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Review
GENETIC BREEDING OF GUAVA (*Psidium guajava* L.)

Revisión bibliográfica
Mejoramiento genético de guayabo (*Psidium guajava* L.)

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ABSTRACT. Guava (*Psidium guajava* L.) is native to the tropical regions of America, but it has been introduced in different countries of the world where is nowadays naturalized. It is one of the most valuables tropical and subtropical fruit tree, because it is a natural source of vitamins and mineral salts. Due to its seed propagation, there are heterocigotic populations in which there is a correct genetic variability for the selection of desirable commercial types. At the foundation of the Cuban Fruit Tropical and Subtropical germplasm bank in 1965 (nowadays Tropical Fruit Research Institute, IIFT), was established the biggest collection of guava in the country. This germplasm collection has been characterized by using morphological markers and techniques of Amplified Fragment Length Polymorphism (AFLP) and Simple Sequence Repeats (SSR). The evaluations allowed the selection of elite genotypes for commercialization, breeding and the establishment of crossing programs. They are able to build linkage maps using molecular markers and over 50 quantitative trait loci were detected, as a basis for marker assisted selection. These results, in conjunction with the use of tissue culture techniques for the conservation and propagation of germplasm, have allowed to propose a methodology for developing crop improvement program in the country.

Key words: fruits, germplasm, molecular markers, plant breeding

RESUMEN. El guayabo (*Psidium guajava* L.), es oriundo de la región tropical de las Américas, pero fue introducido a otras regiones del mundo, donde actualmente se encuentra naturalizado. Es considerado como uno de los frutales tropicales y subtropicales más valiosos, pues resulta una fuente natural de vitaminas y sales minerales. Producto de su propagación por semillas, se cuenta con poblaciones heterocigóticas, en las que existe una adecuada variación genética para la selección de tipos comerciales deseables. Con la fundación del Banco de Germoplasma de Frutales Tropicales y Subtropicales en 1965 (actualmente Instituto de Investigaciones en Fruticultura Tropical, IIFT), se creó la colección más grande de Cuba. Esta colección de germoplasma del cultivo, ha sido caracterizada con el empleo de marcadores morfológicos y técnicas de Polimorfismo de la Longitud de Fragmentos Amplificados (AFLP) y de Secuencias Simples Repetidas (SSR). Las evaluaciones realizadas permitieron la selección de genotipos élites para propósitos de comercialización y mejoramiento genético. Además, se lograron construir mapas de ligamiento utilizando los marcadores moleculares y se detectaron más de 50 *loci* de caracteres cuantitativos, como base para la selección asistida por marcadores. Estos resultados, junto al uso de técnicas de cultivo de tejidos, para la conservación y propagación de germoplasma, han permitido proponer una metodología para desarrollar el programa de mejoramiento del cultivo en el país.

Palabras clave: frutales, germoplasma, marcadores moleculares, mejoramiento genético de plantas

INTRODUCTION

Guava (*Psidium guajava* L.), is one of the tropical and subtropical fruit of major economic importance, which is grown commercially in over 60 countries (1). It belongs to the Myrtaceae family, which is constituted of over 133 genera and 3800 species (2), few of which produce edible fruits (3).

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It is considered, within the genus Psidium, as one of the most important representatives from the commercial point of view, mainly due to the high nutritional value of its fruits, which are rich in vitamins (A, B and C) and minerals, which are important for health and human diet, and so profitable cultivation (4). It is a tropical and subtropical fruit of great importance, which has large areas dedicated to industry and marketing in fresh.

Although the flower morphology of this species favors self-pollination, also 35 to 40 % cross-pollination was reported, which together with the propagation by seeds since the beginning of domestication has allowed have heterozygous populations, where there is adequate genetic variation for selection of desirable commercial rates (5).

Breeding programs of the crop are in development in countries like India, Brazil, Cuba, Venezuela, Thailand, Mexico, Pakistan, among others, where the guava tree represents a growing economic importance (6); these programs are at different levels of development and different goals. In several of these countries they have made significant contributions over the years.

Although selective breeding of guava cultivars began almost a century ago, ease of propagating plants through seed, prevents to preserve improved cultivars without significant changes in their attributes (7). There are probably more than 400 cultivars of guava in the world, but only a few are grown commercially.

Based on the above antecedents, the objective of this review is, to present the importance and advances in breeding programs guava in Cuba and the rest of the world.

