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Papaya Germplasm and Breeding in Hawaii

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Abstract

The papaya improvement "program" in Hawaii consists of several projects involving collaboration between researchers at the University of Hawaii, the USDA, and mainland institutions. The program aims to collect and evaluate papaya germplasm from Central and South America, as well as address specific breeding objectives. In addition to conventional techniques, the methodologies of molecular biology are being applied to the germplasm collection to reveal the history of papaya domestication, and to commercial cultivars to increase the speed and effectiveness of breeding efforts.

Germplasm

The UH Department of Horticulture and the USDA/ARS National Clonal Germplasm Repository (Hilo) are collaborating in a joint program to collect, maintain, and evaluate accessions of papaya and other *Carica* species. Our objectives are to use the papaya germplasm for 1) clarifying the genetic relationships among accessions to better understand the history of papaya domestication, and 2) improving papaya cultivars for the local Hawaiian papaya industry. In pursuing these objectives over the last two years, we have collected a total of 166 papaya accessions from Central and South America, as well as 75 accessions encompassing eleven other *Carica* species (Table 1). Part of the collection is currently under evaluation for morphological and fruit quality factors on Oahu.

In addition to the germplasm, our travels have provided us with experience bearing on the origin of the domesticated papaya, about which some controversy exists. Some reports place the center of origin of papaya in the eastern slopes of the Andes Mountains in South America, primarily because that region is the center of diversity of the genus *Carica* (15). Approximately 20 species exist in the genus, and the majority of them have distributions in this area (1). Others suggest the center of origin to be farther north along the Caribbean coast of Central America (2, 17).

Germplasm observed during our travels revealed the geographic range

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of the wild papaya to correspond quite well with the homeland of the ancient Mayan civilization. Plants which most resembled truly wild papayas were located in the region from Yucatan south through Belize and the Peten region of Guatemala. The wild type plants are always dioecious. These trees are weedy, thriving in abandoned fields and disturbed areas, and they have small fruits about the size of a golf ball, typically produced in clusters of 3 to 6 in the axillary nodes. The flesh color is invariably yellow-orange, never red, and the cavity of the tiny fruit is completely packed with seeds. The seeds are small and exhibit a marked dormancy, a trait that has been nearly eliminated in domesticated papayas.

South of this area, in Central America and into South America, papayas outside of cultivation share many traits with domesticated plants, and appear to be more feral than truly wild. In contrast to the wild plants, domesticated papayas are frequently hermaphrodites. The capacity for self-pollination, resulting in better fruit set in isolated trees and greater uniformity and predictability among the seedling progeny, was a character strongly favored by early domesticaters. Large fruit size and red flesh color were other characters emphasized by early growers.

In Central America, feral or domesticated papayas along the eastern or Caribbean coast displayed more of the wild characteristics, such as dioecism and yellow flesh color, whereas along the Pacific side of the Cordillera, papayas were less influenced by introgression with wild plants. Although our collections in the eastern slopes of the Andes were limited, only fully domesticated plants were found in that region. Our preliminary conclusion regarding domestication is that wild progenitors of domesticated papayas are found along the Caribbean coast from southern Mexico to Venezuela with increasing introgression between wild and domesticated types as one progresses from north to south and from east to west. There is no evidence in the germplasm that papayas were domesticated in South America.

Breeding

Within the materials collected in Central and South America there is a wide range of variation for fruit characters and other morphological traits of horticultural importance, including fruit size, sugar content, flesh color, and precocity. These horticultural characteristics show the tremendous plasticity of the germplasm when traits of high heritablity are placed under stringent human selection. However, the degree of variation in papayas for other traits which have not been emphasized by human selection may be quite small. Isozyme markers [as studied by UH graduate research assistant Ms. Maimunah Morshidi (Table 2)] show relatively little variation among accessions, suggesting a rather narrow genetic base for domesticates. As a breeder, it is instructive to remember that many problems in papaya production have arisen as the rather narrowly adapted crop has moved out of its center of origin into new environments. For instance, sources of resistance to *Phytophthora*, a serious soil-borne fungal pathogen that attacks both root and aerial portions of the plant, may be difficult to find in papaya germplasm, because papaya evolved in regions with calcareous soils of high pH in which *Phytophthora* growth is inhibited. In Hawaii, where our weathered volcanic soils are more acidic, *Phytophthora* has a more suitable environment, and papaya plants, having never encountered this pathogen within the native range of the species, lack genes for resistance and suffer accordingly. We can anticipate that breeding for *Phytophthora* resistance will be a long gradual process involving the accumulation of many genes of small effect.

