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Review
AVOCADO (*Persea americana* Mill)

Revisión bibliográfica
El aguacatero (*Persea americana* Mill)

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ABSTRACT. Avocado (*Persea americana* Mill) is the only one commercial important member between edible fruit which belong to *Lauraceae* family, it is originated from Mexico and Central America. It shows a growing value on the international market not only because its nutritional quality but also due to its medicinal uses and in cosmetic industry. This work presents information about the research in this fruit crop and some relevant aspects about germplasm conservation, main diseases affecting avocados and advances in genetic improvement.

Key words: avocado, conservation, diseases, breeding

RESUMEN. El aguacatero (*Persea americana* Mill) es el único representante de importancia económica entre las frutas comestibles de la familia *Lauraceae* y es originario de México y Centro América. Presenta un creciente valor en el mercado internacional no solo por su calidad nutritiva sino también por sus usos medicinales y en la industria cosmética. Este trabajo resume informaciones relacionadas con las investigaciones sobre esta especie y se discuten algunos aspectos relevantes sobre sus vías de conservación de germoplasma, enfermedades así como avances en el mejoramiento genético.

Palabras clave: aguacatero, conservación, enfermedades, mejoramiento

INTRODUCTION

Avocado (*Persea americana* Mill) is a species from Mexico and Central America and the only one of commercial importance, from an economic point of view, of the *Lauraceae* family, which includes about 2 200 species. This family includes woody plants, producers of essences producing that grow in warm regions and where the laurel (*Laurus nobilis* L.) are also included, Camphor (*Cinnamomum camphora* Siebold (L.)) and the cinnamon (*Cinnamomum verum* J Presl). This delicious fruit is well known to man for thousands of years, they show the earliest evidence of its consumption from a cave in Coaxcatlán, Puebla, Mexico; with a length of 7000-8000 years (1), and beyond its edible use in fresh and processed has wide applications as a raw material for the oil extraction and cosmetic industry (2).

Mexico is the main producer of this fruit because it produces a third total part of the world and is the leading exporter with 40 % (1), while Cuba, ranks 39th in terms of production (3).

Germplasm banks are a way of conserving plant genetic resources. A variant of these banks based *in vitro* conservation is very useful for the tropical agricultural conservation of crops and food importance. In the same it seeks to maximize the collected specimens' diversity from population in the field or in its center of origin (4, 5). Regarding gene banks, at present the number of accessions superpass six million (6), which represent a great potential for genetic improvement.

This fruit species has recalcitrant seeds, which do not support the dehydration, so it must be stored in a humid environment and retain its germination capacity only for a short time, so it should pay attention to ways of preserving them (7).

This crop is attacked by many diseases, among which are the root rot caused by the oomycete *Phytophthora cinamonni*, although it is reported to other species of *Phytophthora (Phytophthora heveae* Thompson, *Phytophthora citrus* Sawada and *Phytophthora*

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palmivora) as causal agents this condition (8, 9). Other diseases affecting this fruit are: black spot (*Cercospora purporea* Cooke) and anthracnose (*Colletotrichum gloeosporioides*) (10).

Avocado genetic improvement by conventional hybridization methods is extremely difficult, so genetic reported studies have been limited in this regard (11, 12). Additionally, extensive juvenile period and large areas needed for tree growth expensive breeding programs.

In this paper, considering the avocado importance, it will deepen its importance as a fruit, main forms of cultivar conservation, perspectives and main diseases of this species. Also as a fundamental problem, it aims to provide an updated view of its breeding and conservation.

AVOCADO HISTORY

The avocado is native to tropical and subtropical regions, Central America and Mexico (13). Archaeologists found *Persea* seeds in Peru, which were buried with Incan mummies dating to 750 BC and there is evidence that its cultivation in Mexico is as early as 1500 BC after the arrival of the Spaniards and the conquest of America, the species has spread to other parts of the world (8).