TAXONOMIC CLASSIFICATION

The taxonomic position of guava according to recent phylogenetic studies is (8):

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Rosidae
Order: Myrtales
Family: Myrtaceae
Subfamily: Myrtoideae
Tribe: Myrteae.
Genre: *Psidium*
Species: *Psidium guajava* L.

The Myrtaceae family, which is the guava tree, is represented by 133 genera and 3800 species of trees and bushes which thrive in tropical and subtropical areas of the world (2). *Psidium* gender, included in this family is composed of about 150 species (9); some of the most important are: *P. cattleianum* Sabine, *P. fredrichsthalianum* (Berg) Nied and *P. guajava* (10).

P. guajava BOTANICAL DESCRIPTION

Psidium guajava L. is an evergreen tree or bush or deciduous, reaching 5-10 meters high on average, but if properly handled pruning, not to exceed three meters and a diameter at breast height of up to 6 x 2,10 m. His cup is irregular[A], with simple decussate leaves, oblanceolate, oblong or elliptical, margin entire; bright green to brownish; arranged in alternating pairs along the branches have thin and soft hairs on both sides, with a central rib and several secondary protruding eye. They have a specific scent to be starry, which comes from an essential oil and smell depends on the cultivar.

The trunk is usually twisted and highly branched, with thick branches, bottom-up and twisted (11).

The outer crust is flaky in smooth, thin and irregular pieces, reddish brown and gray scales. The inner bark is fibrous, slightly bitter, creamy pink or pinkish brown, changing to dark brown. Total thickness 5 x 10^{-3} m to 8 x 10^{-3} m (12). The flowers are solitary or in cymes, axillary; sweetly scented, actinomorphic; sepals 4-5, green on the outside and white inside; four to five petals, white (12). The style is filiform, smooth, and yellowish-green. Flowering can be maintained all year, if the plant breeding conditions are good, and the environmental conditions permit it (11).

Fruit shape, the color of the pulp and skin depend on the variety. The most common is the fruit berries up to 8 x 10-2 m in diameter, globose to ovoid, with persistent calyx at the apex, fleshy, yellowish beige to pink and fragrant. The flavor varies from sweet to acidic or very acidic.

The outer skin is thin, yellow; the fruit containing many seeds (11). Seeds are small, kidney shape, with smooth edges and a hard crust. They can be numerous in the fruit (112 to 535), although some are seedless guava or have very few (13).

[A] Union for the Protection of New Varieties of Plants. *Guidelines for the conduct of test for distinctness, homogeneity and stability. Guava* (*Psidium guajava* L.) [en línea]. no. TG/110/3, Geneva, Switzerland, 1987, p. 29, [Consultado: 7 de enero de 2016], Disponible en: <http://www.upov.int/en/publications/tg-rom/tg110/tg_110_3.pdf>.

The root system is very shallow, but the tree makes up for the size and number of roots, which go beyond the projection of the cup. This is what increases your chance of surviving in areas where often problems with cyclones have and allows him to develop in almost all types of soils.

FLOWERING AND FRUITING

Guava flowers and fruits at different times in different places where it is grown, depending on the climatic characteristics and handling. The flowers are usually arranged in inflorescences with two or three flowers, although more than three flowers may appear, but not all produce fruit. Flowers located between the middle and the base of the branch is more likely to bear fruit (14).

The full development of the flower from the flower bud differentiation until the opening of the cup takes about 30 days (15). The opening of the cup usually happens 24 hours before flower opening (7, 14).

When flower buds reach their maximum development, sepals begin to break down at various points, indicating the start of anthesis. The start time of anthesis may vary depending on the daytime temperature. This feature is invaluable for evaluating collections or genebanks genotypes, due to its close relationship with the productive characteristics of plants. Observation and knowledge of these characteristics, can guide breeding programs of the crop, especially those focused on obtaining new cultivars from the recombination of genotypes (14).

Guava is a predominantly self-pollinated fruit (16) with the help of external agents, but pollination can also occur cross-25,7- 41,3%, with an average of 35,6%.

IMPORTANCE AND USES OF THE CULTIVATION

Currently, the guava has a well-established market in more than 60 countries, because of their hardiness, proliferation, high in vitamins and high economic return (17). The fruits of guava are widely used for fresh consumption and as industrial raw materials juices, nectars, ice cream, jellies and sweets (18). A recently developed market “guatchup” is a bittersweet mojo sauce prepared with guava, as a substitute for ketchup, among other products reported (19).

It is a fruit of great nutritional value, in which its high content of vitamin C. stands. This natural antioxidant varies in content between 200-400 mg/100g fresh weight, substantially higher values than those found in lemon juice (*Citrus limon* L.) (46 mg 100 g^{-1} juice) and orange (*Citrus sinensis* L. Osb.) (50 mg 100 g^{-1} juice), fruit commonly associated as excellent sources of vitamin C (20).

