REPORT

*Genetic resources and breeding of guava (***Psidium guajava *L.)* in Cuba**

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ABSTRACT

In this document, research on genetic resources and breeding in guava during the last 50 years in Cuba is included. The morph-agronomic characterization of 395 accessions and a catalogue with 18 of them, together with a new illustrated descriptor with more than 70 descriptors are offered. The discrimination power and information on morph-agronomic characters and AFLP and SSR as dominant and co-dominant molecular markers respectively were compared. The use of discrimination power (*D*) for morph-agronomic characters facilitated the key elaboration to distinguish among genotypes. Also, the effectiveness of AFLP for discrimination (*D j* = 0.991) and SSR for diversity estimation (H_{ep} = 0.60) was determined. The selection of "Enana Roja Cubana", (Red Cuban Dwarf) and EEA 1-23 cultivars due to their low size and high yield is documented. The combination of morph-agronomic characterization and diversity analysis was effective to design conservation strategies, to establish a crossing program, to increase the current cultivar composition and to develop genetic linkage maps. An integrated map from "Enana Roja Cubana" x N6 cross was constructed using AFLP and SSR molecular markers. The eleven linkage groups and 50 quantitative trait loci (QTLs) detected constituted the starting point for gene cloning and the future implementation of marker assisted selection in guava. Moreover, the 176 candidate sequences identified for resistance (RGL) and plant development (MADS-box and HOMEO-box) genes could contribute to linkage map saturation. All these results, along with the use of tissue culture techniques for germplasm conservation and propagation have permitted to propose a methodology to develop a guava breeding program in Cuba.

Keywords: fruit genetic resources, genetic breeding, guava, crop propagation, national program

*I*ntroduction

Guava (*Psidium guajava* L.), belonging to Myrtaceae family, is native from the Americas, but was introduced to other regions of the world where it is cultivated nowadays. Guavas constitute one of the tropical and subtropical fruits of great nutritional value, basically due to their vitamin and mineral contribution. Medicinal properties have also been attributed to them and have been widely used in traditional medicine in different countries. These characteristics, together with the low cost of cultivation, have allowed gaining consciousness of their great economic importance in various tropical and subtropical regions of the world.

Taking into account that guava constitutes a fruit tree of significant importance both, national and internationally, the present paper resumes the principal results related with genetic resources and breeding of this crop under Cuban conditions.

*R*esults

Guava collection, establishment. Cultivars and characterization

Prospection, conservation, evaluation and rational management of phytogenetic resources constitute one of the principal tasks to face for developing countries, with the objective to ensure the correct use of resources. These phyto-resources not only include wild plants, but also the cultivated ones, which constitute the biggest percent used by men as aliments. In this sense, a guava collection was established in the Scientific Technological Unit from Alquízar, adscript to the Institute for the Research of Tropical Fruits. Different ways were employed for its fomentation and enrichment: prospection (2%), foreign material introduction (5%) and selection of individuals coming from open and controlled pollination (93%) (Figure 1). Although the number of accessions has increased for long, part of the original variability has been lost due to biotic and abiotic factors. The rescue of local prospection is recommended as an important way to broad genetic diversity in this species, in spite of the great variability observed in 395 accessions using qualitative and quantitative characters. This permitted the identification of 26 interesting genotypes which can be useful in breeding programs and commercialization, paying attention to interesting characteristics such as tree height, fruit form and uniformity, seeds number, vitamin C content, total soluble solids, acidity level, external pulp thickness and fruit surface relieve [1].

Cultivars catalogue of Cuban guava collection

The document presents the characterization of 18 cultivars, where the ones used in productive areas are also included. For each of them, 40 descriptors were evaluated (25 quantitative and 15 qualitative). The cultivars were: Belic L-123, Belic L-207, Belic L-213, BG 76-10, BG 76-18, BG 76-19, BG 76-23, 'Cotorrera', EEA 1-23, EEA 18-40, EEA 38-4, Homero No. 1, Ibarra, "Indonesia Blanca", "Microguayaba", N6, "Selección Seychelles" and "Suprema Roja". In additional notes, information available for each cultivar was included. The information is offered

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1. Valdés-Infante J. Utilización de caracteres morfoagronómicos y de marcadores de ADN para el desarrollo de una metodología que contribuya al mejoramiento genético del guayabo (*Psidium guajava L.*) en Cuba. Tesis en opción al grado científico de Doctor en Ciencias Biológicas. Facultad de Biología, Universidad de La Habana, 2009.