The papayas grown commercially in Hawaii are "solo" cultivars. They are small, averaging about half a kilogram, and reputedly received their name from Portuguese sugarcane laborers who found they could eat a whole one in a single sitting. They are gynodioecious and produce fruits with high soluble solids (14-16%) and mild flavor. The Hawaiian papaya breeding program has focused on improving disease resistance, since the inbred solo cultivars are generally considered to be the quality standard, but are quite susceptible to most papaya pathogens.

As mentioned previously, there is very little useful variation in papaya germplasm for *Phytophthora* resistance. Screening of papaya germplasm for resistance was begun by Drs. Henry Nakasone and Minoru Aragaki (Table 2) in the late 1970s and has continued through the present (11, 12). The tolerant 'Waimanalo' cultivar and line 40 that have been identified through screening are now being used in a phenotypic recurrent selection program involving crosses with other solo cultivars. Because this program is expected to require years of effort, we are interested in the potential for quicker solutions through genetic engineering, specifically whether synthetic "elicitin" genes may be useful as resistance factors (13).

Papaya ringspot virus is another important pathogen for which only limited resistance exists in papaya germplasm. This aphid-transmitted potyvirus attacks the canopy and fruit, resulting in loss of production. Starting with germplasm supplied to Dr. Nakasone from the Florida breeding program of Dr. Robert Conover (Table 2), a PRV tolerant line called 356 was selected in the early 1980s. The dioecious line 356 was crossed with several solo cultivars to introduce genes for PRV resistance from the former into the higher quality,

Table 1. Source and number of *Carica* accessions discussed in the text.

Geographic region	*Carica papaya*			Other *Carica* species
	Cultivated	Feral	Wild	
CENTRAL AMERICA	54	30	38	5
Mexico	6	5	5	—
Belize	1	3	9	—
Guatemala	25	14	19	—
Honduras	6	7	4	—
Costa Rica	13	1	—	—
Panama	3	—	1	*C. cauliflora* (5)
SOUTH AMERICA	40	4	0	71
Venezuela	9	2	—	*C. cauliflora* (1) *C. microcarpa* (1)
Colombia	16	—	—	*C. cauliflora* (1) *C. crassipetala* (2) *C. goudotiana* (20) *C. microcarpa* (1) *C. pubescens* (10) *C. sphaerocarpa* (3)
Ecuador	15	2	—	*C. x heilbornii* (7) *C. microcarpa* (8) *C. pubescens* (11) *C. pulchra* (1) *C. stipulata* (4)
Brazil	—	—	—	*C. glandulosa* (2)

gynodioecious background of the latter. This program is being continued through recurrent selection for population improvement. Though still in the early stages of the program, some plants with improved PRV resistance and near acceptable fruit quality are appearing. The efficiency of screening segregating materials has been improved by Dr. Steven Ferreira (Table 2), who mechanically inoculates large seedling populations with a paint sprayer to identify the most tolerant individuals, which are then evaluated for horticultural merit in the field.

The search for resistance to PRV has also been extended to wild relatives of papaya, particularly *C. pubescens* and *C. quercifolia*, which have high levels of resistance. Interspecific hybrids have been produced between papaya and these species by Dr. Tim Wenslaff (Table 2) in the mid-1980s, then an MS candidate at UH (9, 10). Tim used embyro rescue to obtain plants that could be evaluated for resistance. Under field conditions that resulted in infection of papayas within 6-9 months, the interspecific hybrids remained symptomless. The major difficulty with these materials is that they are quite sterile, and there is virtually no viable pollen produced by the hybrids. We have managed to obtain one backcross generation in both hybrids, but in both cases, the resulting plants were sterile sesquidiploids, produced by fertilization of unreduced egg cells in the interspecific hybrid.

Recently, the University of Hawaii, Cornell University, and The Upjohn Company have collaborated to produce genetically engineered papayas with virus resistance, using the coat protein-mediated approach that has been successful with a number of other crop-pathogen systems. Dr. Dennis Gonsalves (Table 2) isolated and cloned the coat protein gene of PRV at Cornell, and Dr. Jerry Slightom (Table 2) at Upjohn inserted the construct into a plant transformation vector that allows its expression in plant cells. We used the gene gun, devised by Dr. John Sanford (Table 2) and others, to transform embryogenic cultures obtained by 2,4-D treatment of immature zygotic embryos (5) or seedling hypocotyl sections. The regeneration method was developed by Dr. Maureen Fitch (Table 2) as her doctoral dissertation research at UH from 1987 to 1991 (4, 6). We were able to recover transformed papaya plants, and these have shown a range of reactions to PRV inoculation (7). The most resistant transformants have remained symptomless and ELISA negative for more than 18 months

Table 2. Projects and affiliations of individuals involved in papaya improvement in Hawaii.