The fruits have a content of 0,1-1,9 % pectin, which increases during maturation and decrease in mature fruits super. They are also rich in iron, calcium and phosphorus (21). Manganese is present in combination with phosphoric, oxalic and malic acids (22).

Fruits also contain saponins, piranosideous, flavonoids, guaijaverin, oleanolic acid and quercetin (23), very used by man for its medicinal properties and important for the plant in its metabolic functions

Pharmacological studies have shown a large number of medicinal uses in guava, such as antimicrobial, antigenotoxic, anti-inflammatory, antiallergic, antiplasmodic, hepatoprotective and for the treatment of diarrhea and diabetes (24). Its consumption has also been associated with lowering cholesterol, triglycerides and blood pressure (25).

This species has been cultivated as an ornamental in various parts of the world and its flowers are very important for beekeeping (26). Its wood is moderately heavy (density 0,8 x 10^{-2} kg x 10^{-6} m), tough, elastic, moderately durable, is used as firewood, charcoal, for tool handles, in aircraft construction, among other uses (27). It is also useful in controlling pollution, it serves as a bioindicator and bioaccumulative. It is essential in mixed plantations for the recomposition of degraded areas (27).

PLOIDY LEVEL IN THE *Psidium guajava* L SPECIES

In general, representatives of the Myrtaceae family, have a base number of 11 chromosomes, although (28) stated that can be found species whose basic chromosome number varies between 6 and 9. The polyploidy is more common in the Myrtaceae with fleshy fruits in species that have dry fruits[B] (29).

In *P. guajava* L. cytological variation is high, there have been diploid, triploid, tetraploid and aneuploid (disploides) forms.

[B]Costa, I. R. *Estudos cromossômicos em espécies de Myrtaceae Juss. no sudeste do Brasil.* Tesis de Maestría, UNICAMP, Campinas, S.P., 2004.

Polyploid genotypes are chromosomal races or cytotypes (29). Most commercial varieties are reported as diploid with a chromosome number of 2n=2x=22 (30), although it have found seedless guava cultivars, which had 33 chromosomes. In addition, (31) it found triploid cultivar of guava, and suggested that it might be a autotriploid.

Cytological studies in the *P guajava* species, based on the structure and behavior of chromosomes indicate that meiosis proceeds normally, with the formation of 11 bivalents in diakinesis and proper distribution of chromosomes in the final stages of the process (10, 15).

The absence of seeds character is related to several factors, of which chromosomal abnormalities and self-incompatibility are the main ones. Iyer and Subramanyan (32) stated that the production of triploid was useless, since the formation of triploid fruits was highly irregular and poorly finished, due to the different size of the seeds.

In India a cross between a triploid seedless crop and one diploid (‘Allahabad Safeda’) was performed to develop a cultivar with fewer seeds and increased yield potential. This author obtained in the F1 generation, 26 diploid plants, trisomic nines, five double trisomic and 14 tetrasomics (30). In this progeny, variations were observed in growth habit and characters of flowers and fruits.

There is a few research focused to know the conduct of the trisomic, tetrasomic and aneuploid in improving the species, even though it is known that trisomic and other aneuploid can be used to overcome the problem of incompatibility in certain interspecific crosses (31). In Cuba, works were carried out to obtain some seedless cultivars like ‘Gonzalo No.1’, ‘Gonzalo No. 2’, ‘Ibarra’ and ‘N7’ seedless. However, it should not be forgotten that, even if the absence of seeds is a very important commercial attribute, has been published in poor literature germination of pollen grains and low percentage of mooring of the fruits, which also have few viable seeds; as well as low yield (33).

OBJECTIVES FOR THE GUAVA TREE IMPROVEMENT

The objectives for crop improvement are varied and differ in countries where this fruit is grown. Improvement strategies generally considered promising cultivars, which leads to a narrow genetic base. Introgression of traditional varieties or genotypes without characteristics of commercial interest to the gene, it is not widely accepted because of the possible incorporation of unwanted characters. Inventorying, collecting, characterization and evaluation ensure the maintenance and use of a genetic heritage for future generations, with the aim of having a more broad-based improvement programs, making it possible to respond to the demands the market and to the incidence of pests and diseases (34).

