1) Introduction

Papaya, also called *papaw* or *pawpaw*, is an edible melon-like fruit of a tropical softwood tree (*Carica papaya*) of the family Caricaceae. It is native to Central America and has spread to many other tropical and sub-tropical lands including India, the Philippines, and parts of Africa by way of early trade routes. Today it is cultivated throughout the tropical world and in the warmest parts of the subtropics. Papaya is considered one of the most economically important and nutritious fruits, being a rich source of antioxidant nutrients such as carotenes, vitamin C and flavonoids; the B vitamins folate and pantothenic acid; the minerals potassium and magnesium; and fiber. In addition, papaya is the source of the digestive enzyme *papain*, which is an industrial ingredient used in brewing, meat tenderizing, pharmaceuticals, beauty products and cosmetics.

Global papaya production averaged 5.78 million tons in 1998-2003, with an annual average increase of 4.1% during the last five years. Global trade in papaya averaged US$ 113 million across the same period. Brazil, Mexico, Nigeria, India and Indonesia have consistently been the top producers, contributing more than 71% of the total world production (FAOSTAT, 2004).

2) Importance of papaya in Southeast Asia

Papaya is one of the major fruit crops in Southeast Asia. It is an important traditional part of the household diet of families in countries like Indonesia, Malaysia, the Philippines, Thailand, and Vietnam. Subsistence farmers grow papaya trees in their backyards or in small plantings for their own consumption and sell modest surpluses in local markets. With the exception of Malaysia, where nearly 50% of the production is exported to Hong Kong and Singapore, papaya is consumed domestically with significant trade taking place within countries, particularly for urban needs (ISAAA, 1999).

3) Papaya in the Philippines

Papaya ranks 6th in production area and 5th in volume among fruit crops in the Philippines with 94% of production used for food and 6% for feed. Although only 2% is exported, it has substantial economic value because of its varied food and industrial uses (BAS, 2003). The papaya industry generated average foreign exchange earnings of US$ 5 million in 2001 and 2002 (FAOSTAT, 2004). Papaya is grown all over the country with the three regions of Luzon being most important: Southern Tagalog contributes 15% of the national total, the Bicol, 13% and Ilocos 9%. Outside Luzon, Western Visayas contributes 9%, Southern Mindanao 12%, and Western Mindanao 8% (BAS, 2001). Area planted to papaya has been between 5000 and 6000 hectares over the last decade (FAOSTAT, 2004). Most papaya production is on a small scale in backyards and gardens, mainly for local consumption. About 80% of all growers have 0.25-3.0 hectares. The two major commercial growers, Del Monte Philippines and Dole Philippines, have 2.5% of the total production area.

Many varieties of papaya are grown throughout the country to cater to varied consumer preferences. The Solo variety enjoys good demand in the foreign market but the major cultivars grown for the local fresh fruit market are still the traditional varieties such as Cavite Special and Morado. More recently, the hybrid cultivar Sinta, the first Philippine-bred hybrid released by the papaya breeding program of the Institute of Plant Breeding of UPLB, has found a growing niche in the local market with potential for international sales through franchising arrangement with East-West Seed Company. Demand for high-papain varieties like red Solo, used for beauty products, is increasing because of their high value in that application.

4) What are the major production constraints in papaya production?

Papaya crops suffer from several diseases and pests, the most ubiquitous and widespread of which is papaya ringspot virus (PRSV). This virus affects production and productivity in every region of the world by decreasing photosynthetic capacity of the plants, which subsequently display stunted growth, deformed and inedible fruit, and eventually, plant mortality. When plants are infected at the seedling stage or within two months after planting, the trees will not produce mature fruit. If trees are infected at a later stage, fruit production is reduced and of poor quality because of ringspots on the fruit and a decrease in sugar concentration (Gonsalves, 1998).

The PRSV disease is the biggest constraint to papaya production in the Philippines. In 1982, an outbreak of PRSV in the Philippines that grew to epidemic proportions wiped out the small-scale papaya industry of the province of Cavite on the island of Luzon. By 1994, the disease had spread through the entire Southern Tagalog area and reduced output as much as 80%, worth nearly 60 million pesos (Quebral, et al, 1994). Production fell by over 1% a year from 1981 to 2000 reducing output from 105,420 tons to 74,135 tons and national average yield declined to 12.11 tons per hectare in 2000 from 26.9 tons per hectare before the outbreak of the disease in 1988 (Laude, 2002;FAOSTAT, 2004).

5) How do farmers control and manage PRSV?