The avocado has more than 100 cultivars and clones classified into four horticultural breeds: Guatemalan (*P. americana* var *Guatemalensis*) Antillean (*P. American* var. Drymifolia), Mexican (*P. americana* var American) (14) and Costa Rican (*P. americana* var. *Costaricensis*) (fifteen). Additional aspects related to diversity and domestication of avocado in America can be reviewed in other conducted studies (16).Specimens of *P. American* originated in highlands of central and eastern Mexico, generate the Mexican breed. The ones from highlands of Guatemala generate Guatemalan breed and the Antillean breed comes from the first plants found in the Antilles. Regarding to the origin of the Antillean breed there are discrepancies because it is possible that the first specimens of this species existing in the Antilles have been introduced from Mexico by the Spaniards or the English during colonization (17). The most commercialized internationally varieties are those of Guatemalan and Mexican origin or especially 'Hass', 'Fuerte' and 'Nabal' (18).

BOTANICAL CHARACTERISTICS

On average, the avocado tree can reach a height of 20 meters; However, when it grows in commercial plantations, it is not grow more than 5 m, to facilitate phytosanitary control practices, harvesting, pruning and foliar fertilization. This plant species has thick trunk and elongated leaves, with several branches that generate dense foliage. It is considered a perennial crop because it is grown throughout the year (10).

The fruit is a drupe, pear-shaped, light green to dark green and purple to black, rough skin with a yellowish green flesh and a large central bone. There are approximately 400 varieties, so we can find fruits of different shapes and weights, which may through 150 to 350 gr (19).

Some aspects of the species related to systematic *Persea americana* Mill are (8):
Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Laurales
Family: *Lauraceae*
Genre: *Persea*
Species: *Persea americana* Mill

GENETIC CHARACTERISTICS

The number of chromosomes (karyotype) of avocado has only been studied in some species: *P. americana*, *P. nubigena*,

P. Borbonia, *P. longipes*, *P. Floccosa*, *P. palustris*, *P. cinerascen*, *P. schiedeana*, *P. indica*, *P. donnell-smithii* and *P. pachypoda* all with the chromosome number of 2n=24 and has identified only one tetraploid species, *P. hintonii* (20), originally from Temascaltepec and Tejupilco in Mexico. However, the same author identified some types of tetraploid and triploid *P. American* from San Juan de la Vega, in Guanajuato, Mexico.

Perhaps the wild progenitor of cultivated avocado has been a polymorphic tree covering a wide geographical area from the east and central highlands of Guatemala through Mexico to the Pacific coast of Central America (21). Neolithic peoples selected early domesticated descendants (wild forms) from these populations, replacing completely the wild ancestor of the *P. americana* species for three ecotypes well characterized for their possible centers of origin, as the horticultural breeds already mentioned. Ethnobotanical data (22) and genetic marker studies (23) suggest that these three breeds were domesticated independently and they were not contacted until the sixteenth century with Europeans.

In the late 1800s avocado improvement gained momentum through interracial hybridization between Guatemalan cultivars and the Mexican germplasm in California and Florida from Cuban germplasm (Antillean breed) and the Guatemalan breed (24). A long period of open pollination and interracial hybrids has resulted in modern cultivars with a mixture of the three horticultural breeds that are complex and often wrongly characterized (23). Its phylogeny has been difficult to study, so that relationships within the family have not yet been fully defined, and therefore, its taxonomy and nomenclature are unclear (25).

The recent suggestion of two genetic populations based on latitudinal differences in the highlands of Mexico will encourage the realization of future genetic analysis in Mexico and Central America. The avocado is one of the first domesticated trees in the Neotropics and it can be used as a model for the domestication of trees in areas with high biological diversity (26). The ability to unravel the complex hybrid origin of several cultivars should provide a useful guide for those who manage genetic resources (27).

Due to, as already explained, this species has a high degree of hybridization, assessing genetic relationships to distinguish different taxa and identify promising material for breeding programs (25).