Most guava tree improvement programs include the following objectives: (a) collect, enter, characterize and select genotypes of guava tree with defined characteristics and suitable for the production; (B) selecting genotypes with high productive potential and mechanisms of resistance to pests and diseases; (C) establish important botanical descriptors for the guava tree, trying to eliminate redundancies in the collection; (D) maintaining the collection of genotypes of guava in areas of strategic development; (E) select and disseminate guava genotypes for the formation of commercial orchards and to supply material to other elite breeding programs and nurseries (6, 14).

Crops that have fruit with many seeds are indicated for controlled hybridization and the progeny obtained by open pollination, may be suitable for development programs cultivars (10). These authors argue that the selection criteria to be considered are:

Fruits: a) fruits of large size (200 x 10^{-3} kg-340 x 10^{-3} kg.), with few seeds and thick pulp; b) white pulp for fresh consumption and dark red to pink for industry; c) characteristic flavor and aroma of the fruit; d) total soluble solids content greater than 10 %; e) acidity of 1,25-1,50 % for the fruits used in industry, and 0,2-0,6 % for those who consumed fresh; f) vitamin C content less than 300 x 10^3 mg x 10-3 g) minimum number of stone cells; h) good quality post-harvest; i) resistance to pests and diseases damage the fruit.

Plant: a) vigorous trees with wide canopy and low height; b) resistance to pests and diseases; c) high production; d) rootstocks with reducing effect as height.

Others authors (35) also published a list of goals for improving the guava tree, which includes:

- External Appearance of fruit: over 100 × 10-3 kg average weight not thinned plants, oval, halos medium or small size, color yellow-green bark or yellow when ripe fruit, resistant to transportation and with good quality storage.
- Internal appearance of the fruit: pulp color, pink or red, the weight ratio of the pulp and over 70 total weight, thickness greater than 100 x 103 m pericarp, absence or few spots in the pericarp, absence of stone cells, few seeds and small seeds.
- Organoleptic characteristics and content of the fruits: content of total soluble solids greater than 10° Brix, total soluble solids /AT ratio greater than 11,7, vitamin C content about 100 mg of ascorbic acid in 100 g of pulp, flavor and nice aroma to stay in industrial products.
- *Plants*: minimum productivity of 30 t ha^{-1} yr^{-1}, low and open canopy, resistant to P. psidii.

INHERITANCE IN GUAVA TREE

In perennial crops such as guava, it is difficult to obtain accurate information, because they are highly heterozygous, large areas and work for cultivation are needed, spread by seeds have a high adaptability and genetic variability, and it is necessary to assess great populations for this type of study, which can be derived little information related to culture heritage (7).

This is one reason why, about guava there is little information regarding the inheritance of quantitative traits, however it has been studied a little inheritance of qualitative characteristics.

It is known that the ovoid shape of the fruit is dominant over the rounded and pyriform types. In Brazil, some authors observed that the red pulp is dominant over the white pulp (26). In India others demonstrated that this attribute is governed monogenetically and the gene coding for the flesh color is linked with the gene that controls the seed size (15).

The heritability in general includes all types of gene action, such as dominance, additivity and epistasis (30), while the narrow sense heritability only has the contribution of additive genetic variance to phenotypic variance into account. The effect of additivity is the most important because it is the main cause of the resemblance between relatives, determinant of the observable genetic properties in the population and the positive response to selection (36).

Considerable efforts have been made to estimate the heritability of traits of agricultural importance in the guava tree.

It has been observed that some commercially important, such as characteristics: yield, fruit size, certain types of disease resistance, vitamin C, pectin and acidity, are often low heritability estimates. None of these characteristics is determined exclusively by major genes, although basic genes subject to polygens modifying effects have been identified for some qualitative characteristics such as crust color and acidity.

In India, the F1 progeny obtained four crosses (37), among 'Apple Colour' cultivar with 'Arka Mridula', 'Chittidar', 'Beaumont' and 'Allahabad Safeda' cultivars were studied. These authors found that the genotypic variance and coefficient of genetic variation were lower than the phenotypic variance and coefficient of phenotypic variation, respectively, in the characters evaluated (content of total soluble solids, weight, length, width and volume of the fruit). The low values of genetic variation coefficients indicated a low degree genetic variability in half-brother evaluated populations, while the highest values of phenotypic variation coefficients imply greater manifestation of those characters.

Variation coefficients indicate only the variability in different characters but not its heritable portion (37), so it is necessary to also determine the heritability thereof. They estimated the values of narrow sense heritability of traits evaluated and estimated average obtained therein; the highest values of heritability were observed in characters: length of the fruit and total soluble solids content. Therefore, the selection can be made in order to improve yield components, these characters being controlled by genes with additive effects. These authors also calculated the genetic and phenotypic correlations between characters and determined that the fruit weight showed positive correlation with length, width and volume of the fruit, while the correlation with soluble solids content was negative.

