The Papaya Story: A Special Case or a Generic Approach?

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The approach of developing virus-resistance in plants by expressing the coat-protein gene of the target virus is one of the truly great contributions in plant pathology and agriculture in general. It can be used for nearly all groups of plant viruses. Yet, since its development in the mid-1980s, only two virus-resistant transgenic crops have been commercialized in the United States: squash and papaya. In this short article, I will examine the papaya case and elucidate the factors that drove the investigators towards commercialization, and ask the question: Was this a unique case or might it be a generic approach?

PAPAYA AND PAPAYA RINGSPOT VIRUS IN HAWAII UP TO 1992

The Hawaiian papaya industry is based on the 'Hawaiian solo' variety, which is derived from a line that was introduced to the state about a century ago. Its characteristics are its small size (typically about 1 lb in weight), a sweet and pleasant flavor, and good shipping quality. 'Kapoho' became the dominant cultivar, grown nearly exclusively on Hawaii Island in the Puna district. The Puna district, in which papaya has been grown since the early 1960s, is unique in Hawaii because it has largely a lava-based soil, and, although it has about 100 inches of rain per year, it enjoys lots of sunshine. Thus, a combination of good drainage, abundant rain and sunshine, the availability of large tracts of land for lease at a reasonable price, and absence of papaya ringspot virus (PRSV) made the Puna district ideal for raising 'Kapoho' papaya. In fact, in 1992, Puna was producing 95% of Hawaii's papaya.

The ringspot virus is the most important viral disease of papaya worldwide. It was discovered in Hawaii in the 1940s by Jensen who coined the name. In the 1950s, the virus began to cause severe problems on Oahu Island, where Hawaii's commercial plantings were located. Some have surmised that the virus discovered by Jensen was milder than the strain that caused severe damage in 1950s. The papaya industry was forced to relocate in the 1960s to Puna, where it flourished.

Although free of PRSV, Puna was potentially in danger of getting the virus, because it was found in the early 1970s in Hilo, a town located about 19 miles from the main papaya growing areas in Puna. The state recognized this potential problem and the Hawaii Department of Agriculture (HDOA) utilized a small crew to rogue PRSV-infected trees in Hilo and surrounding areas. Much credit is due to HDOA for delaying the spread of PRSV.

Research was started in our laboratory in 1978 to develop PRSV-control measures, given the potential that the disease would inevitably move to Puna. The virus was purified, characterized, and antiserum produced allowing rapid detection. Cross-protection was the first approach we used, resulting in a mild mutant being produced and tried on Oahu Island. The approach provided economic returns to farmers on Oahu, but was not adopted widely because the mild strain caused distinct symptoms in the winter months. Some cultivars, such as ‘Sunrise,’ showed significant symptoms, and rather involved logistics were necessary to produce the mild strain and to inoculate plants. In the mid 1980s, we started developing transgenic papaya that expressed the coat protein of the mild PRSV mutant that we had developed in our cross-protection approach. A lesson to be learned—as will be seen as the papaya story unfolds—is that, when possible, one should work towards solving a problem before the problem occurs in the target region.

Industry in Crisis—Transgenic Papaya Commercialized: 1992–1998

The research team consisted of Jerry Slightom (molecular biologist), Richard Manshardt (horticulturist), Maureen Fitch (then a graduate student of Manshardt and an expert in tissue culture), and me (virologist). Our primary goal was to develop a timely practical solution for controlling PRSV and we had the good fortune of obtaining moderate but consistent support from special USDA grants that were earmarked for improving agriculture in the Pacific region. The PRSV coat-protein gene was cloned, engineered into the transformation vector, and transformations of embryogenic cultures were initiated with the gene gun in 1988. We also had the distinct advantage of having the counsel and use of the facilities of John Sanford, the co-inventor of the gene gun.