2. Rodríguez NN, Valdés-Infante J, González G, Fuentes V, Cañizares J. Genetic resources of guava (Psidium guajava L.) in Cuba: germplasm characterization and breeding. Proceedings of the Second International Symposium on Guava and Other Myrtaceae; 2009, Nov 10-19; Mérida-Aguas Calientes, México. Acta Horticult 2010;849:341-48.

as a catalogue, which presents photos of young branch and fruit for each cultivar [2]. This document constitutes a manual of obliged consultation for researchers, producers, professors and students.

Illustrate descriptor for guava

A document in both languages, English and Spanish, was elaborated to contribute to guava morph-agronomic characterization. The strategic objective of the descriptors is to offer an easy and quick discrimination among phenotypes, as well as to bring an important way to standardize guava characterization worldwide. The descriptor includes high heritable characteristics which can be easily detected visually. In addition, these may contain a limited number of additional traits thought desirable by a consensus of users of the particular crop. The document offers more than 70 descriptors, many of them accompanied with illustrations to help in the election of the different states of each variable (Figure 2). Classes for quantitative characters are established to permit the evaluation of them as both types of data: quantitative and qualitative. Values from one to nine are used to designate the states inside each descriptor. This contributes to have a database and facilitates data processing. A group of minimum descriptors with high discrimination power is emphasized. The number of variables recommended for accessions clustering and the total of variables contained in this guava descriptor increased in relation to the ones offered by UPOV in 1987 [3].

Genetic diversity of the active collection from Cuban guava germplasm bank

Different genetic markers were compared attending to the discrimination capacity and informativeness and the diversity contained in the active collection of Cuban guava germplasm bank were evaluated using morphagronomic characters and DNA markers. Morph-agronomic characterization permitted to detect the variability and germplasm potentialities for breeding and conservation purposes. The utilization of discrimination power (*Dj*) for morph-agronomic data facilitated the creation of a key to differentiate the accessions. AFLP primer combinations contributed with the identification of all the accessions, which corroborates the values of discrimination power (*Dj* = 0.991) found. The heterozygosis value (0.60) and the seven diversity groups detected for SSR indicated an acceptable level of diversity in the active collection. The combination of both types of genetic markers permitted an integral analysis of this collection. Then, they are recommended as important complementary tools to have into account to design conservation strategies and to contribute to the genetic breeding program in this species [1, 4-7].

Classical methods of genetic breeding for guava in Cuba

Classical methods for genetic breeding of guava in Cuba had its beginnings in the 1950´s. The first attempts were the introduction and local prospection of genotypes, which, together with mass selection, permitted to count on 140 materials selected and preserved in the germplasm bank. The evaluation of 82 of these materials contributed to the pre-selection of

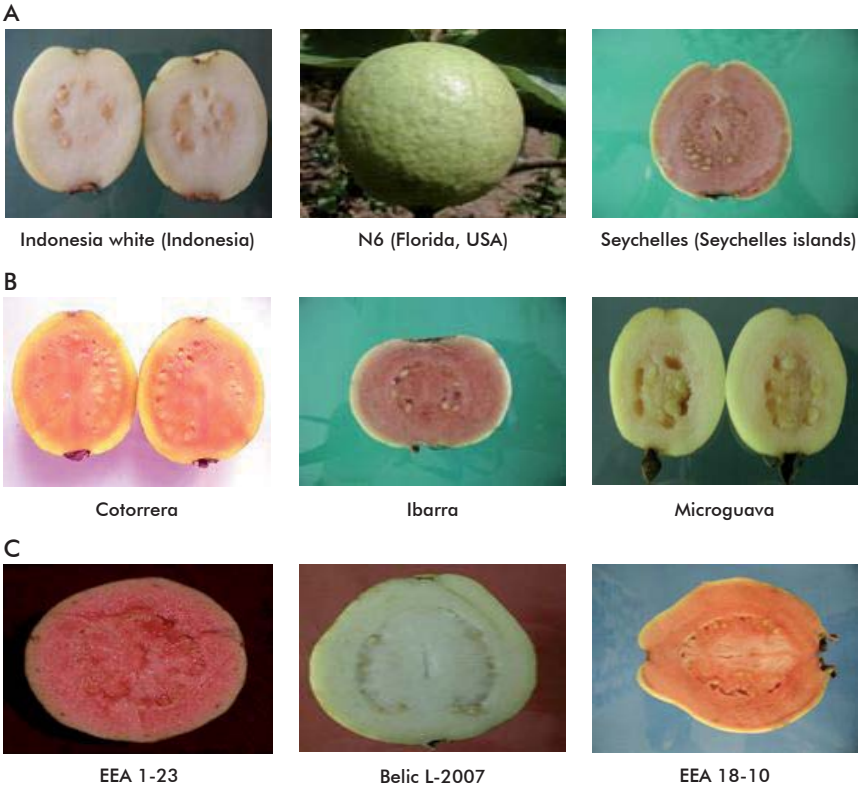

Figure 1. Accessions included in Cuban guava germplasm: A) Accessions with different geographical origin. B) Wild guava accessions collected in different Cuban localities. C) Accessions from open and controlled crosses, obtained during the Cuban genetic breeding program.