The control methods used by farmers to help manage PRSV include (Swain and Powell, 2001 and references therein):

- Delayed introduction through quarantine
- Burning all infected plants and nearby ground plants and destroying all papayas within a large surrounding area once the virus appears
- Using PRSV-tolerant varieties, pesticides, and anti-aphid netting for the first three months, when available
- Pruning young plants heavily to delay flowering and to build plant strength
- Using heavy and frequent anti-aphid sprays for the first year to minimize exposure
- Introducing mild strains of the PRSV virus into plants to prevent the symptoms of more severe strains (cross-protection)

Since the outbreak of the disease in the Philippines, several of these measures have been undertaken, including a quarantine to restrict the movement of papaya from the island of Luzon to the rest of the country. Despite this, PRSV infection has been reported recently in the Visayas and Mindanao, potentially threatening the rest of the major papaya production areas of the Philippines (Eusebio et al, 1997; Herradura et al., 2001).

6) Why genetically engineer PRSV resistance into papaya?

Traditional strategies like those mentioned above have had limited success. First, the virus is very efficiently spread from plant to plant by about 60 species of aphids and transmission is "non-persistent," meaning the aphids need to feed on a PRSV-infected plant for only a few seconds to pick up the virus. This makes it virtually impossible to control the virus by insect control or by discarding diseased plants. Second, there is no known natural resistance to the disease, although tolerant varieties manifesting reduced symptoms have been identified. In the Philippines, a tolerant hybrid cultivar, Sinta, has been a commercial success. Unfortunately, breakdown of resistance and loss of its effectiveness have been reported as early as two years after the release of the hybrid. Third, production under netting is an option but this requires large initial capital investments, which most small-scale producers can barely afford. Fourth, cross-protection has been used in Hawaii, Thailand, and Taiwan, with limited success. Protection is virus strain specific and never complete. Cross-protection, as well as quarantine and even eradication are only temporary solutions. Finally, considerable variation exists in the genomic sequences of various strains of PRSV. Because of these limitations, scientists have turned to genetic engineering and the use of the PRSV "coat protein resistance" strategy to develop transgenic PRSV-resistant papaya.

7) What is transgenic PRSV-resistant papaya?

Transgenic PRSV-resistant papaya is a papaya that has been genetically engineered to contain a virus gene that encodes for the production of the coat protein of the virus. As a major component of viruses, the coat protein's primary function is to protect viral genetic information.

Expression of this gene in the resulting papaya line renders the plants resistant to the virus. In other words, a gene from the pathogen is used to fight against the pathogen itself. In much the same way vaccines immunize humans against different diseases, modern biotechnology allows scientists to insert small fragments of plant viral DNA into crops. This develops natural protection or immunity against those viruses.

8) Is PRSV-resistant papaya safe to eat?

Papaya varieties genetically engineered to resist PRSV have been accepted for food use in the US since 1997 and in Canada since 2003. As part of the safety assessment, the nutritional composition, toxicological implications and allergenic potential were examined by the US and Canadian regulatory authorities.

Nutritional data
Because papaya is a tropical fruit crop that is normally consumed fresh and is valued as a rich source of vitamins A and C, samples of fruit from transgenic and non-transgenic control plants were analyzed for nutritional composition. Scientists found the nutritional composition of transgenic papaya to be equivalent to conventional varieties by the analyses of vitamins A and C and sugar content.

Toxicity
The white milky juice ("latex") of green papaya tissues contains a toxin called benzyl isothiocyanate (BITC) that has been linked to incidents of spontaneous abortions in pregnant women and to a higher incidence of prostate cancer in Japanese men over the age of 70. Because ripe papaya fruits have no latex, they also have virtually no BITC content. The levels of BITC in transgenic papaya and conventional papaya varieties were measured and found to be much lower in ripe fruit compared to immature fruit. There were no differences between transgenic papaya and conventional papaya from either immature or ripe fruit.

Allergenicity
There is a history of safe consumption of the PRSV coat protein derived from papaya fruit infected naturally by PRSV, and there is no indication that the form of the coat protein expressed in transgenic papaya is materially different in any way that would affect its potential for toxic or allergenic effect. Additionally, the PRSV coat protein does not possess any of the properties normally associated with protein allergens or toxins, such as heat stability and resistance to digestion by simulated gastric fluids. Also, there were no regions of similarity when the deduced amino acid sequence of PRSV CP was compared to the amino acid sequences of known protein allergens or toxins.

It is important to note that humans have been eating PRSV-infected papaya fruits for many years and these fruits have higher concentrations of the virus coat protein than what is expressed in the transgenic papaya. There has been no evidence of adverse effects linked to the consumption of virus infected fruit.

