Proc. Fla. State Hort. Soc. 117:182-187. 2004.

CURRENT STATUS OF THE SUBTROPICAL AND TROPICAL GERMPLASM REPOSITORIES OF THE NATIONAL PLANT GERMPLASM SYSTEM

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Additional index words. Genetic resources, collections, NPGS, accessions, genebank

Abstract. **Germplasm collections are viewed as a source of genetic diversity to support crop improvement and botanical research, as well as conservation efforts. The United States Department of Agriculture's National Plant Germplasm System (NPGS) is responsible for managing plant genetic resources in the USA. This paper examines the benefits generated by the genetic resources held by the three subtropical and tropical clonal germplasm repositories that are part of the NPGS. They are the Subtropical Horticultural Research Station (SHRS) in Miami, FL (4,744 accessions), the Tropical Agriculture Research Station (TARS) in Mayaguez, PR (715 accessions), and the Tropical Plant Genetic Resource Management Unit (TPGR-MU) in Hilo, HI (675 accessions). The NPGS research programs are dedicated to answering questions that help curators conserve and manage genetic resources in a more effective and cost—efficient manner. In addition, the research on preserving these collections, the cost of maintenance and the procedures for distributing germplasm are discussed here.**

The maintenance of *ex situ* germplasm collections for cultivated plants has become a primary means for preserving genetic diversity. As environmental degradation continues, the associated loss of species diversity makes *ex situ* collections, in some cases, the last reservoir for genetic variation. Additionally, these collections are the only place where material is catalogued and evaluated, thus providing basic material in an organized manner for plant improvement programs. Controlling the number of genotypes by careful evaluation ensures that the maximum amount of genetic variation is maintained with the least number of individuals. If duplicated accessions with different names can be removed through the use of molecular and/or phenotypic markers, the costs of field gene bank maintenance will be reduced. Genetic mechanisms can be used to solve a large number of agronomic problems, thus making a significant contribution to sustainable agriculture while also preserving part of our botanical heritage.

Tropical and Subtropical National Germplasm Repositories

Production of many of the tropical fruit, ornamental, beverage, sugar, medicinal, and tropical forage crops are based on a few commercial cultivars. This reliance on select genotypes, which are often closely related, has developed because of consumer demands for quality products and the difficulties associated with breeding long-lived crops. Genetic vulnerability is potentially a problem. Collections maintained by the Agricultural Research Service (ARS) at the National Germplasm Repositories (NGR) in Miami, FL, Mayaguez, P.R. and Hilo, Hawaii are currently the major genetic reservoir in the Western Hemisphere for ameliorating potential problems caused by genetic uniformity in farmers' fields in the tropics and subtropics. These three Repositories maintain approximately 6,200 accessions under field, screen house and greenhouse conditions for distribution and genetic evaluation.

Developing a greater array of commercial types from the germplasm collection by collaboration with other ARS, university, and private scientists involved in breeding, pathology, and other improvement programs should increase the stability of these industries. Information generated by these collaborations increases the value of the collections as more complete characterization data are generated, such as the breeding value of a given accession or its disease resistance status. Collaborations also help to ensure that quality information is provided to users of the material.

Management

The ability to maintain tropical/subtropical germplasm in the United States is limited to Hawaii, Puerto Rico, and Florida. The clonal repositories are unique in the NPGS because accessions must be maintained as plants in the field. In some cases, *in vitro* backup cultures are also maintained (Westwood, 1989). The subtropical and tropical locations maintain crops with recalcitrant seed that limits options available for the preservation of genes. Research needs to be focused at the National Center for Genetic Resource Preservation (NCGRP) to develop long-term preservation of these tropical species (Towill, 1989). Until seed or *in vitro* preservation is a reality, costly backup collections at alternative sites must be maintained.