By 1990, we had obtained seventeen transgenic plants of ‘Sunset’ and ‘Kapoho.’ Sunset, a sib of ‘Sunrise’ that has red flesh as compared to the yellow-flesh ‘Kapoho,’ is grown extensively in Brazil, but not in Hawaii. Our preferred target was ‘Kapoho,’ but it was more recalcitrant than ‘Sunset’ in regenerating plants from transformed embryos. Clones of the transgenic lines were developed and tested for resistance to PRSV by mechanical inoculation under greenhouse conditions in 1991. To our joy, we identified a resistant line, designated “55-1,” which was resistant to a Hawaiian isolate, PRSV-HA. Following our practical philosophy, plans were quickly made to do a field trial at the Waimanalo field station of the University of Hawaii on Oahu. The field test consisted of clones of 55-1, other susceptible transgenic lines, and nontransgenic 'Sunset.'

The field test was started in April 1992, and, coincidentally, PRSV was discovered in Puna in May 1992. This discovery sticks out in my mind because I happened to be in Hawaii when it was reported, and Steve Ferreira, a plant pathologist with whom I was collaborating on cross-protection, and I were among the first to see the pocket of PRSV-infected trees. The image of the small pocket of infection and the devastation that would occur within only two years helped to spur our efforts to control PRSV. Another lesson in the papaya story is that we went straight to testing clones of transgenic plants in the field. We could have, and we did later, obtain seeds from the transgenic R0 line and then tested the R1 plants in the field as is normally done. However, this approach would have delayed the initial field trials by about two years—disadvantageous considering the speed at which PRSV spread throughout Puna.

As the virus spread rapidly in Puna, we moved to determine the resistance of line 55-1 under field conditions on Oahu Island. The HDOA's massive efforts to suppress the spread of PRSV by roguing infected trees helped to slow the spread of the virus, but by October 1994, it had spread to such an extent that further control efforts were abandoned. This further accelerated the spread, because infected fields served as continuous sources of virus spread by aphids.

By 1995, the papaya industry in Puna was in crisis. This translated to the industry in Hawaii as a whole, because Puna was growing 95% of the state's papaya. In the meantime, the field trial in Waimanalo provided convincing evidence that line 55-1 was resistant to PRSV. Manshardt developed two cultivars that would be subsequently commercialized, 'SunUp' and 'Rainbow.' 'SunUp' is transgenic line 55-1 'Sunset,' which is homozygous for the single coat protein gene insert. 'Rainbow' is an F1 hybrid of 'SunUp' and nontransgenic 'Kapoho'. The development of 'Rainbow' proved decisive because it is yellow-fleshed, which is preferred by consumers and farmers in Hawaii, it has hybrid vigor which results in increased yield, and it has suitable shipping qualities.

While 1995 was a crisis year for the papaya industry, it was also a pivotal year in our research efforts because we were able to establish a large field trial on a commercial papaya farm that had gone out of business because of PRSV. The field trial was important because it was to be done in the area where we wanted to control PRSV; it allowed us to test 'SunUp and 'Rainbow' under the target climatic conditions; it served as a basis for farmers to observe the plants and fruit since they would need to be convinced of the resistance and of horticultural qualities; and it gave us a firm basis for observing the transgenic papaya as we initiated the process of deregulation by APHIS, EPA, and FDA. Steve Ferreira joined the team and directed the field trial that was started in October 1995. It provided convincing evidence to researchers, farmers, and packers that the transgenic papaya was, indeed, resistant to PRSV under severe virus pressure, had horticultural qualities that farmers and packers liked, and that productivity of ‘Rainbow’ was very high. Under severe virus pressure, the calculated yield of ‘Rainbow’ was about 125,000 lb/acre, as opposed to 5,000 lb/acre for nontransgenic ‘Sunrise.’ The latter yield was low because of early infection by PRSV.

A lesson learned from the field work is that trials that serve several purposes—such as obtaining critical scientific data, simulating commercial conditions, and serving as forums for observations by farmers, packers, and consumers—are very useful in shortening the time required for commercialization. From 1996 to 1997, petitions were prepared and submitted to APHIS and EPA to deregulate the transgenic papaya, and documents were prepared for consultation with FDA on food-safety aspects. The transgenic line 55-1, which was the parent of ‘SunUp’ and ‘Rainbow,’ was deregulated and consultations were completed by the end of 1997.

