# Update on Genetically Engineered PRV Resistance

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Genetic engineering for resistance to papaya ringspot virus (PRV) is another approach to controlling PRV in papaya. Unlike the cross-protection strategy, which Dr. Ron Mau indicated is now being implemented commercially, genetic engineering for PRV resistance is still in the research phase. Since I have discussed the procedures involved in creating genetically engineered plants at several previous HPIA meetings, I will only review these briefly here, before moving on to present the latest results from our field trial, mention the current plans for incorporating genetically engineered PRV resistance into commercial cultivars, and talk about some regulatory problems which have to be overcome before seed of genetically engineered plants can be distributed.

The genetically engineered papaya plants are resistant to PRV because they contain a foreign gene from the PRV virus itself that interferes with normal replication of the virus in the host papaya. The gene codes for the viral coat protein that surrounds the virus particle. The coat protein gene (CP) was isolated by Dr. Dennis Gonsalves, a virologist at Cornell University, and it was manipulated by Dr. Jerry Slightom of the Upjohn Company to permit the papaya to produce the PRV coat protein. Dr. Maureen Fitch, then a PhD student at UH and now with the USDA Sugarcane Technology Lab at the Hawaiian Sugar Planters' Association, put the CP gene into cells of specially prepared papaya tissue cultures and regenerated plants that produced the coat protein. One of the genetically engineered papaya plants has demonstrated a high level of resistance to PRV in greenhouse tests at Cornell and in Hawaii. Over the last year, a tissue cultured clone of this plant has been tested for PRV resistance in the field at Waimanalo, and it is the result of this test that I will present today.

The most promising resistance chanced to occur in a genetically engineered 'Sunset' plant with the identification code 55-1. This plant was cloned to produce 20 replicates, which were planted in the field along with 20 replicates of a 'Sunset' plant that was genetically engineered with genes other than the CP gene (the CP gene control), and 20 normal 'Sunset' seedlings (the genetic engineering control). The objectives of the field trial were to (1) test the effectiveness of the CP gene as a PRV resistance factor and (2) determine whether the method of virus inoculation (manual versus natural aphid vectors) affected disease resistance or symptom severity. The experimental design was a split plot with 10 replicates, and the plants were manually inoculated in July 1992. Disease reactions in the inoculated plants were assessed on four occasions during the last year (November 1992, February 1993, April 1993, and September 1993), using a disease symptom rating scale (1 = no symptoms, 2 = mild, 3 = moderate, 4 = severe) and ELISA (enzyme linked immunosorbant assay) serological test.

The results of the field trial to date are very clear and as good as we could have hoped for. All control plants showed disease symptoms and high ELISA values within one month of the date of manual inoculation, or within four months if inoculation was left to aphids that are the natural vectors of PRV. In contrast, the 55-1 plants containing the CP gene have been completely free of PRV for 14 months, in spite of two manual inoculations and continuous exposure to local aphid populations (Table 1). Growth and vigor of the 55-1 plants, as measured by trunk diameter, was significantly better than in the controls (Table 2), and there did not appear to be any detrimental side effects of genetic engineering as far as reproductive fertility, fruit size, or sugar content were concerned. The method of inoculation had no effect on severity of symptoms or degree of resistance in any of the plants (Table 1). These initial results indicate a great success for genetically engineered PRV resistance, but the field test will be continued for a full two years to see if the protection persists.

Although several genetically engineered 'Kapoho' plants were produced and tested in this program, none of them proved to be as resistant to PRV as the 55-1 clone of 'Sunset'. The reason for this is not clear, but it probably has nothing to do with the cultivar differences between 'Kapoho' and

**Table 1. Effect of inoculation method (manual vs. aphid vector) and papaya genotype (transgenic CP+ [55-1], transgenic CP− control [62-1], and seedling CP− control) on PRV symptom expression.**

| | Nov. 11, 1992 | Feb. 9, 1993 | Apr. 13, 1993 | Sep. 8, 1993 |
|---|---|---|---|---|
| Inoculate method | n.s. | n.s. | n.s. | n.s. |
| Papaya genotype | ** | ** | ** | ** |
| 55-1 vs. controls | 1.03 : 2.89** | 1.03 : 2.36** | 1.00 : 2.45** | 1.00 : 2.79** |
| 62-1 vs. seedling | 2.90 : 2.88 n.s. | 2.25 : 2.47* | 2.40 : 2.49 n.s. | --- |

PRV rating scale: 1 = no symptoms, 2 = mild symptoms, 3 = moderate symptoms, 4 = severe symptoms
n.s. = not significant; * = significant (0.05 > P > 0.01); ** = highly significant (P < 0.01)

**Table 2. Effect of PRV CP gene expression on susceptibility of papaya to PRV (measured by ELISA) and on trunk diameter.**

| | ELISA range $O.D._{405}$ | Trunk diameter (cm at 45-cm height) |
|---|---|---|
| Nov. 11, 1992 | | |
| Transgenic (CP+) | 0.010 – 0.017 | 8.85 |
| Control (CP−) | 0.681 – 1.914 | 7.33 ** |
| Feb. 9, 1993 | | |
| Transgenic (CP+) | 0.020 – 0.084 | 12.14 |
| Control (CP−) | 0.868 – 1.891 | 9.55 ** |
| Apr. 13, 1993 | | |
| Transgenic (CP+) | 0.000 – 0.005 | 13.28 |
| Control (CP−) | 0.157 – 2.138 | 9.73 ** |
| Sep. 8, 1993 | | |
| Transgenic (CP+) | 0.000 – 0.014 | 14.49 |
| Control (CP−) | 0.387 – 0.993 | 8.87 ** |

** = highly significant (P < 0.01)

'Sunset'. Most likely, the success of the product is dependent upon where in the set of nine papaya chromosomes the CP gene becomes inserted, with some regions being better than others for expression of the resistance factor. Since insertion appears to be random, it may simply be poor luck that we did not produce a more resistant 'Kapoho' on our first attempt. Dr. Gonsalves at Cornell has agreed to continue our collaboration in a new project, and we are again attempting to produce a PRV-resistant 'Kapoho'. In the meantime, the quickest way to use the resistance in 55-1 is to make conventional hybrids between it and other commercially important cultivars, such as 'Kapoho' and 'Kamiya'. These hybrids will have yellow or orange flesh color and should be acceptable to growers and consumers. Preparations are being made to produce hybrid papaya seed incorporating the PRV resistance from 55-1.

The developments described above are mostly good news for papaya growers. The not-so-good news is that it may be awhile before genetically engineered papaya seed is available for commercial release. The U.S. Department of Agriculture considers genetic engineering, in which genes from one organism are moved into and expressed in another organism, to be a technology that has more potential dangers than conventional breeding. Consequently, the distribution of genetically engineered products is regulated by the USDA. The chief concern is that the competitive advantages conferred upon genetically engineered plants might allow them to persist in the agricultural environment and become serious weed problems. It now appears that, before seed can be commercially distributed, we must provide the USDA with data showing that the genetically engineered papaya is no more a weed threat than a normal papaya. It is not clear at this point what kind of data are required, but in the worst case, if several generations of seedling survival observations have to be accumulated in different environments, we are talking about years of work. There is some reason for optimism in that other crops will be passing over these deregulation hurdles before papaya, and they may set precedents that will permit speedier clearance in our case.