Researcher	Affiliation	Program
Minoru Aragaki	Plant Pathology, U. of Hawaii, Honolulu, HI	*Phytophthora* Resistance
Robert A. Conover	Plant Pathology, U. of Florida, Homestead, FL	Ringspot Virus Resistance
Stephen Ferreira	Plant Pathology, U. of Hawaii, Honolulu, HI	Ringspot Virus Resistance
Maureen M. M. Fitch	USDA Sugarcane Production Lab, Aiea, HI	Ringspot Virus Resistance
Dennis Gonsalves	Plant Pathology, Cornell U., Geneva, NY	Ringspot Virus Resistance
Richard M. Manshardt	Horticulture, U. of Hawaii, Honolulu, Hi	Papaya Breeding and Genetics
Maimunah Morshidi	Horticulture, U. of Hawaii, Honolulu, HI	*Carica* Systematics
Henry Y. Nakasone	Horticulture, U. of Hawaii, Honolulu, HI	Papaya Breeding and Genetics
Robert Paull	Plant Molecular Physiology, U. of Hawaii, Honolulu, HI	Postharvest Improvement
John C. Sanford	Horticultural Sciences, Cornell U., Geneva, NY	Ringspot Virus Resistance
Jerry L. Slightom	Molecular Biology, Upjohn Company, Kalamazoo, MI	Ringspot Virus Resistance
Suresh Sondur	Horticulture, U. of Hawaii, Honolulu, HI	Chromosome Mapping
John I. Stiles	Plant Molecular Physiology, U. of Hawaii, Honolulu, HI	Mapping, Postharvest Improv.
Timothy F. Wenslaff	Hawaiian Sugar Planters' Association, Aiea, HI	*Carica* Interspecific Hybrids
Francis T.-P. Zee	USDA National Clonal Germplasm Repository, Hilo, HI	*Carica* Germplasm

after inoculation with a Hawaiian PRV strain under field conditions in Hawaii. The resistance appears to be narrowly based, however, since strains of PRV from regions outside Hawaii have overcome it, as demonstrated by Dr. Gonsalves in greenhouse experiments at Cornell (18). The UH-Cornell collaboration is continuing in a project which has the objective of developing stable multigenic virus resistance using new and different gene constructs from Dr. Gonsalves' lab to transform our current resistant cultivar in Hawaii. Among the new constructs will be functional and non-translatable versions of the PRV replicase gene and coat protein genes from other non-Hawaiian PRV strains.

As an aid to the breeding program, the UH Departments of Horticulture and Plant Molecular Physiology (PMP) are nearing completion of the first phase of a joint project to map the papaya genome using randomly amplified polymorphic DNA markers. Over the last four years, Dr. John Stiles of the PMP Dept. and Mr. Suresh Sondur, a Horticulture doctoral candidate (Table 2), have produced a genetic map with eleven linkage groups and an average distance between markers of about 20 centiMorgans. This program has moved along rapidly because of the relatively small size of the papaya genome, the ease with which RAPD markers can be determined, and the power of computerized analysis programs. Suresh has scored an F_2 population that is segregating for many economically important traits, as well as for molecular markers. He is examining the correlations between markers and traits in an effort to locate chromosome segments that contribute significantly to the expression of particular quantitatively inherited horticultural characters, such as precocious flowering, tendencies toward carpellodic anther formation and carpel abortion in hermaphrodite plants, and PRV resistance. Because RAPD technology has become so convenient, it may be practical to use molecular markers to augment field evaluations in selecting the best segregants in a breeding population, since markers can be scored unambiguously. This methodology has also been applied to determining the genetic relationships between solo and various other papaya lines through multivariate analysis of RAPD polymorphisms (16).

