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Research Project to Develop Taro with Increased Fungal Disease Resistance

Lead Researcher

Susan Miyasaka is a crop scientist at the University of Hawaii's College of Tropical Agriculture and Human Resources. She has been working on taro since the 1970's when she earned her Masters of Science (MS) degree at the University of Hawaii. Her research project involved the study of calcium deficiency as a possible cause of 'guava seed' disease in taro. Susan was born and raised in Hawaii, and has been eating poi and luau leaf all her life.

This current research project to genetically engineer Chinese taro is just one of several grants of Susan Miyasaka that are focused on increasing taro production. Another grant involves research on the management of green manure crops to reduce root knot nematodes in dryland-grown taro. A third grant seeks to evaluate new, conventionally-bred taro hybrids for both poi quality and increased resistance to the fungal disease, **leaf blight** caused by *Phytophthora colocasiae*.

Research Goals

Taro yields in Hawaii have been declining over the past 30 years, with steep decreases in the past 5 years. The overall, steady decline may be due to decreased soil fertility, but the recent dramatic decreases are probably due to **fungal diseases** (such as leaf blight or pocket rot) and apple snail. See Fig. 1 below:

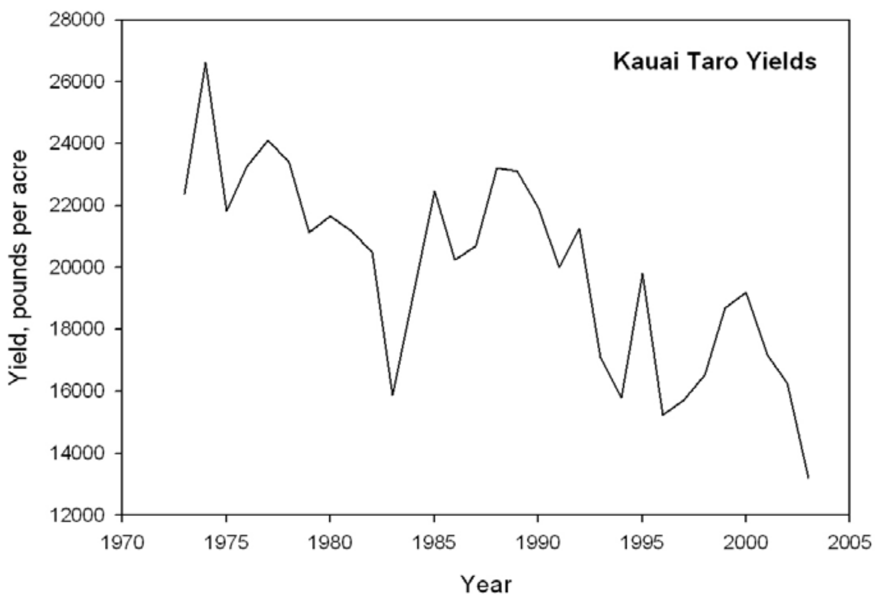


Figure 1: Kauai Taro Yields (1970-2003). Data source: Hawaii Department of Agriculture and U.S. Department of Agriculture Statistics.

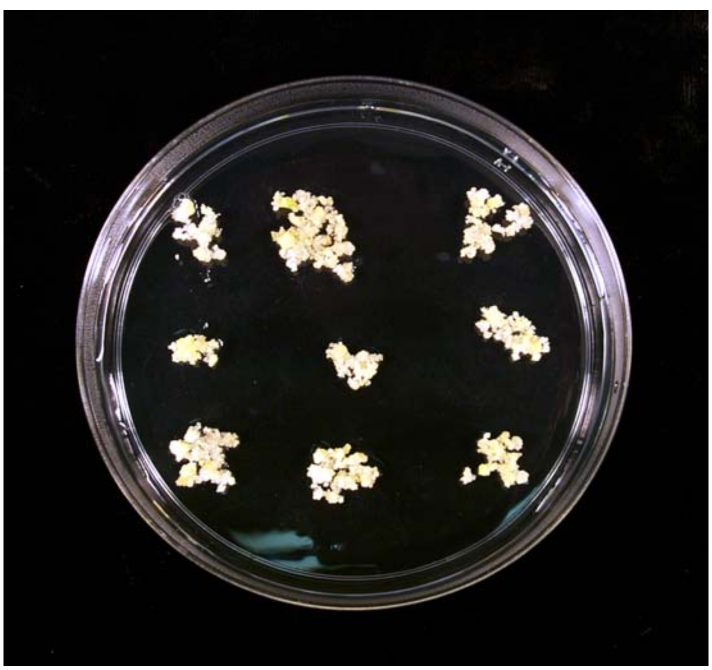
Estimates of corm (kalo) losses due to disease range from 25 to 50% in Hawaii. Also, it is not a question of *if* but *when* new diseases will arrive into Hawaii. The fungal agent that causes **leaf blight** arrived in Hawaii during the early 1910's and probably resulted in the loss of susceptible, ancient Hawaiian varieties at that time. After it arrived in the Samoan islands during the early 1990's , this disease has reduced yield of traditional, susceptible varieties by 95%. Only discovered recently in Hawaii, **pocket rot** is caused by a new species of *Phytopthora*.

A level of *Phytophthora* leaf blight resistance has been found within the taro germplasm. Another UH researcher is conducting conventional crosses between commercial taro varieties and wild relatives to breed in disease resistance that is found in wild relatives. However, it is a slow and costly process, and the hybrids produced will no longer be the original variety. In addition, the disease resistance existing in wild relatives breaks down under weather conditions that favor the disease organism.