9) Are there any risks to the environment from PRSV-resistant papaya?

In order to asses the impact PRSV-resistant papaya may have on the environment, the following findings (determinations) were required by US regulatory agencies:

- the introduced genes, their products, and the added regulatory sequences controlling gene expression do not present a plant pest risk
- the transgenic organism should not increase the likelihood of new plant viruses (resistance buildup) emerging
- the transgenic organism is no more likely to become a weed than the non-transgenic forms
- out-crossing and hybridization between the transgenic organism and its relatives are unlikely to produce hybrids that would persist in the environment and become weeds
- the transgenic organism should not be harmful to beneficial organisms including bees
- the transgenic organism should not cause damage to processed agricultural commodities

After a thorough assessment addressing the points above, it was concluded that the PRSV-resistant papaya developed in Hawaii did not pose a direct or indirect plant pest risk and did not have environmental impacts any different from that of non-transgenic papaya.

10) The Hawaiian experience with transgenic PRSV-resistant papaya (Gonsalves et al., 2004)

In 1992, Hawaii's papaya industry faced economic disaster when PRSV was discovered in the Puna District of the Hawaiian Island where 95% of the state's papaya was grown. By 1995, PRSV was widespread in Puna and the industry was in crisis. Research into developing transgenic papaya resistant to PRSV started in the late 1980s and it was not until May 1998 when two lines ('SunUp' and 'Rainbow') of transgenic papaya were commercially released. Since then, transgenic papaya has fulfilled the hope of the Hawaiian papaya industry to control PRSV and to restore the supply of papaya to nearly the level that existed before PRSV entered Puna in 1992. The resistance of the transgenic papaya allowed farmers to directly reclaim their farms without first clearing their land of all infected papaya trees. The percentage of Hawaii's fresh papaya production produced in Puna has increased from a low of 65% in 1999 to 84% in 2002.

Challenges Facing Hawaii's Papaya Industry

Although a major constraint to papaya production in Hawaii was eliminated with the introduction of PRSV-resistant transgenic papaya, Hawaii's papaya industry still faces a number of challenges. They include serving the markets in Canada and Japan, the durability of the resistance of transgenic papaya, and growing non-transgenic papaya for niche markets.

Canadian and Japanese markets. Japan and Canada are large markets for the Hawaiian papaya industry. Currently, Japan accounts for 20% of Hawaii's export market, while Canada accounts for 11%. Canada approved the import of 'SunUp' and 'Rainbow' transgenic papaya in January 2003, and transgenic papaya shipments continue to Canada. However, the sale of transgenic papaya in Japan has not yet been approved. Thus, it is critical that papaya shipments to Japan are not intermixed with transgenic papaya. Several steps are being taken to minimize mixing.

At the request of Japanese importers, Hawaii's Department of Agriculture (HDOA) adopted an Identity Preservation Protocol that growers and shippers must adhere to in order to receive a certification letter from HDOA that accompanies the papaya shipment. This is a voluntary program. Papaya shipments with this certification are allowed to be distributed in Japan without delay during the time Japanese officials are doing spot-testing to detect transgenic papaya in imported shipments. In contrast, papaya shipments without this certificate must remain in custody at the port of entry until Japanese officials complete their spot checks. Completing the tests may take several days or a week, during which time the fruit lose quality and marketability.

Achieving "durable" resistance. The issue of durability of resistance should also be seriously considered. Studies have shown that 'SunUp' papaya has broader resistance than 'Rainbow', but the reality is that 'Rainbow' is the dominant transgenic papaya grown in Hawaii. So far, no breakdown of resistance of 'Rainbow' in Puna or on Oahu has been observed.Nevertheless, scientists need to be on guard for this possibility. The likelihood of new virulent strains developing due to recombination of PRSV strains in Puna with the coat protein transgene of Rainbow is remote.

A more realistic danger comes from the possibility of the introduction of PRSV strains from outside of Hawaii. 'Rainbow' is susceptible to many strains of PRSV from outside of Hawaii, including strains from Guam, Taiwan, and Thailand. Goods imported to or in transit through Hawaii thereby increase the opportunity to also introduce new PRSV strains into Hawaii. Technically, 'SunUp' should be resistant to many strains of PRSV that might be introduced into Hawaii. However, as noted above, the red-fleshed 'SunUp' is not the preferred cultivar in Hawaii.

A potential solution is to develop transgenic papaya that is resistant to a wide range of PRSV strains, although the time frame to commercialize this transgenic papaya may be longer than the time it took to commercialize the previous lines. Thus in the meantime, careful guard must be taken against the introduction of PRSV strains into Hawaii and the usefulness of existing transgenic cultivars must be maximized.