Another area of investigation which is just commencing concerns extending the postharvest shelf life of papaya cultivars. While some genetic variation for postharvest keeping quality exists in papaya germplasm, recent advances in the molecular biology of fruit ripening mechanisms have made it possible to consider genetic engineering as a quick route to cultivars with longer shelf life (14). In a joint project involving the UH Horticulture and Plant Molecular Physiology Departments, the ACC synthase gene responsible for autocatalytic ethylene production in papaya is being cloned, with the intent to suppress endogenous ethylene through antisense gene technology. Since ethylene is the phytohormone that triggers and maintains the ripening processes in fruit, this should result in papayas that do not ripen when physiologically mature, but which will ripen in response to exogenously supplied ethylene. These papayas might be harvested at maturity, shipped in a firm green condition, and ripened to the correct degree at the market destination. Once removed from exposure to ethylene, the fruit should remain at that stage of ripeness for a substantially longer time than non-genetically engineered papayas. This project is intended to improve the quality of export papayas that currently require up to ten days to reach West Coast and Japanese markets.

The papaya cultivars transformed with the ACC synthase antisense construct may help reduce another major papaya problem in Hawaii, namely,

fruit fly infestation and the injurious postharvest treatment that it necessitates (8). Since papayas are not attractive to fruit flies until ripening begins, those that are harvested and shipped at physiological maturity may be free of eggs and larvae, and consequently may not be required to undergo the hot water or forced hot air treatments that are currently mandated by the USDA for exports to California. The postharvest treatments, though much improved over earlier versions, are expensive and occasionally impair normal fruit ripening, so eliminating the need for such treatments would be a great economic benefit to the Hawaiian papaya industry. To address this problem more directly, preliminary studies have been conducted to identify gene products that might be used to halt the development of fruit fly larvae in infested papayas. The result of one laboratory experiment indicates that nikkomycin (33 uM), a bacterial product that is a potent inhibitor of chitin synthesis (3), completely halts larval development. No formal project exists to transform papayas with genes to make nikkomycin, because these constructs are not currently available.

In conclusion, the breeding program at UH is perhaps more aptly characterized as a papaya improvement program that has focussed on employing new technologies to quickly solve serious problems, rather than on conventional breeding methods for cultivar development. This is a major limitation of the program, but one that is necessary and defendable on the grounds that there is little genetic variability in papaya germplasm for some major problems, such as resistance to PRV, *Phytophthora*, and fruit flies. These problems must be addressed quickly, if the Hawaiian papaya industry is to remain competitive.

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Amazonian Small Fruits with Commercial Potential[1]

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Abstract

Although most native Amazonian fruit species are trees, a few are woody or herbaceous shrubs. The Myrtaceae is especially rich in small fruit species. The araçá-boi (*Eugenia stipitata* McVaugh) was domesticated in western Amazonia for its deliciously flavored, sour pulp. The araçá-pera (*Psidium acutangulum* DC) was managed in swidden second-growth and around village sites for its pleasantly flavored, sour fruit, frequently similar in flavor to the strawberry guava (*P. cattleianum* L.). The caçari or camu-camu (*Myrciaria dubia* (HBK) McVaugh) is a wild species that occurs in monospecific stands in flood-plains. It is an extremely sour, though pleasantly flavored fruit, with up to 4 g of ascorbic acid per 100 g of edible pulp, making it richer in this vitamin than the acerola(*Malpighia glabra* L.). The Solanaceae offers the cubiu or cocona (*Solanum sessiliflorum* Dunal), domesticated in western Amazonia and similar in appearance to the naranjilla (*S. quitoense* Lam.). Its potential yields are enormous and its pleasantly flavored fruits are used for juices or preserves. The Rubiaceae contains the purui (*Borojoa sorbilis* (Huber) Cuatre.) and several relatives. Another sour fruit with a pleasant flavor, the purui appears to have been at least semi-domesticated in western Amazonia also. These species offer the potential for development as processed juices or other products, as they are all too sour for out of hand consumption. This paper describes each species, presents available composition and yield data, and suggests the research necessary to develop them as small fruit crops.

Introduction

Amazonia is the last untapped frontier for the fruit explorer, geneticist and entrepreneur. When Francisco de Orellana descended the Napo and Amazon Rivers in 1540 he was amazed at the variety of fruit crops grown in and around the Amazonian villages (18, 19). Within 100-200 years, however, the native population was decimated by disease (9), and most of their selected fruits had disappeared into the returning forest (5). Nonetheless, the fruit selections and overall diversity that remain are impressive, so much so that they have been used to propose a center of crop genetic diversity in Amazonia (7).

Although most native Amazonian fruit species are trees, a few are woody or herbaceous shrubs. Some are currently collected from the wild, without any apparent management, e.g., *Myrciaria dubia*. Others are volunteers in ecosystems disturbed by humans and consequently are subject to some type of human management and selection, e.g., *Psidium acutangulum*. Some of these may have once been semi-

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