The genotypic correlation was higher than the phenotypic traits evaluated in correlation with the exception of soluble solids, which can be related to the relative stability of genotypes. This happens not only when genes that control characters are similar, but also when environmental factors have similar effects.

In another work done in India high heritability values were obtained on vegetative characters, flowers and fruits of evaluated guava accessions (38). These authors also determined positive phenotypic correlations among the fruit width, the fruit weight, content of soluble solids and days required for fruit ripening with the size of flowers, as well as the number of seeds/fruit and acidity, soluble solids content and acidity and total sugar content. Besides high heritability values were obtained broad sense and the coefficients of genetic and phenotypic variation in characters of fruits and seeds, evaluated in 50 accessions of guava (39).

These results indicated that these characters had an additive genetic effect, so that could make an effective selection of them. These authors also observed positive correlations and phenotypic genotypic among the length, width, fruit weight and number and weight of seeds/fruit, in all their combinations; while were negative correlations among weight, over the fruit weight and number of seeds/fruits with fruit weight/weight ratio of seeds.

From the estimation of components of phenotypic variance in fruit characters evaluated in eight genotypes of guava, high values of genetic variance and error variance (variance component fruits in trees, taken as error (40) were obtained, by the hierarchical structure of experimental design and statistical) analysis in most of the characters, the estimated error variance in some characters being older.

These authors had a significant interaction, as well as, low values for the variance of the interaction. They also obtained low values of the variance component trees within genotypes (replicas of genotypes) and this source of variation was significant only in two of the nine characters evaluated (content of vitamin C and weight of the seed cavity).

With the obtained results (low values of variance of trees within genotypes and high values of the variance of fruits in trees), the authors suggested that fruits harvested from different trees of the same genotype had similar qualities, so effective evaluations of fruit characters could be done in one tree per genotype, and increase the number of fruits to evaluate tree (genotype) was more effective than increasing the number of trees (replicates) per genotype, for accurate genetic estimates. These results could allow the crop improvement program operate more economically and efficiently in field evaluations.

In Cuba, they have also done work with the objective of estimating genetic-statistical parameters in populations of crop, obtained from controlled crosses made among plants of the cultivar Enana Roja Cubana' and 'N6', 'Supreme Roja' and 'Belic L-207' cultivars. In this regard in 2013 very variable values in broad sense heritability and narrow sense in vegetative characters and fruits, evaluated in the three populations[C] they were obtained. These estimates were mostly media, which shows the influence of both genetic factors such as environmental, in the expression of the characters. Values were also obtained mostly means of genetic variation coefficients, results showing the marked environmental influence exerted characters; criterion to be taken into account when adopting new strategies in the breeding program of crop[C].

This author obtained values of the genetic variation coefficients higher than coefficient of environmental variation in most traits evaluated characters. There is a very favorable situation for the gain selection when the relationship between the coefficient of genetic and environmental variation is greater than one, since in these cases the genetic variation is greater than environmental one, indicating that selection for these characters has the best conditions in terms of genetic gain (41).

In this study, the response to selection was also calculated and the highest values in the characters were observed: fruit weight, number and total mass of seeds per fruit, leaf width and height of the plant, which had also means genetic variation coefficients, and estimated heritability in both senses, medium or high. Therefore, these characters have high genetic variability that can be exploited in breeding programs cultivation, because in the same predominant action of genes with additive effect and can make an effective selection (39, 42).

[C] Pérez, L. *Análisis de la variabilidad genética y de la interacción genotipo-ambiente en poblaciones de guayabo* (*Psidium guajava* L.). Tesis de Doctorado, Facultad de Biología, Universidad de la Habana, La Habana, Cuba, 2013, 138 p.

To analyze the association among evaluated characters[C], it was that detected values of the genetic correlations were greater than phenotypic in most correlated characters, indicating the relationship among them it is due to genetic additive factors in the material under study, rather than the existence of environmental effects (43).

Genetic correlations and positive and significant phenotypic between weight, length, width and outer fruit pulp thickness were observed; between the number and the total mass of seeds with the fruit weight, in all their combinations; and between the inner thickness of the pulp, the number and the total mass of seeds per fruit. However, relating these characters with the fruit size, negative genetic correlations between the fruit length with the internal thickness, the number and the total mass of seeds were observed; and phenotypic negative correlations along the fruit between the inner thickness of the pulp and seed number.