The development of new technologies has greatly influenced germplasm management. The Germplasm Resources Information Network (GRIN) is the centralized computer database for the NPGS (White et al., 1989) and maintains records for all ger-

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mplasm repositories. Computer advances such as image storage and transmittal and relational databases are producing a more integrated system capable of managing and sharing extraordinary amounts of information. The GRIN is designed to facilitate the management and operation of the NPGS as well as making available the germplasm and information to scientists and other users (Perry et al., 1988; Mowder and Stoner, 1989).

Developments in molecular biology have produced many new tools. These tools, primarily DNA markers and improved DNA sequencing techniques, both based on the Polymerase Chain Reaction (PCR), have allowed more accurate analysis of genetic diversity with reliability, speed, and at a lower cost than older methods. These new molecular techniques coupled with increased computational abilities are greatly improving our management efficiency by providing better scientific evaluation, preservation, and utilization of genetic resources.

The repositories receive policy advice from the National Plant Germplasm Committee and technical advice from the Tropical Fruit and Nut Crop Germplasm, Woody and Herbaceous Landscape Plant Crop Germplasm, and the Sugarcane Crop Germplasm Committees. These committees meet on a regular basis and provide input on collection structure, new collection expeditions, and overall management of the collections. Regulations are adhered to from the Animal and Plant Health Inspection Service (APHIS), USDA-ARS, Florida Department of Plant Industry and Agriculture, Hawaiian Department of Agriculture, Commonwealth of Puerto Rico Department of Agriculture, and other regulatory agencies controlling introduction and distribution of plant material.

Facilities

The Miami repository is located on Federal land at latitude N25'38.57 and longitude 0'17.51, and is just over 200 acres in size. The facilities include a 6,600 sq. ft. lab building constructed in 1988, three greenhouses, lath houses, a full sun nursery, and an extensive irrigation system for 125 of the 200 acres. A new 36,000 sq. ft. lab facility is currently under construction that will house all the projects at the SHRS. The Mayaguez station is also located on Federal property at latitude N18°12'27.8" and longitude W67°08'18.1"and is 235 acres in size. The repository is housed in a large lab/administrative building dating from 1908 but renovated in 1996. Greenhouses and plot space are used to maintain germplasm at both Mayaguez and the substation at Isabela. The Hilo repository is on the University of Hawaii substation at Waiakea at latitude N19'38 and longitude W155'6. The repository leases 33 acres including a 1,500 sq. ft. lab/administrative building built in 1988 and several greenhouses for pathogen testing, propagation, and maintenance. A new facility is in the planning stage for Hilo that will house the repository and other ARS labs. Construction is anticipated in the next few years.

Maintenance

The most important objective of the NGR is to maintain healthy field collections of current accessions of tropical/subtropical fruit trees, ornamentals, medicinal and beverage species, and grasses. The collections of the subtropical and tropical repositories include approximately 6200 accessions and include over 400 genera and 1400 species from around the world (Table 1). It is recognized that the genetic diversity available in these species is not fully represented in the current collections. Significant loss of biodiversity and increasing difficulty in collecting germplasm in tropical countries makes it imperative that new accessions be collected as quickly as possible.

Implementation of improved horticultural practices to ensure the longevity of current collections by re-propagating old clones on new rootstocks in new fields is always ongoing. Adding new accessions as they become available from foreign collaborators and breeding programs is the most efficient way to increase genetic diversity. It is essential that we support interna-

Table 1. Collections maintained by Miami, Mayaguez and Hilo germplasm clonal repositories.

Miami		Mayaguez		Hilo	
Accession	Quantity	Accession	Quantity	Accession	Quantity
Annonas	72	Artocarpus	4	Artocarpus	40
Avocado	245	Annonas	16	Bactris	15
Bananas	73	Bamboo	98	Camellia	26
Brugmancia	12	Bananas	112	Canarium	25
Cacao	72	Cacao	155	Carambola	25
Carambola	40	Carambola	19	Durio	4
Coconuts	20	Citrus	6	Guava	63
Copernicia	9	Cinammon	5	Litchi	81
Ficus	82	Ficus	14	Longan	23
Garcinia	25	Guava	2	Macademia	36
Hevea	44	Hevea	2	Malpighia	7
Iris	40	Litchi	10	Nephelium	60
Jackfruit	3	Longan	11	Papaya	143
Lagerstroemia	18	Mamey sapote	26	Passiflora	64
Litchi	18	Nephelium	9	Miscellaneous	23
Mamey	25	Palm-lily	6		
Mamey	40	Sapodilla	25		
Mango	286	Miscellaneous and ornamentals	231		
Plumeria	108				
Pouteria	40				
Sugarcane	1700				
Tabebuia	12				
Tamarind	35				
Tripsacum spp.	239				
Miscellaneous and ornamentals	1500				
Total	4796		751		635