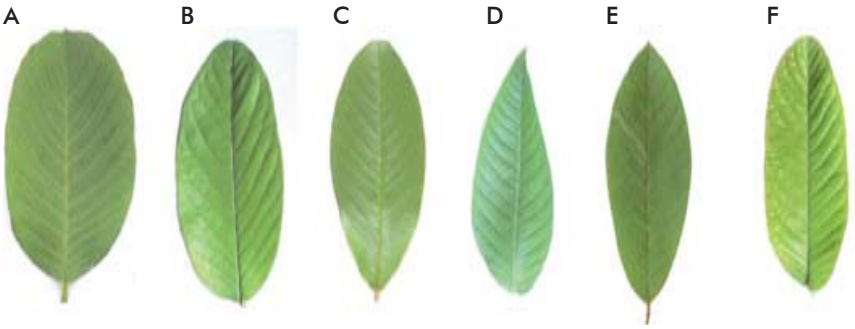

Figure 2. Mature leaf shape. A) Rounded. B) Ovate. C) Obovate. D) Trullate. E) Obtrull. F) Oblong.

the cultivars EEA 18-40; Belic L-207; EEA-27; EEA 28-26; EEA 1-23; Belic L-215; Belic L-97; EEA 21-3 and EEA 28-44 with productive purposes. From them, EEA 18-40 or "Enana Roja Cubana" and EEA 1-23 were emphasized due to their low size and high yield [8, 9]. The latter constituted the first Cuban cultivars recommended for commercial purposes. Taking into account the current approaches for elite genotypes selection, a morphagronomic characterization was combined with a diversity study, employing different genetic markers for such purposes, and also DNA-based molecular markers. This allowed the selection of parental "Enana Roja Cubana", N6, "Suprema roja" and Belic L-207 for intra-specific crosses deve-

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4. Valdés-Infante J, Becker D, Rodríguez NN, Velásquez B, González G, Sourd D, et al. Molecular characterization of Cuban accessions of guava (*Psidium guajava* L.), establishment of a first molecular linkage map and mapping of QTLs for vegetative characters. J Genet Breed 2003;57:349-58.

lopment [1]. Their evaluation allowed identifying 25 low size hybrids, which showed variability in productive potential and fruit quality. They will contribute to increase the cultivar composition for commercial purposes [10]. On the other hand, the search for plants tolerant to the principal pests and diseases has been limited in Cuban conditions. In this sense, the presence of sources tolerant to *Colletotrichum gloeosporioides* in *Psidium guajava* "Enana Roja Cubana" has been referred [11]. Also, from moderate to high susceptibility of *P. friedrichsthalianum*, *P. guineensis* and *P. guajava* species to *Meloidogyne* genus nematode, one of the principal pests affecting this crop, has been detected [12].

Biotechnologies as tools for propagation, conservation and genetic breeding in guava

Biotechnologies contribute positively and significantly in the propagation, conservation and breeding programs of many plant species. Tissue culture is an important tool for both basic and applied studies, as well as for commercial purposes. A technique for *in vitro* multiplication of wild guava was standardized in three culture phases: establishment, multiplication and rooting. This technique constituted a useful way for propagation, germplasm conservation and genetic breeding in the specie. A method for short-medium term conservation was also standardized. This included to culture lateral buds at 21 ºC on MS basal medium, reducing the mineral composition 50%, and adding 15 g/L of sucrose, 0.25 mg/L of 6 bencil aminopurine, 0.025 mg/L of 1- naftil acetic acid and 1% of activated charcoal. Guava did not tolerate conservation temperatures inferior to 9-10 ºC [13]. On the other hand, a genetic linkage map was constructed for the specie using 'Enana Roja Cubana', N6 and 82 descendants from the intraspecific cross. For this, 74 AFLP and 72 SSR primers combination were used. The 11 groups of the genetic linkage map and the 50 QTLs related with vegetative and internal/external fruit characters constitute the starting point for genes cloning of agricultural interest and the future implementation of markers assisted selection in guava [4, 5, 14]. The 176 candidate sequences for resistance-gene-like (RGL) and plant development (MADS-box and HOMEO-box) genes detected can be of great usage in linkage map saturation, variability studies in this crop, as well as in the solution of problems related with yielding and resistance to biotic and abiotic stresses [15].