These results are of great importance, because one of the characters to improve in the Guava is the number of seeds, for breeders wishing to obtain cultivars with few seeds that are more profitable, both for industry and for fresh consumption.

RESULTS OBTAINED IN THE GUAVA GENETIC IMPROVEMENT

Historically, Guava has spread from seeds and obtained plantations are highly variable due to pollination by insects. Seedlings have high rates of segregation, and this is the basis of the variation used worldwide, both by breeders as producers (14).

The flower morphology of guava tree favors self-pollination, but also to be a sort of cross-pollination; it crosses naturally with other cultivars, giving rise to different genotypes in appearance, production and quality (44). The above with the propagation by seeds in the beginnings of domestication has generated some variability should be studied and characterized, for purposes of being able to recognize, identify and document the variability within and among cultivars of the species, and analyze their agronomic performance in response to the production system used (45).

In some countries like United States (mainly in Florida and Hawaii) and Israel, where the guava tree is grown for more than a century, the cultures are based almost one commercial cultivar. While in Israel (46) made crosses among different cultivars from Brazil, Thailand and Mexico and obtained five new promising cultivars, with which it began to increase the genetic base of the crop in the country.

In Pakistan they have used unconventional techniques such as mutation induction, to try to increase the genetic variability (47). Resistance to nematode that attack the root is the main objective of the breeding program in Malaysia, and three potential accessions with resistance have been identified (48, 49). In Thailand (40) and Mexico (50, 51) have characterized the crop germplasm collections and has been detected high genetic variability, mainly in fruit characters. In India the main guava tree improvement program is developed, and have been identified and characterized new hybrids and cultivars with different morphological and molecular markers[D] (32, 52, 53). In Brazil, although production has increased in recent years, the crop improvement program has been discontinued for various reasons (35).

In Cuba, the work of breeding and selection began in the early 60s of last century, with the production of 36 open-pollinated cultivars by cultivar 'Indian Pink' at the Agricultural Experimental Station of Santiago de las Vegas. It continued with the study of 108 cultivars in the collection of Germoplasma Bank[E]. It is valid to highlight the work done by Gonzalez and Sourd[F] (54). The first test includes 10 varieties of *P. guajava*, nine of them grafted on the cultivar 'Cotorrera' and on *Psidium friedrichsthalianum*. The second work included the evaluation of 10 selections of guava basis of the physico-chemical characteristics of the fruits.

Another study done (5) the offspring from a cross among the cultivars Enana Roja Cubana' and 'Bangkok' was characterized. The traits evaluated showed very few differences between the test plants; they could be pooled descendants based on plant height, color of pulp and resistance to fungal attack as *Colletotrichum gloeosporioides*.

[D] Aswath, C.; Padmakar, B.; Kanupriya; Vasugi, C. y Dinesh, M. R. ''Genotyping of parental lines in *Psidium guajava* using microsatellite markers''. En: *7th International Symposium on In vitro Culture and Horticultural Breeding*, Bélgica, 2011.

[E] González, G.; Fuentes, V.; Rodríguez, N. N.; Torres, M.; Capote, M.; Cañizares, J.; Lima, H. y Orozco, P. ''Colecciones y recursos filogenéticos en la Estación Nacional de Frutales de Cuba''. En: *Primer Simposio Internacional sobre Fruticultura Tropical y Subtropical*, La Habana, Cuba, 1995, pp. 71-72.

[F] González, G. y Sourd, D. ''Ensayo de variedades clonales en *Psidium guajava* L''. En: *Primer Congreso Nacional de Cítricos y Otros Frutales*, La Habana, Cuba, 1981, pp. 319-336.

Subsequently, an assessment of the diversity of Cuban guava germplasm was performed by morphological markers and techniques of Length Polymorphism amplified fragment (LPAF) and Simple Sequence Repeats (SSR) (55, 56, 57).

Based on the results, the authors were able to recommend elite genotypes for different purposes such as marketing and improvement, and for the establishment of crossbreeding programs, of which 25 short-statured genotypes were selected. In addition, they managed to construct linkage maps using these molecular markers (SSR and LPAF), saturation of molecular linkage map and detection of over 50 quantitative trait *loci,* as a basis for marker-assisted selection. These results, in conjunction with the use of tissue culture techniques for the conservation and propagation of germplasm, have allowed proposing a methodology to develop the crop improvement program in the country (3).