The commercialization of the transgenic papaya was led by the Papaya Administrative Committee (PAC), which consists of growers who have agreed to be in a USDA marketing order that allows the taxing of a percentage of the proceeds that are obtained from the sale of papaya from packing houses. This approach was necessary because the investigators had no backing from commercial companies. The PAC enlisted a lawyer, Mike Goldman, who was successful in obtaining the necessary licenses for commercialization.

Seeds were distributed free to growers on May 1, 1998, almost six years to the day on which PRSV was discovered in Puna. Since seeds were not available in sufficient quantities for all farmers to have what they desired, the distribution were based on the degree of PRSV damage experienced and the acreage grown. All of the farmers had to view a video on the transgenic papaya and sign an agreement that the seeds would be not be planted outside of Hawaii. Essentially, the transgenic papaya could be grown only in Hawaii, but fruit could be sold wherever transgenic papaya was legally accepted wordwide.

Commercial Transgenic Papaya in Hawaii: 1998–Present

A survey on adoption of the transgenic papaya by farmers was taken, starting in 1998 until the end of 1999. Results showed a rapid adoption rate (adoption being defined as the obtaining and actual planting of transgenic seeds) by a great majority of the farmers in Puna; ‘Rainbow’ was by far the dominant transgenic cultivar, and resistance to the virus held up in commercial orchards. It was also observed that transgenic papaya adjacent to highly infected fields remained resistant, which allowed growers to directly reclaim infected fields without prior elimination of the orchards.

The stability of resistance under field conditions was always a concern, to growers and to investigators. This was especially true for the widely planted hemizygous ‘Rainbow,’ because our greenhouse tests had shown susceptibility to isolates of PRSV from Guam, Taiwan, and Thailand. Fortunately, inoculation tests showed that ‘Rainbow’ was resistant to numerous isolates collected in Hawaii. Nevertheless, a program to consistently monitor for resistance-breaking Hawaiian strains was started and continues to the present. Hawaii is cognizant of the need for constantly monitoring to help ensure the long-term stability of the resistance of the transgenic papaya. In particular, it is crucial that care be taken against the introduction of exotic strains.

The impact of transgenic papaya has been dramatic and measurable in yield. In 1992 when PRSV was discovered there, the region was producing 95% of Hawaii’s papaya, amounting to 53×10^6 lbs of fresh papaya being marketed locally and abroad. The virus caused production to decline to 26×10^6 lbs annually in 1998, when transgenic seeds were released. However, by 2001, Puna was producing about 46×10^6 lbs primarily due to the planting of ‘Rainbow.’

The impact is also apparent in allowing the growth of non-transgenic papaya in Puna. Non-transgenic papaya is crucial to Hawaii’s industry because Japan, an important export market, requires it. Japan has not yet approved the introduction of transgenic papaya. Numerous observations had clearly shown that planting of non-transgenic papaya next to infected fields results in almost no economic return. Although spatial isolation can result in increased economic returns from non-transgenic plantings, prior to the release of the transgenic papaya, isolation from infected fields was virtually impossible in Puna. After release of the transgenic seeds, large tracts of transgenic papaya—along with destroying abandoned infected orchards—served as effective isolation from PRSV to allow the economic regrowth of non-transgenic papaya in Puna. Thus, Hawaii continues to produce nontransgenic ‘Kapoho’ to satisfy the Japan market. Without the transgenic papaya, it is my opinion that this practice would not have been possible.

Although transgenic papaya is now an entrenched component of Hawaii’s fruit production and is a dominant reason why resurgence of PRSV has not occurred to a large extent in Puna, the industry is not without its problems. In the past, overproduction has resulted in oversupply and lower prices to farmers. In contrast, production of papaya can be limiting because, although it is produced year-round, the crop has troughs in production during which demand cannot be met. Lastly, as a consequence of PRSV, Hawaii lost much of its market share during the 1990s. All of these factors affect the well-being of Hawaii’s papaya industry. If not for the transgenic papaya, however, production would have continued to decrease in Puna, perhaps resulting in the demise of the industry, which is second only to pineapple in Hawaii.