Methodology for the development of guava genetic breeding program in Cuba

Taking into account the importance acquired for this fruit tree in the country, a methodology is proposed as a guide for the development of a genetic breeding program in guava. The integration of all the results coming from different scientific works allowed proposing a methodology for management and efficient use of genetic resources of this crop. The combination of morpho-agronomic characters and DNA molecular markers constituted a novel tool of great utility to characterize guava germplasm, estimate diversity level and parentage relationship among accessions, and also to recommend genotypes with conservation and breeding potential. These results established the basis to develop crosses, to pre-select hybrids of desirable characteristics and to develop a genetic linkage map, where QTLs were associated using variables of agricultural interest. On the other hand, candidate sequences to resistance (RGL) and plant development (MADS-box and HOMEO-box) genes were detected, which can be employed to saturate the map referred for this crop. Also, tissue culture techniques are biotechnological methods which can contribute to the propagation, conservation and genetic breeding in this fruit tree. They constitute essential tools to break natural incompatibility barriers, to generate spontaneous or induced variability, as well as for the introgression of genes that can be identified throughout the QTL and linkage map developed [1].

Relevance

The morpho-agronomic characterization of the Cuban guava collection was made for the first time at national scale. Also, more exhaustively, some accessions including economically relevant cultivars were well described in a catalogue accompanied with photos of young branch and fruits, to facilitate the identification of each one of them.

A new illustrated descriptor was elaborated for morpho-agronomic characterization of this species, which is more complete than the one published by UPOV in 1987.

This work also constitutes one of the most complete diversity studies internationally referred for this crop nowadays. For the first time in Cuba, morph-agronomic and molecular characterization were combined to determine parentage relationships and to establish diversity groups in guava germplasm bank; as well as to select promissory genotypes for diverse purposes.

The comparison of the discrimination power and information for morph-agronomic characters and DNA markers presents an important scientific novelty. These constitute the first studies in guava, both national an internationally.

Moreover, a quantitative trait loci and a genetic linkage map was constructed. On the other hand, candidate sequences to resistance (RGL) and plant development (MADS-box and HOMEO-box) genes were detected in guava. All these results constitute the first report for Science on this crop.

An *in vitro* tissue culture technique was established for the first time for wild guava propagation in Cuba, as well as a method for short-to-medium term conservation.

The integration of all these results allowed elaborating a new methodology, based on scientific results, to establish a guava genetic breeding program in Cuba.

Also, new hybrids selection referred herein have a potential economical impact, taking into account the possibility of increasing the number of cultivars used for productive purposes in the future.

The results presented here allow facing the challenges related with preservation, use and rational management of guava, using a novel scientific point of view and technologies having a significant environmental impact in the genetic base of this crop for future generations.

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Conclusions

The morph-agronomic characterization of the collection suggests rescuing prospection as an important source to collect wild materials. Nevertheless, a great variability was observed for many of the qualitative and quantitative characters analyzed, which permitted to identify 26 promissory genotypes as to relevant characters. The catalogue elaborated allows in cultivar description and identification. It constitutes an important manual for producers, researches, professors and students and includes an exhaustive characterization of economical important accessions. The illustrated descriptor supports an easy and quick discrimination among phenotypes, and an important way for the standardization of guava characterization worldwide. Additionally, it represents an improvement compared with the one reported by UPOV. Moreover, the morphagronomic characters and the DNA markers constitute complementary tools for an integral analysis of guava germplasm bank and to design strategies for guava conservation and breeding. In this sense, highly heritable characters and AFLP markers are very useful for cultivar identification, while SSR are more effective for diversity studies in this crop.

Genotypes introduction, local prospections and mass selection are important tools to start breeding programs in guava using classical methods. The 25 hybrids derived from this program and pre-selected due to its low size, productive yielding and fruit quality will increase the cultivar composition for commercial purposes. In this sense, the use of biotechnological methods such as tissue culture, linkage maps and genes sequences identification represent an important step to assist guava propagation, conservation and genetic breeding.

The methodology proposed can serve as a guide to develop a genetic breeding program in Cuba and other countries which cultivate this crop. Its application will support a more rational and efficient use of guava genetic resources for futures generations.

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