USE OF MOLECULAR MARKERS IN BREEDING

The morphological markers have traditionally been used to evaluate genetic variability. To supplement and refine descriptions based on morphology, they began using enzymatic markers (58). Subsequently, DNA-based markers provided a new option for genetic studies and showed significant advantages in relation to morphological and DNA biochemical markers[C]. DNA markers have become very important for their use in the construction of genetic linkage maps, the analysis of comparative mapping, marking genes economics significance, marker-assisted selection and map-based cloning. They also provide genetic information in the main areas of germplasm conservation, both ex situ and in vitro (59).

There is great potential for the application of molecular markers in perennial crops of tropical and subtropical fruit. In fact, fruit these tools could be of difficult application due to factors such as self-incompatibility, apomixis, dioecy, the absence of seeds, mature embryo, heterozygosity and long juvenile periods (60).

In the guava tree different techniques based on PCR to verify quality of DNA, DNA f to establish individual accessions, evaluate genetic diversity, and construct linkage maps and to identify economically important genes for marker-assisted selection have been used. Among these it can mention the Random amplification of polymorphic DNA, Amplification Fragment Length Polymorphism; Reps reverse sequences marked and Simple Sequence Repeats.

DEVELOPMENT OF SSR TO *Psidium guajava* L

With the purpose to develop the potential offered by simple sequence repeats (SSR), an enriched microsatellite bank with sequences $(GA)_n$ and $(GT)_n$ was developed to boost genetic studies and marker-assisted selection in guava and their related species (61).

In order to determine the usefulness of primers, DNA samples of guava from diverse origins (Cameroon, Colombia, Cuba, Florida, Hawaii and Martinique) as well as other species such as *Psidium acutangulum* D.C., *Psidium cattleianum Sabine* var *lucidum* and *Psidium friedrichsthalianum* Nied were used. For other species *Psidium* except for four loci, amplification revealed reliable SSR patterns.

This bank is the first one referred to guava tree and can be used to genotype identification, pedigree analysis, studies germplasm diversity and genetic mapping. Moreover, it constitutes a potential for investigations of genetic resources and improvement in the genus (61) .Investigations involving the use of this marker for different purposes also referred obtain SSR reliable patterns both species, and other representatives of the family. They have been developed in countries such as India (62, 63), Cuba (64, 65); Mexico (66); Brazil (67), Venezuela (68, 69), among others.

DNA MARKERS FOR IDENTIFYING GENOTYPES

It has been suggested for many crops using molecular markers in order to establish individual DNA fingerprints accessions (70). In addition, the International Union for the Protection of New Varieties of Plants (UPOV) promotes the introduction of new methods of stable tests, uniforms and distinctive (DUS), and overcome the legal implications of certain changes to the protection of plant varieties (71). In this sense, the use of four isozyme systems (α-esterases, β-esterases, acid phosphatase and peroxidases) was an approach to distinguish intra- and inter-specific variations in species of *Psidium*; however, it was not observed a clear identification of genotypes (72). Sometimes insufficient isozyme markers exhibit polymorphism. In addition, space temporary and experimental variations (73) may occur. Therefore, its use for identification purposes is restricted to a local germplasm if one taking into account that the isoenzyme profiles are not transferable.

Several studies have confirmed the usefulness of LPAF, ISTR, microsatellite and RAPD markers for identification purposes in countries where this species is grown: Cuba[G] (5, 74, 75, 76); México[H] (50, 51, 77); Bangladesh (78); Colombia (79); India (80, 81, 82), Brazil (67, 83, 84); USA (85), among others. In the case of RAPD markers, although its reproducibility among different laboratories remains under discussion (71), they have shown good results so they can be recommended for this purpose.

More recent studies have ventured in the using of other molecular markers. Such is the case in the region 18S of ribosomal RNA, regions of spacer sequences internal transcribed (ITS) of the ribosomal DNA and regions intergenic spacer sequences (ISM) of chloroplast DNA, which have been used in Taiwan for identifying accessions guava and other representatives of the genus *Psidium* (86).

Similarly, the usefulness of the Regions Internal Spacer Sequences (ISSR) has been evaluated for discrimination among genotypes belonging to different species of the genus Psidium. This technique combines the use of a single primer RAPD as reliability and reproducibility of SSR, making it more potent for these purposes (53). This type of molecular marker has also been used in the estimation of genetic fidelity among regenerated plants by somatic embryogenesis and mother plants from accessions of guava, providing very good results confirming the uniformity of the obtained material (87, 88, 89).