tional collecting expeditions with appropriate benefit sharing. After quarantine requirements have been satisfied, the new accessions will be maintained in field plantings. Backup core collections are established at one of the other repositories.

Alternatives to long-term storage of clonal materials are an integral part of the Repositories' goals. Cooperative research with the National Center for Genetic Resources Preservation, Ft. Collins, Colo. (formerly National Seed Storage Laboratory) in the areas of low temperature and cryogenic storage of seeds, clonal buds, and scions are being pursued currently or planned for the future.

The overall costs to the repositories for the maintenance of this germplasm are approximately $2.9 million with an annual cost of accession maintenance of approximately $480. At least three plants are maintained for each accession and this price includes all evaluation research, database management, quarantine requirements, elimination of diseases, distributions, and administrative costs associated with management of these collections.

Acquisitions

Acquisition and distribution of plant germplasm go hand-in-hand to provide and assure the maximum amount of genetic diversity for to the United States. The objective of the NPGS distribution policy is to provide quality germplasm to both domestic and foreign scientists in a timely manner and to develop strong relationships with the requestors for future exchanges and research. For crops and select species covered by the International Treaty on Plant Genetic Resources (ITPGR), direct but facilitated exchange with the country will occur without conditions beyond those specified in the ITPGR. However, for crops and select species not covered by the ITPGR, the Convention on Biological Diversity is abided, with its negotiated terms and benefit sharing.

New international treaties like the Convention on Biological Diversity (CBD, 1992), International Treaty on Plant Genetic Resources (ITPGR, 2001), and the World Trade Organization (WTO) through its Trade-Related Aspects of Intellectual Property Rights (TRIPS) agreement are reshaping the exchange of genetic resources for the future. Free and open exchange will move toward controlled and facilitated exchange. The ITPGR is oriented toward agriculture, while the CBD is controlled by Ministries of the Environment. Some specifically identified crops and their progenitors will be more easily exchanged under the multilateral ITPGR, while others default to the terms in the bilateral CBD. The U.S. government has signed the CBD, but is one of the few nations that have not ratified the CBD as yet. The U.S. has signed and is discussing ratification of the ITPGR. Regardless, other nations that participate in plant germplasm exchange expect the U.S. to abide by the conditions and terms of these agreements. Because not all countries behave uniformly in response to use of protected genetic resources, NPGS curators will need to follow regularly updated guidance from ARS administration on these issues.

Distributions

Plant germplasm is distributed to scientists, educators and other *bona fide* research and education entities from NPGS germplasm active collection sites in accordance with current USDA, ARS, NPGS procedures. Germplasm of mangoes, bananas, plantains, sugarcane, avocado, lychee, papaya, mamey sapote, sapodilla, *Annona* spp., macadamia, acerola, rambutan, *Pulasan*,passion fruit, guava, pineapple, tea, palms, longan, plumerias, tabebuia, and iris from the repositories are available free of charge. Distributions from each repository for last year are listed in Table 2 and are representative of distributions over the past few years. Due to the limited amount of clonal material available at any given time, quantities distributed are limited and shipping costs are to be paid by the requestor. Where possible tissue cultured germplasm is distributed as disease free material which may not require extensive quarantine requirements. While we do sometimes provide germplasm to amateurs and hobbyists, we usually only do so for varieties that are not easy to find in the nursery trade. We encourage hobbyists to visit their local nursery or to search for varieties that interest them on the web before requesting material from us. Access to collection information and requests for germplasm can be found by contacting the National Germplasm Resources Information Network (GRIN) at www. grin-ars.gov. To contact any of the repositories call (808) 959-5833 (Hawaii), (305) 254-4484 Miami, Fla., and (787) 831-3435, Mayaguez, P.R. Successful implementation of a distribution policy will be dependent on informed participation by germplasm program leaders, research leaders, curators, and scientists engaged in germplasm evaluation and crop improvement. Each must follow and know the current international issues and ramifications of laws, precedents and evolving practices. Sound judgment is required to rationally apply the distribution policy to the diversity of species and types of germplasm affected by the various treaties.