ECONOMIC IMPORTANCE

Production and avocado trade in the world are mainly based on its use as food, because its pulp is a valuable source of energy, protein and minerals (28).

This fruit also has medicinal properties; many crops have antirachitic qualities and anthelmintic high power. The oil extracted can be used in friction to alleviate gout and rheumatism. The leaf infusion is used against fever, menstrual cramps and migraine. Additionally, it is used in cosmetology for skin and hair with excellent results (29).

It has recently been shown that the plant is avocado containing more carnitine, an amino acid involved in the metabolism of the heart muscle, which is being used in the treatment of heart disease and lack of appetite (30).

Mexico is the leading producer of avocados, surpassing a million tons annually, followed by Chile and Dominican Republic (table), while the Americas accounted for 60 % of global plantations of this fruit (3).

The main importing country is France, with 39 % of the total imported volume, while other important buyers are USA (10 %), United Kingdom and Belgium, the latter two with 6,5 % each (3).

GERMPLASM CONSERVATION

Today, the society, among other things, has a growing concern for the conservation of biodiversity, sustainability and equity in agriculture. Genetic diversity in the plant heritage of each nation is the essential tool of plant breeder so receives special attention, including the establishment of gene banks to conserve and exploit agronomic qualities of species (31). Additionally, gene banks include other functions such as documentation, characterization, and assessment of genetic variability, phylogenetic studies and the multiplication and distribution of germplasm (32).

The conservation of phytogenetic resources of a country must be oriented according to resources available and the period for which it is desired to preserve the germplasm. If it is for a short term, it is desirable to establish seed banks; if it is for the medium term, the field conservation and preservation *in vitro* is recommended; and if you want to keep for a long period, it is best to use methods of cryopreservation. Considering advantages and insufficiencies presented by each method as well as the botanical characteristics and agronomic species and gender are considered to preserve the optimum is to use several of these methods that complement efforts (33).

Because of the constant loss of wild species of avocado (*P. americana*) due to the devastation of man in different origin places in Mexico and Central America, currently the genetic diversity of this species is threatened (34).

The destruction of the tropical forest with taxa Lauraceae, including relatives of avocado, constantly increasing (35).

Table. Main productors of avocado, 2011 (tons x 1000) (3)

Countries	Production (tons x 1000)
Mexico	1 264,14
Chile	368,56
Dominican Republic	295,08
Indonesia	275,95
Colombia	215,32
Peru	212,85
United States	205,43
Kenya	201,47
Brazil	160,37
Rwanda	143,28
China	108,50
Guatemala	91,47
Spain	83,42
Republic of Congo	83,21
Venezuela	81,59
Israel	75,28
South Africa	75,23
Cameroon	69,53
World total	(more than) 3 200 00

During the last three decades, native and semi-wild avocado genotypes have been disappearing rapidly, like other native species (15). In Ecuador, *Persea theobromifolia* has been reported as nearly extinct (Gentry, 1979, cited by 36); as these habitats are altered, an unknown number of species is disappearing before being studied and therefore without recognizing them. For all proposed routes preserving this culture, and the study and characterization of pests and diseases are highly relevant.

METHODS OF GENETIC CONSERVATION OF AVOCADO

● CONSERVATION *IN SITU*

In situ conservation is the conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of different species. In the case of cultivated or domesticated species conservation also covers around the place where they have developed their distinctive properties. A dynamic process of conservation is considered as plants continue evolving with changes in the environment, making it very favorable for studies of genetics and evolution (33). This variant conservation presents drawbacks that the plant material is vulnerable to natural disasters and the human devastation, plus it is not readily accessible for use. It also requires appropriate management regimes and high levels of supervision and monitoring (37).