To overcome these difficulties, the research team wanted to find out whether modern molecular biological techniques could increase fungal disease resistance (above that found in wild relatives of taro) and yet preserve all other characteristics of the original taro varieties.

Findings:

Fact 1: The research team was successful in developing a tissue-culture method to produce callli (undifferentiated cells) that could subsequently develop into a whole plant for the Chinese variety, *Bun long.* Plant shoots are placed into tissue-culture and some plant hormones are varied in the medium so that calli are produced. Then, the hormones in the media are varied again so that a whole plant is recovered.



Calli of Chinese taro variety (Bun long) are capable of regenerating whole plants.

The research team was not successful in developing such a method for the Hawaiian variety, Maui Lehua. Since the method of genetic transformation requires a regenerable calli, *no attempt* was made to insert a gene construct into a native Hawaiian taro variety.

Fact 2: The research team inserted a disease resistance gene (chitinase gene) from rice into the Chinese taro variety, *Bun long*. Chitin is a component of the cell walls of fungi; it isn't found in plants. Chitinase is an enzyme that digests chitin, and increased production of this enzyme has been related to increased disease resistance in many plants.

In a recent test of one transgenic Chinese taro line, the research team found no increased resistance to *Phytophthora* leaf blight. This transgenic line will be tested for increased resistance to other fungal diseases, such as *Pythium* rot or *Sclerotium* blight. In addition, more transgenic lines of Chinese taro will be tested for resistance to leaf blight and other fungal diseases.

Fact 3: There *have not been any* releases of genetically engineered taro outside the laboratory. At this time, no field trials are planned, because there are no lines of transgenic taro that exhibit increased fungal disease resistance.

Are there potential risks of genetically engineered (GE) taro?

1. Possible Allergenicity: Proteins that result in allergic reactions in sensitive individuals could be produced by the transferred chitinase gene. This is a remote but real possibility, since some plant chitinases (but not all) have been found to be allergens. Methods for testing for allergenicity are well established and Federal rules require that allergenicity and nutritional values be evaluated before any GE crop is released. If any taro lines are genetically improved for disease resistance with chitinase or any other gene, then these traits will be carefully tested.

2. Transfer of Antibiotic Resistance: Could antibiotic resistance genes from a GE taro be transferred to bacteria in the stomach or intestines of animals or humans? This scenario is extremely unlikely, because enzymes in the digestive system would break down any released genetic material. A recent assessment of the safety of genetically engineered foods ("Safety of Genetically Engineered Foods", The National Academy of Sciences, 2004) has found no evidence during the past decade or more of GE foods for the transfer of antibiotic resistance to intestinal bacteria. In addition, taro is typically cooked and processed prior to being eaten, making it even more unlikely that any genetic material will be transferred. If consumers continue to be worried about this issue and if a favorable GE taro is produced, new molecular techniques to remove the antibiotic resistance genes will be investigated.

3. Gene flow Issues: Could transfer of gene constructs occur to non-genetically engineered taro varieties or weedy related species? The risk of accidental gene transfer from a genetically modified *Bun long* is very low, because this variety doesn't flower naturally under the environmental conditions in Hawaii. In addition, genes to produce sterility are available and could be considered if the disease resistance trait proves successful. Such genes could prevent the accidental transfer of gene constructs to other taro varieties via pollen and yet wouldn't hurt taro production since this crop is vegetatively propagated.

4. Environmental Impact: Could adverse environmental effects occur in unintended targets? In a recent report by the National Academy of Sciences ("Environmental effects of transgenic plants", 2002), the committee concluded that: a) the transgenic process does not present any *new* category of risk compared to conventional methods of crop improvement; and b) although genetically engineered plants could contain genes transferred across species, there is no increase in environmental risk as the genetic relationship between the donor and recipient of the gene gets farther apart. The committee concluded that environmental risks could occur in GE plants (as well as in conventionally bred plants), but each plant needs to be evaluated on a case-by-case basis.

5. Public Acceptance: Would lack of consumer or farmer acceptance make the release of a GE taro commercially non-viable? If taro farmers in Hawaii refuse to grow GE taro and if consumers in Hawaii refuse to eat GE taro, then obviously there is no potential for commercialized GE taro in Hawaii. However, the lack of acceptance of GE taro in Hawaii will not stop genetic transformation of taro across the world. In 2000, scientists in Japan were the first to genetically transform a Japanese variety of taro, *Eguimo*, using a marker gene.

<u>Potential benefits of GE taro</u>?

At this point in the project, only possible benefits can be speculated, because it is too early to determine whether insertion of disease resistance genes results in increased fungal resistance in taro.

Other researchers have inserted a rice chitinase gene into tobacco, and found increased resistance to damping off caused by *Rhizoctonia solani*. In addition, transgenic canola plants were resistant to stem rot caused by *Sclerotinia sclerotiorum*. Apparently, the chitinase gene is effective against a broad range of fungal pathogens, indicating that a transgenic plant could have increased resistance to several fungal pathogens.

Any new technology comes with risks and benefits. This project seeks to determine the possible benefits of insertion of disease resistance genes into taro, so that you (the farmer or consumer) could make an educated decision about whether to accept this new technology.

Farmers should ask themselves whether they would accept transgenic taro if yields were improved by 30% to 50% due to increased disease resistance, without loss of quality. Consumers should ask themselves whether they would accept GE taro if it has been tested according to Federal government regulations and shown to be safe for human consumption. Native Hawaiians should ask themselves whether insertion of disease resistance genes in taro is acceptable if used to preserve traditional varieties that might be lost due to introduced diseases.

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Think about the current downward trend in taro yield over the past 30+ years. **Ask yourself, will there be taro for future generations to enjoy?**