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Transgenic Papaya in Hawaii and Beyond

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Papaya ringspot virus (PRSV) is often a limiting factor in the production of papaya worldwide. In 1992, PRSV was discovered in the district of Puna on Hawaii island, where 95% of Hawaii's papaya was grown. Within two years, PRSV was widespread and causing severe damage to the papaya in that area. Coincidentally, a field trial to test a PRSV-resistant transgenic papaya had started in 1992, and by 1995 the 'Rainbow' and 'SunUp' transgenic cultivars had been developed. These cultivars were commercialized in 1998. 'Rainbow' is now widely planted and has helped to save the papaya industry from devastation by PRSV. Transgenic papaya have also been developed for other countries, such as Thailand, Jamaica, Brazil, and Venezuela. Efforts to have these papaya deregulated in these countries are ongoing.

Key words: Hawaiian papaya industry, papaya ringspot virus, pathogen-derived resistance, Rainbow, SunUp.

Introduction

Papaya (*Carica papaya*) is widely grown in the tropics and has been grown in Hawaii for over a century (Gonsalves, 1998). A major production constraint for papaya worldwide is papaya ringspot virus (PRSV), a potyvirus that is rapidly transmitted by a number of aphid species in a nonpersistent manner (Gonsalves & Ishii, 1980). Although PRSV was first reported in Hawaii in the mid-1940s, it did not become a major problem to the Hawaiian papaya industry until the 1950s. At that time, the Hawaiian papaya industry was located on Oahu island, but by the late 1950s and early 1960s, PRSV had severely affected papaya production on Oahu. Fortunately, the industry was able to relocate to the Puna district on Hawaii island. Puna had many advantages for raising papaya: (a) the area had an abundance of rather inexpensive land to lease; (b) the area has plentiful rainfall and sunshine; (b) the excellent 'Kapoho' papaya cultivar was uniquely adapted to the region; and (d) PRSV was not present in the region. Consequently, Hawaii's papaya industry expanded and prospered; by the 1970s, about 95% of Hawaii's approximately 2,500 acres of papaya were located in the Puna district on Hawaii island.

However, things changed for Hawaii's papaya industry when PRSV was discovered in Puna in May 1992 (Gonsalves, 1998). The virus spread very rapidly; by late 1994, the papaya industry was in a crisis situation. This brief article provides a snapshot of the development and impact of the transgenic papaya on helping the papaya industry in Hawaii.

Timely Development of the Transgenic Papaya

In retrospect, it is rather surprising that PRSV was not discovered in Puna earlier. By the 1970s, PRSV had established itself in the backyards of residential homes in Hilo, which was about 19 miles away from the main papaya-growing areas of Puna. This potential threat did not go unheeded; a small task force was employed by the Hawaii Department of Agriculture to contain the spread of PRSV. They searched for and destroyed infected papaya trees in Hilo and surrounding areas. Furthermore, my laboratory started research in 1978 to investigate the potential of cross protection to control PRSV in Hawaii (Yeh & Gonsalves, 1984). A nitrous acid induced mild strain was developed and shown to be effective in controlling damage by severe strains on Oahu island. However, this technology was not used on Hawaii island because of the lack of virus in Puna and the observation that the mild strain caused significant symptoms on papaya, especially during the winter months.

Efforts to develop transgenic papaya to control PRSV began shortly after news spread that transgenic tobacco expressing the coat protein gene of tobacco mosaic virus was resistant or showed delay in symptoms following inoculation with tobacco mosaic virus. The papaya research team consisted of molecular biologist Jerry Slightom (Upjohn Company), papaya horticulturist Richard Manshardt (University of Hawaii at Manoa), tissue culturist Maureen Fitch (a graduate student of Richard Manshardt), and me, a plant virologist. By 1986, we had cloned the coat protein gene of PRSV, and

Figure 1. Transgenic papaya line 55-1 showing resistance to PRSV HA compared to infected nontransgenic papaya. Photo by D. Gonsalves.

Figure 2. PRSV infected papaya fields in 1994.

in 1988, we started on the transformation of embryogenic cultures of papaya using the biolistic approach. We worked on transforming commercial cultivars of 'Sunrise', its sib 'Sunset', and 'Kapoho'. These cultivars were excellent candidates because they bred true to type, were commercial, and 'Kapoho' was the cultivar grown almost exclusively in Puna. Transformation of papaya was a pioneering challenge, because no one had yet reported its successful transformation (Fitch, Manshardt, Gonsalves, Slightom, & Sanford, 1990). Nevertheless, using the biolistic approach to transform embryogenic papaya cultures, we obtained 17 independently transformed plants. We immediately set forth to test the resistance of these lines at the R0 stage by propagating each line and inoculating clones with PRSV HA, a severe strain from Hawaii (Fitch et al., 1992; Tennant et al., 1994, 2001).

Fortunately, in 1991 we identified a single line (designated *line 55-1*) that was resistant to PRSV HA in greenhouse inoculations (Figure 1; Fitch et al., 1992). The resistant line was the red-fleshed 'Sunset', which was much less desirable than the yellow-fleshed 'Kapoho'. Confident that we had identified a resistant line of transgenic papaya, we quickly planned a field trial. The purpose of the initial field trial was to observe the resistance under field conditions and to determine if the single resistant line had desirable horticultural properties (Lius et al., 1997). The field trial started in April 1992 in the Waimanalo Field Station of the University of Hawaii on Oahu island.