In situ conservation includes the protected area preservation and on farms and home gardens (37). In Mexico there are protected areas hosting species of *Persea,* including the Sierra de Manantlán Biosphere Reserve where *Persea hintonii* (38) grows. However, it was reported that only reserves of Los Tuxtlas and El Triunfo include mesophilic forests that are home to *Persea Americana* (39). In the last century it was thought that the conservation of avocado wild relatives was best done *in situ* or natural populations in their place of origin (15), but due to the accelerated destruction of these habitats has become necessary to proceed to their rescue for preservation by utilizing *ex situ* conservation means.

● CONSERVATION *EX SITU*

It represents the conservation of biological diversity outside its natural habitat. It involves sampling, transfer and storage of plant material from the collection area to the place where it will be preserved. It is divided into different techniques, such as seed storage, field conservation, *in vitro* conservation, cryopreservation, pollen storage and DNA storage (37).

The objective of *ex situ* conservation is to keep unchanged collections in their genetic constitution (40). Many plants species can be stored for long periods at low temperatures and humidity; however, there are species whose seeds can not be preserved in this way, because they produce “recalcitrant” seeds, such as *Persea* gender. These groups of species are maintained only as *ex situ* field collections, in vitro and cryopreservation, if the species permits.

There are many *ex situ* collections include accessions of wild and cultivated avocado, with largest collections in the United States (California and Florida) and in some states of Mexico.

In Mexico, the Autonomous University of Chapingo maintains an *ex situ* collection of some 180 accessions of *Persea* established in two locations at different altitudes (41), while another collection is located at the National Research Institute of Forestry and Livestock in Guanajuato. In general, 500 specimens belonging to 12 species of the *Persea* genre and four related species are preserved *ex situ*. The diversity of this collection consists of creole and regional selections wild types; some of them started in the 80s and to date are preserved accessions Mexico, Central America, Israel and Chile. *Persea Americana* is the most diverse preserved species represented by his four botanical breeds; var. Drymifolia, var. Guatemalensis, var. jacket and var. Costarricencis (42).

Other *ex situ* collections including *Persea* accessions exist in Guatemala, Honduras, El Salvador, Nicaragua, Costa Rica and Panama, which together with Mexico formed the Mesoamerican Network of Plant Genetic Resources. Other countries maintain collections of interest include Spain in the Experimental Station “La Mayora” in Malaga (43) and the Volcanic Center in Bet Dagan, Israel (14).

Other countries where avocado has high demand have also established *ex situ* collections. In Cuba, the avocado germplasm collection was established in 1965 and drew on old collections, including cultivars supplied by farmers, to complete 210 genotypes, from which a catalog that provides the description with 101 descriptors of 19 cultivars of interest for production (44) was published.

Ex situ conservation in the field is costly and vulnerable to climatic factors that could eliminate good intentions; as a safer alternative has been proposed, conservation through alternative methods such as *in vitro* method to short-medium term and long term cryopreservation (45).

● Conservation *in vitro*

Most *in vitro* gene banks specialized in tropical plants are associated with international research centers or conservation and to a lesser extent, universities. An example is the Tropical Agricultural Research and Higher Education Center (CATIE) in Costa Rica, where it has worked on the development of appropriate protocols for storage in vitro zygotic embryos, somatic embryos, tips and seeds of different coffee genotypes (*Coffea arabica* L.) and cell suspensions of the same species and *Musa* spp. This has also facilitated the exchange of plant genetic resources with producers and international organizations (46).

The International Biodiversity Group (Biodiversity International, BI), formerly known as "The International Board for Plant Genetic Resources" (IBPGR) with its centers in America, Asia, Pacific, Oceania, Europe and Africa, has focused on conservation *in vitro*, by the slow growth of cacao stems (*Theobroma cacao* L.), mango (*Mangifera indica* L.), banana (*Musa* sp.), avocado (*P. americana*) and some forage (*Cynodon* spp. And *Digitaria* spp.) (46).