Characterization

The first step in germplasm characterization after establishment of an accession involves the proper identification and/or verification of each species or cultivar. Initial verification is done by classical botanical and horticultural methods. Descriptor lists have been developed by the International Plant Genetic Resource Institute (IPGRI) for use in characterizing accessions. These include phenotypic characters such as flower color, leaf shape, growth habit, and other qualitative traits (Alercia et al., 2001). Information for these records is obtained from field observations of repository personnel and by cooperating research personnel at various institutions. Descriptive data pertaining to our accessions or their evaluations is stored in the USDA-ARS Germplasm Resources Information Network (GRIN), a national database of all plant germplasm. Access to GRIN information is available to the general public through the World Wide Web at www.grin-ars.gov. Digital images are now commonly used to document many accessions on the GRIN database, an example for a mango accession at Miami and cacao, banana, mamey sapote and bamboo at Mayaguez are given in Figs. 1 and 2, respectively. Several characteristics can be obtained from this image such as fruit size, seed size, fruit color, and flesh color and these are useful for verification of accession identification.

Phenotypic evaluation

Horticultural performance and quality data from the evaluation of germplasm provide information that facilitate the use of genetic resources by breeders and enable more effective management of the collections. For example, phenotypic evaluation of the sugarcane collection helped confirm genetic groups and identify accessions useful in the breeding program

Table 2. Germplasm distributions (domestic and foreign) from Miami, Mayaguez and Hilo, for the years 2001-2003.

		Miami						Mayaguez						Hilo					
		Domestic			Foreign			Domestic			Foreign			Domestic			Foreign		
Genus or Common Name	Year	01	02	03	01	02	03	01	02	03	01	02	03	01	02	03	01	02	03
Ananas		—	—	—	—	—	—	—	—	—	—	—	—	35	12	—	50	—	16
Annonas		2	—	9	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—
Artocarpus		—	—	1	—	—	—	1	2	1	—	—	—	9	—	—	—	—	—
Avocado		11	78	19	4	—	13	3	—	1	1	—	—	—	—	—	—	—	—
Bamboo		—	—	—	—	—	—	8	8	13	—	—	—	—	—	—	—	—	—
Bananas		—	5	—	—	—	—	2	5	—	2	—	—	—	—	—	—	—	—
Cacao		19	18	59	—	—	—	10	5	6	—	—	1	—	—	—	—	—	—
Camellia sinensis		—	—	—	—	—	—	—	—	—	—	—	—	58	195	224	—	—	3
Carambola		—	—	5	—	—	—	3	3	1	—	—	—	5	—	25	3	—	—
Carica		—	—	—	—	—	—	1	—	1	—	—	—	30	17	23	16	1	1
Coconuts		3	2	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Coffee		—	—	—	—	—	—	1	—	—	—	1	—	—	—	—	—	—	—
Dimocarpus		1	—	3	—	—	—	—	—	—	—	—	—	8	—	10	—	2	—
Ficus		1	6	11	—	1		—	—	—	—	—	—	—	—	—	—	—	—
Garcinia		—	—	—	—	—	—	4	9	8		1	—	—	—	—	—	—	—
Litchi		8	3	6	—	—	—	—	—	—	—	—	—	11	2	14	—	1	—
Malphigia		—	—	—	—	—	—	—	—	—	—	—	—	10	—	—	3	—	—
Mango		52	22	16	—	—	19	1	2	—	—	—	—	—	—	—	—	—	—
Passiflora		—	—	1	—	—	—	—	—	—	—	—	—	3	2	1	26	4	—
Pouteria		—	1	2	—	—	—	1	1	—	—	—	—	—	—	—	—	—	—
Psidium		—	—	4	—	—	—	—	—	—	—	—	—	20	53	79	—	—	—
Sugarcane		93	44	5	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—
Syzygium		1	—	4	—	—	—	—	—	—	—	—	—	5	1	4	1	—	—
Tripsacum		27	90	25	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Vasconcellea		—	—	—	—	—	—	—	—	—	—	—	—	11	2	4	11	4	1
Zingiber		—	—	—	—	—	—	—	—	—	—	—	—	—	3	2	—	—	—
Ornamentals		78	41	97	—	—	—	7	14	18	—	—	3	—	—	—	—	—	—
Total		218	269	174	4	1	33	43	51	52	3	2	4	210	287	394	110	12	22