1992: Start of the Transgenic Field Trial; PRSV invades Puna

It is rather rare that a potential solution is coincidental with a potential disaster. Such was the case with the transgenic papaya and PRSV in Puna. In May 1992, PRSV was discovered in the papaya orchards in Puna on Hawaii island, only a month after the field trial of line 55-1 was started in Waimanalo on Oahu island. By December 1992, results from the field test showed that all of the nontransgenic plants were infected while the line 55-1 plants were resistant. In Puna, massive efforts to contain PRSV were started, but the virus kept spreading, although a bit slowly at first. However, by October 1994 PRSV had spread to such an extent that efforts to contain PRSV by rouging were abandoned, causing more rapid spread of PRSV in Puna (Figure 2). Hawaii's papaya industry was in a crisis.

A major benefit of the 1992 R0 field trial in Waimanalo was that it gave us a head start in developing cultivars that might be useful for growers in Puna. Indeed, two new transgenic cultivars were developed—'SunUp' and 'Rainbow'. 'SunUp' is a transgenic red-fleshed Sunset that is homozygous for the coat protein gene.

Figure 3. Transgenic papaya test field in Puna. Yellow plants are nontransgenic papaya; plants on right are transgenic 'Rainbow' papaya. Photo by S. Ferreira.

Figure 4. Aerial view of transgenic papaya test field in Puna, showing block of healthy transgenic 'Rainbow' surrounded by severely infected nontransgenic 'Sunrise' papaya. Photo by S. Ferreira.

'Rainbow' is a yellow-fleshed F1 hybrid developed by crossing 'SunUp' and nontransgenic yellow-fleshed 'Kapoho' (Manshardt, 1998). As noted above, 'Kapoho' was the nearly exclusive variety grown in Puna. Thus, the 1992 trial confirmed the field resistance of the transgenic line and helped to speed up the development of the transgenic cultivars.

In October 1995, a crucial field trial of 'Rainbow' papaya (along with smaller numbers of 'SunUp') was started in Puna to determine resistance of the cultivars to severe virus pressure and to obtain horticultural data on the cultivars in the Puna, which is climatically different from Waimanalo. The field trial was led by Steve Ferreira of the University of Hawaii. The results conclusively showed that the transgenic 'SunUp' and

Table 1. Papaya production in Hawaii, 1992-2001.

Year	Total	Puna
1992[a]	55,800	53,010
1993	58,200	55,290
1994	56,200	55,525
1995	41,900	39,215
1996	37,800	34,195
1997	35,700	27,810
1998[b]	35,600	26,750
1999	39,400	25,610
2000	50,250	33,950
2001	53,000	40,290

[a] *PRSV was first observed in Puna in May 1992.*
[b] *Transgenic seeds were released to farmers in May 1998.*

'Rainbow' were resistant (Figures 3 & 4) and that these cultivars were of commercial quality. In fact, data showed that 'Rainbow' yielded about 125,000 pounds of marketable fruit per acre per year, whereas the nontransgenic 'Sunrise' (all of which became infected by PRSV) yielded 5,000 pounds of fruit per acre per year (Ferreira et al., 2002). The papaya growers in Puna were especially impressed by 'Rainbow' because it was yellow-fleshed, bore mature fruit a few months earlier than 'Kapoho', and gave much better yields than 'Kapoho'.

Efforts to move the transgenic papaya line 55-1 through the deregulation processes of the United States Department of Agriculture's Animal and Plant Health Inspection Service and the US Environmental Protection Agency, and the consultation process with US Food and Drug Administration, were started in late 1995. The processes were completed in 1997, and licenses to commercialize the transgenic papaya were obtained in April 1998. Seeds were distributed free to growers in May 1998, seven years after the initial experiment that showed line 55-1 was resistant to PRSV HA.

Farmers quickly planted the transgenic papaya seeds, which were nearly all 'Rainbow' because the farmers in Puna favored this transgenic cultivar. Harvesting of 'Rainbow' started in 1999 (Figure 5), and grower, packer, and consumer acceptance were widespread. The papaya industry had been spared from disaster. Table 1 shows the impact of the transgenic papaya on stemming the destruction caused by PRSV. Since 1992, when the virus was discovered in Puna, the yearly amount of fresh papaya sold from Puna had gone from 53 millions pounds in 1992 to 26 million pounds in 1998. In 2001, Puna papaya production rebounded to 46 million pounds of fresh market papaya. Another important impact has been the dramatic reduction of PRSV

Figure 5. Commercial transgenic 'Rainbow' papaya field. Photo by D. Gonsalves.

inocula in Puna, because infected fields have been replaced by the resistant transgenic papaya, and because many abandoned infected fields have since been destroyed. These conditions, along with judicious isolation and rouging of infected plants, have enabled growers to continue to produce nontransgenic papaya, especially to supply the Japan market, which does not yet allow the importation of transgenic papaya. In January 2003, Canada allowed the importation of transgenic papaya. Yet another benefit is that papaya acreage has expanded on Oahu due to the use of PRSV-resistant transgenic 'Rainbow' (or new hybrids that have been derived from 'Rainbow').

Brief Remarks on Efforts Beyond Hawaii

Following the breakthrough in Hawaii, my laboratory started concerted efforts to collaborate with interested countries to develop PRSV-resistant transgenic papaya for their countries. The countries included Brazil, Jamaica, Venezuela, and Thailand. Efforts have recently started with Bangladesh, Uganda, and Tanzania. Efforts in the first four countries have resulted in the development of resistant transgenic papaya that is suitable for their country (Cai et al., 1999; Tennant, Ahmad, & Gonsalves, 2002). In fact, some of the transgenic papaya are well advanced in field trials (Figure 6) and are moving through the process of deregulation. How fast the process will move, in the light of the current GMO climate, is not known. However, it is clear that the PRSV-resistant transgenic papaya is a practical solution for controlling PRSV, as has been shown in Hawaii.

Figure 6. Transgenic papaya field of R3 plants in Thailand in 2002. Work led by Vilai Prasartsee of the Thailand Department of Agriculture. Photo by Vilai Prasartsee.

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