There have been numerous studies in avocado (*P. americana*) for the establishment of *in vitro* germplasm banks. Among these it worked on *in vitro* conservation of axillary buds of the Mexican race and significant differences between growing areas used for storage was found to be test tubes (2,0 cm diameter x 15 cm height) where budding, number and length of these sprouts were minimal (47). As for the temperature response was noted that a low temperature (5 °C) is suitable for the optimal preservation of axillary buds, and in causing sprouting inhibition. Also, it was shown that a light intensity of 80 luxes, managed to keep dormant buds, because virtually no sprouting, but buds survived *in vitro* culture period.

In another investigation, Vargas (48), with axillary buds creole avocado of Mexican race, obtained plant growth in a Murashige and Skoog (MS) (49) medium containing brassinosteroids (BA), but survived after being treated at low temperatures. The concentration of 4 mg L^{-1} was the one that showed a greater effect on survival and the concentration of 8 mg L^{-1} most influenced growth; compared to sprouts which were grown in medium containing BA only (control), which failed to survive. Additionally, electron microscopy tests demonstrated a clear effect on the preservation, physiology and integration of grown sprouts and maintained in Brasinolide (48).

● Cryconservation or cryopreservation

Cryopreservation is a technique long term based on the reduction and subsequent stop of metabolic functions of all explants, including cell division by the liquid nitrogen effect (-196 °C); This is a method of preservation and long term, cost effective safe for species with recalcitrant seeds or vegetatively propagated, as in the case of avocado (*P. americana*) (50).

However, for efficient cryopreservation protocol is required in principle define the most appropriate techniques, such as tissue dehydration and the use of cryoprotectants (51).

In studies with axillary buds of Mexican and Guatemalan crops (52), dehydration time with sterile air, cryoprotective solutions and the freezing process were evaluated and it was determined that the most important parameter to be measured was the average survival; further testing chemical cell viability by direct observation were performed in epifluorescence microscope, after staining with fluorescein diacetate (FDA test). Although it failed to succeed in sprout regeneration, other information viability test was obtained; explants with less drying time showed a low viability percentage, making it convenient to reduce the water content of the plant material reduce effects of ice formation in the freezing. Results indicated that the best time of dehydrated was 120 minutes from viability percentages (fluorescence) were the most uniforms.

In another study, six times of dehydrated to avocado axillary buds of native Mexican race produced *in vitro* (51), with sterile air in laminar flow hood for 30, 60, 90, 120, 150 and 180 minutes and two cryoprotective solutions were evaluated PVS2 (30 % glycerol+15 % ethylene glycol+15 % DMSO+ medium with 0,4 M sucrose) and PVS4 (35 % glycerol+20 % ethylene glycol+ medium with 0,6 M sucrose). Results indicate that with 60 minutes of dehydration and keeping buds in a PVS4 solution for 30 minutes at 0 °C, plants with normal development, with sprouts, dark green shapely leaves and 100 % survival were obtained at 30, 45 and 60 days after setting buds through budding.

GENETIC IMPROVEMENT

Avocado improvers have to face numerous challenges, such as the great difficulty of obtaining controlled crosses, a long state of immaturity that limits the progress improvement, large tracts of land needed for experiments; in fact, the large number of labors in farming made this an expensive process. From a biological point of view, of the millions of flowers produced only a small fraction gives rise to fruit that achieve maturity and of these some tend to abort and fall; other hand, hand pollination is unreliable, while controlling pollination using cages, has had limited success. From the genetic point of view, as already mentioned, the avocado is highly heterozygous, so the behavior of the offspring is unpredictable and positions produced by a single tree are extremely variable. Among few advantages of this culture is the ability to propagate by grafting, desirable genotypes (53).

Open pollination and hybridization followed by the selection of promising materials in the progeny has been the only method available to avocado breeders (54). The need for a broader range of cultivars prompted the elite breeding programs to assess thousands of positions in search of both tolerant rootstocks to root rot caused by *Phytophthora*, as soil salinity and soil calcareous (55).