Mangifera indica 'Irwin'
MIA-17452 / PI-277476

Notes from 1959:

Mangifera indica L. Anacardiaceae. Mango.

Plants growing at the United States Plant Introduction Station, Miami, Florida.
Numbered November 29, 1961.
Budwood received May 1959, from the Subtropical Experiment Station, Homestead.
277476. P.I.S. 17452. 'Irwin'.
From PI Books.

Fig. 1. Digital images (mango) commonly used to document many accessions on the GRIN database.

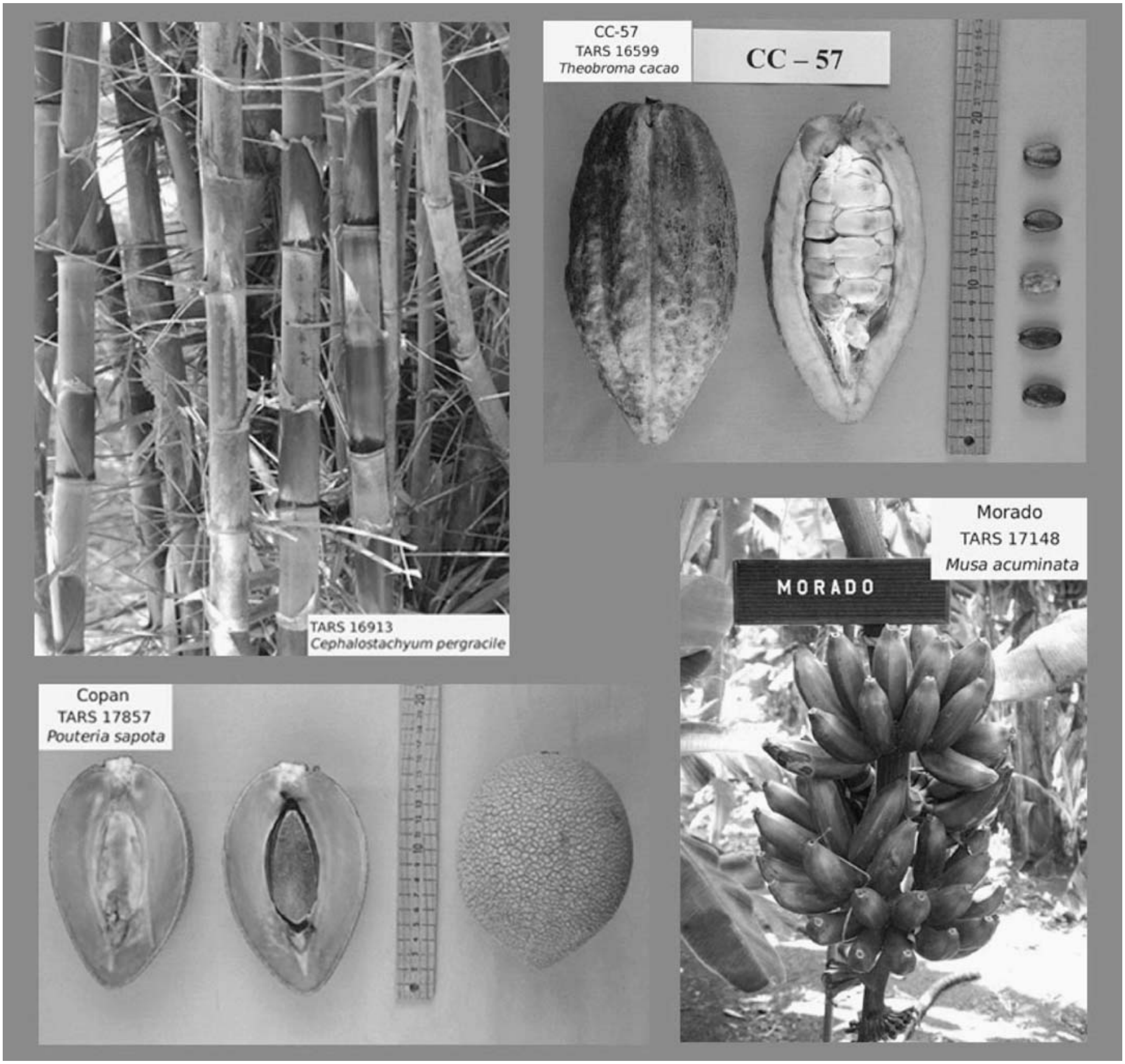

Fig. 2. Digital images of bamboo, cacao, mamey sapote, and banana commonly used to document accessions of this germplasm on the GRIN database.

(Brown et al., 2003). Another example of phenotypic evaluation of flowering phenology was reported by Schnell and Knight (1998). Differences between mango accessions were significant and repeatable, R = 0.73, 0.88, and 0.77, for days to bloom, days in bloom, and days in bloom and flowering, respectively. These types of evaluations provide useful information for scientists studying the basis for differences in these characteristics.

Cultivar development

One aspect of the new ornamentals program at the SHRS in Miami is the development and release of new cultivars of subtropical/tropical ornamentals on a regular basis. To date, the SHRS has released a dwarf cultivar of African iris (Meerow et al., 2001) and four cultivars of *Iochroma* (Meerow et al., 2004). Two breeding programs with tropical geophytes (*Hippeastrum* and *Alstroemeria*), begun at the University of Florida, are continuing at the SHRS. The program has so far yielded four patented cultivars (Meerow and Meyer, 1998; Meerow, 2000). Development of the 'Fiji Dwarf' cultivar of *Cocos nucifera* as an outstanding landscape palm is being undertaken hand-in-hand with