A Unique Case?

An analysis of the papaya case reveals that it is unusual in comparison with other commercialized transgenic products in that it was developed and commercialized outside the direct influence of companies. Our primary objective was simply to develop a control measure for a potentially severe problem, rather than to create a profit. The urgency of obtaining a commercial solution became apparent after PRSV was discovered in Puna, by which time we had already identified a resistant transgenic papaya. Also, papaya is not a major crop and thus is of little interest to large companies. Although we have not calculated the cost of the project, it was rather modest because it was carried out mainly as a normal university-type project in which a series of grants was obtained to fund long-term research. The project was never a full-time endeavor of the research team. The blending of expertise definitely helped in making progress through all phases.

The timely start of the field trials was a major factor in moving us quickly to our goal. Several important aspects were contributory. The first was the practical attitude of the team in moving directly to a solution rather then being unnecessarily side-tracked in investigating interesting observations. The second crucial condition was the willingness of APHIS to work with us. We also benefited by being able to analyze the squash petitions that had been recently submitted by Asgrow Seed Company, with whom I was collaborating. And, in retrospect, one can say that the climate surrounding GMOs was largely "friendly" during our efforts to deregulate the transgenic papaya.

The deregulation of the transgenic papaya by EPA entailed the development of rational arguments and the generation of data that the coat protein was already present in infected plants at higher levels then in transgenic plants. Furthermore, the cross-protection approach had utilized thousands of mild-strain-PRSV-infected plants from which the cross-protected fruits were consumed, with no reported ill effects. Consultations with FDA went smoothly because our analysis of the plants showed that the transgenic papaya differed from the nontransgenic counterpart only in its disease-resistance.

It can be argued that the papaya case is unique in that it addressed a crisis in which there were no other practical and timely solutions. In my opinion, solving a problem involves a spectrum of cases that provides incremental steps as well as the final key that provides the solution. The papaya case is more towards the latter but by no means does it remain a final solution, especially since we know that there are strains of PRSV that can overcome the resistance possessed by 'Rainbow.' Perhaps a more germane question is: should a biotechnology approach be taken only when all others have failed? I feel that biotechnological approaches constitute just one set of tools among many that may be used, and not necessarily as a last resort or in dire circumstances. Again, when we took up the papaya case, the biotechnology approach provided a rational solution within our capability, no more no less.

It has been mentioned that the amount of data that we had to present to the regulatory agencies was rather small, and that we should have tested the transgenic papaya for a longer period of time before releasing it for commercialization. There are no simple answers to such assertions, except to realize that risk is a relative term that is weighed in light of the potential benefits of the solution. As noted, the Hawaiian papaya industry was in crisis in 1995, and things would have gotten worse if the transgenic lines had not been released. It might be argued that if the transgenic papaya were tested for 10 years before being released, people would feel safer, but it might have been too late to save the Puna papaya plantings from total demise. One will never know. However, we followed the proper steps in moving through the deregulatory process. Five years have passed since commercialization, so we have had at least that much time to observe consequences and more as each day goes by.

Time has shown that transgenic crops must be examined on a case-by-case basis, and that different countries have different requirements. For example, we have had to generate more information on the transgenic papaya in developing our documents for Japan. The same is true for the regulatory stages that transgenic papaya has to go through in Jamaica, where we have a technology-transfer program. Perhaps the information we have gleaned from our work with papaya in Hawaii will help us address future questions relating to transgenic papaya elsewhere.

SUMMATION

This communication has briefly described the rationale that we used to develop and commercialize transgenic papaya in Hawaii. The impact is obvious five years after seed release. In 2003 the GMO debate is much more intense than it was in 1996–1998, when we worked towards deregulating and commercializing the transgenic papaya. Will the transgenic papaya for Hawaii remain a unique case? Time will tell. It is my personal opinion that progress on developing rational approaches for using GMOs will come only through continued dialogue, showing respect for all views and a willingness to work towards safe but realistic solutions.