Additionally, the behavior of the avocado industry has been based on few cultivars, which suggested that only part of the genus *Persea* genetic resources have been developed, so it is very important to protect and develop the wild germplasm of the crop. The development of new cultivars and employers should contribute to this purpose. In this sense, breeders are interested in selecting cultivars with high and stable yields, but also with resistance to biotic and abiotic stresses (53).

Molecular markers, diversity, genetic mapping and assisted selection in avocado

Technologies of molecular markers may attend as useful tools to conventional breeding programs for analyzing genetic relationships, identification and selection of genotypes of interest, and the germplasm conservation. In avocado, the first molecular markers used were isoenzymes to explore aspects of its durability and cropping systems as well as the identification of varieties. In Cuba they were used at the beginning of this century in a study of diversity in cultivars of Antillean and Guatemalan origin and their hybrids (56).

Because of the importance of hybridization in avocado and its reticulate evolution, the genetic relationships should be studied with methods that capture the genetic diversity and include the historical inference.

The development of DNA-based markers has had an impact on research with avocado. Markers based on the length polymorphism of the restriction fragments (Restriction Fragment Length Polymorphism, RFLP) were used in evolutionary, phylogenetic (57) and genealogical studies (58). Other markers such as minisatellites were used for similar studies and with the same results regarding the identification of the three horticultural races already described (59), which was also confirmed by markers based on the random amplified polymorphic DNA markers (Random Amplified Polymorphic DNA RAPD) and microsatellites (54).

Other markers used successfully are microsatellites (SSR). Some authors (60) estimated at 45 000 the number of those present in the genome of this culture and these markers were developed to study genetic relationships among cultivars (61). Also, as markers based on amplified fragment length polymorphisms (Amplified Fragment Length Polymorphism, AFLP) are also useful, due to its high discriminatory power despite their low levels of heterozygosity. 12 AFLP primer combinations were used successfully and 16 of SSR developed for avocado to determine genetic distance estimates and identify unequivocally, 23 cultivars of commercial interest in Cuba (62), while using AFLP is indicated the need for more specific markers such as SSRs to differentiate cultivars of Mexican race avocado (63).

In this sense SSRs markers were used or simple inter repeated sequence (Inter Simple Sequence Repeats) in 77 collections (231 individuals) of *Persea americana* Mill. Mexican race, existing in the gene bank of INIFAP-CFAP_ Uruapan, Michoacan (64) and they found between 82,28 to 95,39 % of polymorphism allowing to form two large groups of diversity, without duplicates material, so it is suggested to keep the collection intact with breeding purposes.

To further explore the genetic relationship of the first three races identified Gross-German and Viruel (65) 25 microsatellites were used and 15 primers corresponding a marked SSRs expressed sequence (Expressed Tag Sequences, EST-SSRs) in a group of 42 accessions and the existence of three groups or subpopulations on the basis of botanical races confirmed: Mexican, Guatemalan-Mexican hybrids and Antillean (65), while Guatemalan-Mexican subpopulations were the only heterozygous individuals showed more than expected by the balance Hardy-Weinberg equilibrium (F=-0,14), pointing to its hybrid nature. Finally, results indicated that the Mexican and Guatemalan races are closer together than the Antilles ones.

25 microsatellites markers were developed to differentiate 35 avocado cultivars and two wild related species (23). The average heterozygosity was higher (60,7 %) and concluded the existence of ancient hybridization or botanical races was more originated recently than previously thought. Following this line of research 75 avocado accessions using 16 microsatellites previously developed in this species (43) were characterized, obtaining 156 different amplified fragments with an average of 9,75 alleles per locus and developed a dendrogram where analyzed genotypes classified within three groups, being the biggest differences in the origin of races.