Another system widely used DNA markers is based on retrotransposons, of the type called Inter Primer Binding Sites (iPBS), which are designed from conserved regions of the primer binding sites within the retrotransposon. For discriminating accessions of guava from open-pollinated, this type of marker is used with very good results with the application of IPBS for these purposes (90). This study demonstrated that the IPBS is a powerful tool, as well as enabling the identification of genotypes when there is no a priori knowledge of the sequences to be analyzed, have a low cost and are efficient and easy to handle.

Moreover, the selection of a given marker is a balance between the level of polymorphism that can detect (information) and its ability to identify multiple polymorphisms (91). The parameter D (discrimination power) evaluates the efficiency of a primer for identifying varieties (the probability that two randomly selected individuals have different patterns) and also can be used to compare different types of labels knowing only the allele frequencies (92).

In this regard, studies determined that the most efficient for discrimination in the accessions of guava forming the Cuban germplasm are LPAF primers such as: E32 x M33, E32 x M35, E32 x M32, E33 x M39, E32 x M36, M32 x E33, E32 x M34, given by D parameter values calculated (65, 93). These works, before diversity studies, to assess the potential of each molecular marker in order to identify what is the most appropriate application of the same, that is, which provide more reliable information to map out future strategies in the program improvement of the species.

CONSTRUCTION OF GENETIC LINKAGE MAPS

For efficient use of polymorphism detected by genetic markers, it is necessary knowledge of their individual location in the genome, and can be achieved through the construction of a genetic linkage map. Therefore, the genetic linkage map represents the adjustment or arrangement of the many *loci*, which include morphological, biochemical and nucleic acid markers, through the chromosome (70).

For the future implementation of marker-assisted selection to boost breeding program in Cuba three mapping populations they were obtained by cross-pollination

[G] Rodríguez, N. N.; Valdés-Infante, J.; Becker, D.; Velázquez, B.; González, G.; Sourd, D.; Rodríguez, J.; Billote, N.; Risterucci, A. M.; Ritter, E. y Rohde, W. ‟Characterization of guava accessions by SSR markers extension of the molecular linkage map and mapping of QTLs for vegetative and reproductive characters”. En: *Proceedings of the First Internatinal Guava Symposium*, Lucknow, India, 2005, pp. 201-215.

[H] Hernández, D. S.; Martínez, J. L.; Padilla, J. S. y Mayek, N. ‟Diversidad genética de *Psidium* sp. en la Región Calvillo-Cañones, México”. En: *1er Simposio Internacional de Guayaba*, Aguascalientes, México, 2003, pp. 71-83.

of three individual trees 'Enana Roja Cubana' as the female parent, pollen donors 'N6' (1 = mapping population MP1), 'Supreme Red' (MP2) and 'Belic L-207' (MP3). The MP1 population was used for an initial identification of the co-segregation of markers in the progeny through the LPAF technique use.

Based on these results, the first genetic linkage map was established with a total of 167 markers mapped on 11 linkage groups which presumably represent the 11 chromosomes of the guava tree (75) haploid genome. These efforts were subsequently extended by the increase in AFLP primer combinations and SSR and the additional mapping of these markers on the linkage map. The high number of common markers allowed the fusion of the individual maps of parental in an integrated a total of 220 markers mapped map. Individual linkage groups contain from 11 to 30 markers each with variations in the length between 104 and 150 cm, resulting in a total of 1379 cM (74).

Similarly, it has worked on the development of two other linkage maps for the crossings 'Red Dwarf Cuban red' x 'Supreme' and 'Red Dwarf Cubana' x 'Belic L-207', based on AFLP and SSR (94-96). This aims to identify common markers to standardize linkage groups for the construction of an integrated map for the species. They have referred to these primer combinations SSR and AFLP have been helpful to achieve this purpose (97).

Recently in India they have also done similar work. Such is the case of a study based on SSR markers two genotypes of guava with contrasting differences for a group of characters of commercial interest. As a result, a linkage map that can be useful for the identification of QTLs, positional cloning and identification of markers linked to characters of importance for breeding and production (98) was developed.

WEAKNESSES AND OPPORTUNITIES

Despite the economic importance of this cultivar in breeding programs, there are still many problems such as:

- Lack of practical implementation of scientific results.
- Insufficient financial support in the Market Free Trade Agreement (MLC) to ensure the required investments and technologies.
- Limited possibilities to develop industrial products with the quality requirements of the international market.
- Outdated and insufficient to postharvest fruits Infrastructure conditioning.
- Obsolescence of industrial plants.
- Lack of a post-entry station that limits the introduction of new species and varieties. However there is a growing domestic market in foreign exchange and an increase in demand in the external market.

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