FURTHER READING

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Q&A

Audience Member: To what extent is Hawaii's crop transgenic today?

Gonsalves: I would say 50/50. It's about 50% transgenic and 50% non-transgenic. Japan occupies 30 to 35% of Hawaii's market and it's a very lucrative market, and so they have to grow non-transgenic papaya. One of the big farmers grows non-transgenic papaya, in isolated locations, essentially surrounded with buffers of transgenic papaya to try to cut down on the virus going in. Now, one may ask if transgenic papaya has been found in Japan? Yes, in a couple of cases one papaya tested positive. I'm not surprised at all. My goodness, with the millions of papaya that we ship, some are bound to get through, but Japan's reaction was very reasonable. They did not ban the papaya. They said that Hawaii had to improve security. Japan has been very fair—very tough—but very fair in our application to get it deregulated.

Audience Member: Viruses have a tendency to mutate. Are you noticing any diminution of effectiveness? Also, have any studies been done to appraise negative health effects from these papayas? Even though they look good, have you fed them to laboratory animals?

Gonsalves: Resistance may not hold up because the virus may change. Recombination between the virus and the transgene may occur and, naturally, this was something we were worried about. So, we went all over the Hawaiian Islands, collected over 300 isolates and inoculated them onto transgenic papayas just to see if we had strains that would break down resistance, and no, none of the strains has done so. Since the release in 1998, only a handful of plants has shown infection so we have no evidence that the transgenic resistance is breaking down. Based on the uniformity of the viral isolates and the mechanism of resistance, I don't think that it will break down unless a strain comes from Guam or from Thailand. We know that the Thailand strain will overcome the resistance of the Hawaiian transgenic papaya. Now, in relation to safety—this is an interesting question. I'll tell you what we did back in 1995, which goes along this line of reasoning. Lots of virus-infected papaya had been consumed for many years and we did not observe any ill effects. Therefore, even though I did not do feeding studies, I had no reason to believe that this transgenic papaya would be harmful because many people had been eating virus-infected fruit. In fact, you will recall, I utilized cross-protection whereas in Taiwan they inoculated millions of papaya trees with the virus and then ate it, and nothing happened. That was the reasoning. Now, did we do any feeding studies? No, we did not do any feeding studies. As far as getting it through EPA or APHIS or FDA, we did not do any feeding studies. In green papaya, there is a natural compound called benzyl-isothiocyanate. Just by hearing the word "cyanate" you know that this is a poison. So, we tested for the presence of this compound, but we did not do any animal feeding. Does Japan require animal feeding? No, but they require extensive characterization of the papaya. I will say that this transgenic papaya, after going through all of the stuff that we have to go through for Japan, is probably one of the most characterized genetic products now on the market.

Audience Member: One of the things that really turned the Europeans around was Dr. Árpád Pusztai's work on genetically engineered potato, and his firing was front-page news across Europe. It really gave the biotech industry a black eye over there. Why not feed papaya to rats and show that there's no problem with it. Then you can take that research to people, label it and have consumer acceptance of it, rather than the suspicion that will arise when such studies are not done which causes consumers to feel that they cannot trust it.

Gonsalves: That's a good point—to prevent criticism why don't we feed the transgenic papaya to rats? Actually that has been done in Jamaica with no ill effects. You know, why we didn't do that? Frankly, I never thought about it. I never thought about doing rat feeding and so forth. Now if there is an established feeding method and if it passes that test then it's safe—I think that's great. Recently there was an article commenting about potential allergenicity of our transgenic papaya, because it has a string of certain six amino acids and so forth. The question of allergenicity did come up and, in my simplistic thinking, I thought, "My gosh we've been eating this stuff for so long and there's been no reaction." But now we have to do it for the Japan application. We have analyzed all this stuff. We have checked it out. We have shown that this protein is digested within 4 seconds. And we are doing that because they say that we have to do it. Same thing with weeds—this thing is virus resistant, it may become a weed. So people ask why we didn't do weed studies. Well I'll tell you our reasoning. Since the early 1900s, the state of Hawaii has been keeping records on weeds. They have very good records on the weeds of Hawaii, and papaya has never been mentioned as a weed. The virus was introduced in 1945 and papaya was never noted as a weed. Should we have done those studies to prove it is not a weed and how long do you do it? That's the kind of "criticism" that might come of our work. I explained this thinking to APHIS. Now you can debate whether we are irresponsible or not.