Retrotransposons also have been used successfully in avocado investigations. In a more detailed analysis of a collection of 17 cultivars of avocado of Cuba collection, using the marked-based and inverse repeated sequences (Inverse Repeats Sequences Tagged, ISTR) polymorphism levels, capacity of discriminating and morphoagronomic character informativeness, AFLP, ISTR, SSR and isozymes (66) markers were compared. Results showed that the discrimination power (D) used for morphoagronomic characters was useful for the identification of genotypes. Only four variables were necessary to distinguish: fruit shape, harvest time, color and thickness of the fruit rind.

Thus, the used markers were powerful techniques for discrimination and varietal certification, but dominant markers were the most efficient. It also demonstrated that with a simple combination of AFLP or ISTR primers, all individuals were identified. On the other hand, higher levels of expected heterozygosity were detected with co-dominant markers, but the value determined with microsatellites exceeded twice or more, those obtained with isoenzymes and dominant markers. The morphological diversity index was a good estimator of the avocado accession diversity when variables high heritability, and in turn, comparable with the expected heterozygosity determined with isozymes and DNA-based markers were used.

The value of this index was similar to those obtained with ISTR and AFLP.

Efficiency ratings of the test (*Ai*) and marker (*MI*) had the same pattern of variation *D*, the average number of states banding patterns or test unit (*I*), the average number of unique patterns bands test unit (*Iu*) and the actual number of test patterns per unit (*P*) for all molecular markers, suggesting that both indices are likely to indicate the capacity of discrimination in the avocado.

The sequencing of the transcriptome of this crop, genetic mapping and partial sequencing of the genome represent a major step aimed at objective is to sequence the entire genome, which is expected to help in improving cultivars and production. In addition, the continued evolution and comparative studies in avocado flowers and fruits of different cultivars can be strengthened with the expression of genes, including comparisons with avocado relatives. This should provide important information concerning the genetic regulation of fruit development in angiosperms (67). This major progress research on avocado has just been informed by researchers who completed the avocado genome sequencing (68); in this respect, estimates suggest that the avocado genome contains about 34,000 genes, 29,345 of them with evidence of transcriptional activity. This result, coupled with the demonstrated broad genetic diversity of this crop can be used to identify genes involved in related to fruit quality and size of plants, among others, by association mapping based approach characters.

Improvement by mutation induction, *in vitro* selection and abiotic stress

The mutation induction techniques are improving alternative methods that have been widely used in major crops of economic interest, ornamental and eventually in perennial fruit crops (69); however, attempts to use avocado have been few and limited to modify the architecture of the plant, to influence the vegetative growth, flowering, fruit set and induce changes in fruiting (70).

Avocado breeding in Cuba today has radiosensitivity curves of two cultivars used as rootstocks, ‘Duke-7’ and ‘Hass’; values of median lethal dose, defined as the dose at which 50 % of full sprouts obtained was estimated at 28 to 27 grays (Gy) respectively for the previous two cultivars. Although results shown by some authors (71) suggested similar gamma radiation sensitivity in both genotypes, it was demonstrated that ‘Hass’ behaves as more sensitive to high doses of radiation. These results have immediate use in generating possible populations of mutants may be useful in enhancing the induction of mutations cultivars of avocado.

As abiotic stress it can be said that salinity affects almost all physiological and biochemical aspects of plants and significantly reduce yields. Salinity stress is one of the environmental stressors that induce changes in growth and morphology of plants and it is known that the avocado is the most sensitive to this stress fruit tree (72), which has limited sensitivity intensive production.

There have been different levels of salt tolerance among the three horticultural races of avocado, where members of the Mexican race are the most sensitive, Guatemalan patterns show an intermediate tolerance and Antillean ones are the most resistant, while several studies have penetrated in assessing changes in concentrations of ions such as magnesium, calcium, potassium, sodium and chlorine (73).

In this regard, the combination of *in vitro* methods and mutation induction can provide valuable results. Additionally, these protocols are relatively easy to use, cheap and become highly applicable methods.

Diseases that affect avocado

Avocado production worldwide is affected by various diseases which can economically limit production and reduce fruit quality (74). Among the most common is the *'sunblotch viroid'*, affecting fruit and leaves, diseased trees often show no symptoms but produce seeds are used as patterns to transmit the disease.