Growing non-transgenic papaya As the demand for non-transgenic papaya is still present in markets like Japan, the Hawaiian papaya industry needs to continue to grow them. However, despite efforts to protect non-transgenic papaya from PRSV, some have observed that PRSV infections are on the rise once again in Puna. As virus concentration builds up in the area, it will become more difficult to produce non-transgenic papaya economically. Therefore, strict attention needs to be paid to planting non-transgenic papaya in as much isolation as possible, doing timely elimination of infected trees, and to plowing under non-transgenic plantings that are no longer in production. The latter will reduce the amount of available PRSV.

11) Biotechnology Regulation in the Philippines

Since 1991, the Philippines has had a regulatory system governing the conduct of all biotechnology activities in the country. Transgenic research in plants and microorganisms has been carried out in the Philippines during the past 12 years. The National Committee on Biosafety of the Philippines (NCBP), a multi-agency and multi-disciplinary body, has overseen the task of implementing all biotechnology regulations. In 2003, the Department of Agriculture (DA) promulgated Administrative Order (AO) No.8 based on the Philippine Quarantine Act, which transferred to the Bureau of Plant Industry (BPI) the supervision for any planned release of transgenic crops in the country. DA AO 8 covers risks assessments for food, feed and environmental safety. As of 2003, the biosafety regulatory bodies of the Philippines have approved 130 contained experiments**,** mostly plants and some microorganisms; five (5) transformation events for field testing in rice and corn**;** two (2) transformation events for propagation (commercial release) in corn; and 17 transformation events for GM crops intended for use as food, feed or processing material in several crops.

In order for the transgenic PRSV papaya to be commercialized, it has to undergo risk assessment analyses required by the regulatory agencies in the country. There are four levels of biosafety assessment in the Philippines: (1) contained research in laboratories and screenhouses, (2) small field trials, (3) large field trials and (4) commercial release. Assessments are guided by the Philippine Biosafety Guidelines formulated by the National Committee on Biosafety of the Philippines (NCBP)

After five years of research, the transgenic PRSV-resistant papaya project of the Philippines is now in its second phase (small field trials). During the first phase, transgenic lines were obtained in the lab and the highly resistant first generation of transformed plants is now at the fruit bearing stage. So far, efficacy has been convincingly demonstrated in a contained environment, but efficacy under Philippine field conditions remains to be shown.

A small-scale field trial will be conducted within the 200 hectare experimental area of the Institute of Plant Breeding in UPLB. An isolated (fenced) field will be established to conform to the requirements of the regulatory agency. Based on the data gathered from the small-scale field trial, the best lines in terms of resistance to PRSV and agronomic performance will be selected for subsequent multi-location field trials.

In addition to the field testing, new transgenic products will be developed to maximize the use of the transgenic PRSV-resistant sources. Selected second-generation transgenic lines will be backcrossed to either parent of the popular IPB-bred hybrid cultivar Sinta and the high papain variety, red Solo.

A regulatory package addressing the requirements of Philippine regulations on the release of transgenic crops will be developed for the transgenic papaya product. While current regulations may opt to consider relevant regulatory data generated in other countries, it is expected that most of the data, particularly for the environmental safety assessment, will be generated within the country. These will include data on molecular characterization of the selected event(s), effects on non-target organisms, gene flow, nutritional analysis and some limited feeding studies. Since transgenic papaya with PRSV-CP technology has already been commercialized in other countries, the regulatory package developed for them will serve as guide.

12) Glossary of terms

Biotechnology - any technique that makes use of living organisms (or parts thereof) to make or modify products, to improve plants or animals, or to develop microorganisms for specific purposes.

Coat protein – a major component of viruses, the primary function of which is to protect viral genetic information.

DNA – a molecule found in cells of organisms where genetic information is stored.

Gene – a biological unit that determines an organism’s inherited characteristics.

Genetic engineering – the manipulation of an organism's genetic endowment by introducing or eliminating specific genes through modern molecular biology techniques.

Genome – the entirety of hereditary material in a cell.

Transformation event –the insertion of a particular transgene into a cell conferring potentially useful traits. The term "event" is often used to differentiate genetically engineered crop varieties.

Transgene – a foreign DNA gene introduced into an organism.

Transgenic plant - A genetically engineered plant created through transformation is sometimes referred to as a transgenic. Often referred to the in the literature as a "genetically modified" (GM) plant, although in reality all plants have been "genetically modified" from their original wild state by domestication, selection, and controlled breeding over many years

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