Audience Member: Would you explain your story in terms of research funds and where they come from? Are they public or private? In connection with intellectual property rights, you indicated that the seed is free—is that still the case? Who controls that part of the papaya industry?

Gonsalves: As I mentioned, the first research fund was $5,000 from the governor's initiative, but basically it was USDA special funds. You might call it a pork-barrel fund. It was Section 406 and now is T-STAR type funding that the University of Hawaii obtains through various means to help this transgenic work. No funds were obtained from companies. The amount of funding actually was very little. In 1988 we obtained a grant of $117,000 for 3 years to support Richard Manzar and me. We had to do what we had to do to get the job done. It wasn't because we didn't get enough funding. It was because there was a problem out there, we were going after it and I'm forever grateful to the University of Hawaii for even allowing a guy from Cornell to work with them on this problem. Now, in relation to intellectual property rights, well, this transgenic papaya has all the genes and the markers that many of the genetically engineered crops have. It has the 35S promoter, it has the *npt*2 gene, and so forth. Well, Monsanto owns the 35S promoter. Jefferson owns the GUS gene. Diatec owns the leader sequence. Fortunately we had patented the coat-protein gene and the gene gun, because it was done when Cornell had the patent. We had to go through the intellectual property right process. Naturally we had no money, so we went to the Papaya Administrative Committee. They pursued it. Now, on purpose, I did not look at other licenses to see how much they cost, but I can tell you it cost hardly anything. Negotiations had to be made with Monsanto, and it did not cost much. The papaya administrative committee controls the licensing. They are composed of growers, so, early on, they had to figure out how they were going to distribute the seeds. They figured out a lottery system, and the seeds were essentially distributed free. The only catch was that they had to sign a license that they would not take the seeds and grow them outside of Hawaii, because part of the license was that the transgenic papaya could be grown only in Hawaii. The farmers had to watch a 30-minute video on what is transgenic papaya and all of that stuff, and after they did that then they could have the seed free of charge. Just recently I heard that the growers association is beginning to sell the seeds because they want to make money so they can raise more seeds. I think it is about $20 an ounce.

Audience Member: Hawaiian farmers adopted this technology because they had a shortage of papaya and they didn't have other options. And you talked about the virus being spread in many different countries. Do you anticipate that in Japan and similar markets there would be a shortage of available non-transgenic plants in the future?

Gonsalves: I don't anticipate a shortage of non-transgenic papaya in Japan. The Philippines is beginning to ship papaya to Japan, so I don't think there will be a shortage.

Audience Member: But you mentioned that the virus is getting worse in many places.

Gonsalves: Oh yeah, it's occurring in the Philippines, so they have started projects to develop genetically engineered papaya. This product is a good sustainable solution. But I don't know whether it will be adopted, because of the factors that we are talking about it. Another thing: the transgenic papaya in Hawaii is better than the non-transgenic papaya. We tried to transform the current cultivar, Kapoho, and we couldn't. We can now, but back then we couldn't. Kapoho is a yellow-flesh papaya which the growers all want. But, we succeeded in transforming a red-flesh papaya. To make red yellow you have to cross with the yellow and the yellow is dominant. We made a cross between Sunset and Kapoho. Now, for years, Hawaii had just inbred Kapoho. When we made this cross—now it's through no brains of ours because we had to get a yellow-flesh fruit, but we got a hybrid that outyields Kapoho and bears 3 months before it. It tastes just as good, so actually people would rather have the transgenic than the non-transgenic. I don't think it's because they lack non-transgenic, no, I think they'd rather have transgenic, plus it's cheap. All papaya is sold the same: 69 cents a pound.