Anthracnose caused by *Colletotrichum gloeosporioides* affects stems, leaves, flowers and fruits, It is the disease that affects postharvest (75,76); nevertheless, it was also identified *C. acutatum* as causal agent of anthracnose in fruits, which it was isolated and identified by morphology and phylogenetic analysis with ITS gene (75). Another postharvest disease is caused by *Lasiodiplodia theobromae* and identified by morphology and gene sequencing of ITS (77).

Other species of fungi also cause damages, for example, species of *Botryosphaeria* (*Botryosphaeria parva, Botryosphaeria dothidea* and *Dothiorella aromatica* (= *F. luteum*) as well as other species, including reported for the first time, that cause cancer in branches and wilt in avocado trees in California (78).

Some authors report to *Neofusicoccum luteum* as a more aggressive pathogen than *Colletotrichum gloeosporioides* or *Phomopsis* sp. but with the same pathogenicity of *N. parvum* to cause wilt in avocado tips (78); it was further informed *N. parvum* causing the same damage in trees from Spain (79) and Mexico (80); as it informs *Neofusicoccum australe* (*Botryosphaeria australis*) causing the same disease in Chile (81).

Laurel wilt has been observed affecting avocado trees (82), it is caused by a new vascular pathogen known as *Raffaelea lauricola*. This disease was documented in Lauraceae on the coast of South Carolina, Georgia, Florida and northwest of Florida since 2003 (83).

Among other diseases is reported wilt by *Verticillium*, caused by the soil fungus *Verticillium dahliae* affects leaves, also diseases caused by species of *Erwinia* associated to anthracnose affecting fruit (84).

Among the bacterial diseases it was reported affectations caused by *Xylella fastidiosa* causing chlorotic mottling, marginal burn, leaf deformation, defoliation, and shortening of wilting branches (85). It has also been found *Xanthomonas axonopodis* causing small, black and angular leaf spot and it is considered the most important bacterial disease of avocado leaves (86).

It is also detected as affections caused by phytoplasma as Stolbur causing leaf curling, leaf, chlorosis venial, followed by an abnormal redness and irregular dwarf (86). While among viroids, viroid of sun stain (Avocado sunblotch Viroid, ASBVd) causing in the fruit yellow and deep veins, that become necrotic or reddish (87).

Nonetheless, the disease that causes more damage in the avocado is the rot caused by *Phytophthora* species. Several species of *Phytophthora* cause canker trunk and branches. *P. cinnamomi* Rands has been described as occasional cause of cankers in Australia, Brazil, California, Cameroon and South Africa.

Heveae P. Thompson, causing the black stripe of rubber in Malaysia, it was found as the cause of bleeding canker and trunk in Guatemalan avocado trees (88) and *P. citricola* Sawada, whose damage is increased in avocado plantings in California (89). It has also been detected *Phytophthora palmivora* Butler (Butler) (8, 9) and *Phytophthora nicotianae* (90) causing root rot in avocado.

The identification of causative agents of disease is particularly important today, climate change can affect the interaction of communities' pathogen with relationships as mutualism, competition, predators and food chain, while some species can adapt faster than other climate change and modify their habits (90). Therefore, the use of tools based on molecular markers allows the accurate identification of many species of pathogens mentioned above; this allows creating appropriate strategies for the handling and control of diseases that affect avocado.

CONCLUSIONS

- The combination of mutation induction and selection in open-pollinated populations as a way of creating variability, in conjunction with the implementation of other biotechnological advances like *in vitro* selection and application of genomic and proteomic approaches, from generation the complete genome sequence of this crop, the basis for the implementation of marker-assisted breeding in avocado.
- The development achieved by molecular markers in the rapid and accurate identification of pathogens that affect this crop can create effective strategies for the management and control of diseases caused in avocado.

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