research on genetic characterization of the variety (Meerow et al., 2002). Scientists at TARS in Mayaguez selected and released in 2000 nine high-yielding clones of cacao (*Theobroma cacao*) which are being distributed to producers in countries where cacao is grown (Irizarry and Goenaga, 2000), and at Hilo a new cultivar of longan was released in 2000 (Ito et al., 2000).

Molecular markers

The development and utilization of molecular markers for accession fingerprinting, diversity analysis, and the mapping of QTL associated with improved horticultural traits and disease resistance has increased quickly over the past five years. A number of publications have documented the utility of molecular markers in germplasm management including macadamia (Steiger et al., 2003), avocado (Schnell et al., 2003), coconut (Meerow et al., 2002), *Iochroma* (Meerow et al., 2004), papaya (Kim et al., 2002), and jackfruit (Schnell et al., 2001). A small collection of cocoyam, *Xanthosoma* spp., maintained at the Mayaguez repository was actually eliminated from the NPGS based on molecular marker data (Schnell et al., 1999) and other duplications have been found and discarded among both seed and clonal collections. Molecular markers have become an integral part of germplasm management in the NPGS.

Summary

It can be said without reservation that the existence of these three tropical and subtropical National repositories are unique within the NPGS. These repositories have been a great asset to the tropical fruits, nuts, ornamentals, and sugarcane industries in the United States and around the world. It is incumbent on all to protect and manage these resources to the benefit of current and future generations.

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