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Genetic Diversity of Mexican Avocado in Nuevo Leon, Mexico

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Additional information is available at the end of the chapter

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1. Introduction

Mexico is considered the center of origin of avocado, and in the mountains of Sierra Madre Oriental in Nuevo Leon the remains of an avocado have been found, which are evidence of the place or origin of *Persea americana* var. *drymifolia*, or the Mexican avocado. Wild creoles have been found growing with native vegetation and have contrasting characteristics with cultivated varieties (improved creoles) and creoles (seedling) found in orchards. All this diversity represents a valuable source of genes and genetic combinations that can be used in *Persea* breeding programs. Therefore, we performed studies in the north and south areas of Nuevo Leon, Mexico, in order to determine the genetic diversity of this crop and implement actions for the characterization and conservation of this genetic resource, which has been disappearing due to changes in soil use, the introduction of improved varieties and the destruction of their habitat.

An analysis of 42 Mexican avocado samples based on the fruits traits as: fruit weight, fruit length, fruit diameter, seed weight, seed length, seed diameter, length seed cavity, the ratio of fruit length/fruit diameter and the ratio of seed weight/fruit weight, was carried out. The results of the analysis showed that the classification of genetic diversity with these fruit traits was not well represented. The characterization of the samples with AFLP molecular markers, allowed the differentiation of five genotypes in the cluster analysis and the separation of those with the same local name, thus demonstrating genetic differences within Mexican avocado

genotypes. When the molecular data were analysed with the morphological data, 11 genotypes were differentiated and the separation of genotypes with the same local name was possible.

At present, we are working with 10 genotypes to determine genomic regions that help us to identify and differentiate genotypes. The research results have identified about 37 improved creoles, 19 creoles (seedling) and nine wild creoles (seedling), all of them with different traits. To preserve this diversity, we are implementing a local germplasm bank together with Mexican avocado growers in order to start a breeding program.

2. Origin of Mexican avocado (*Persea americana* var. *drymifolia*)

Mexico has a wide variety of types of avocado—there are at least 20 different species related to the avocado [1]. The avocado is part of the Lauraceae family, considered by many botanists to be among the most primitive of the dicotyledonous plant; the centre of origin of the avocado is located in the highlands of central and east-central Mexico and the Guatemalan highlands [2]. In Mexico, three races are recognized: Mexican, Guatemalan and West Indian, which were classified as botanical varieties [3]; the possible centre of origin of these races is shown in Figure 1 [4].



Figure 1. Possible origin centres of the three avocado races [4].

Persea americana var. *drymifolia*, or the Mexican avocado, is the oldest variety used as food [2]; seeds of avocados were found in cave deposits (El Riego, Purrón and Coxcotlán) in the Tehuacan Valley, Puebla in Mexico, the oldest cotyledon from the Coxcotlán cave deposit is

dated to at least 7,000 B.C. [4]. In 1966, the geographical distribution of the *P. americana* var. *drymifolia* in Mexico was determined in the mountain forests of the eastern and south-eastern and in the Pacific zone (Figure 2) [5]. The findings of Mexican primitive avocados in the Sierra Madre Oriental of Nuevo Leon show that this area is part of the centre of origin of *Persea americana* var. *drymifolia* [6].

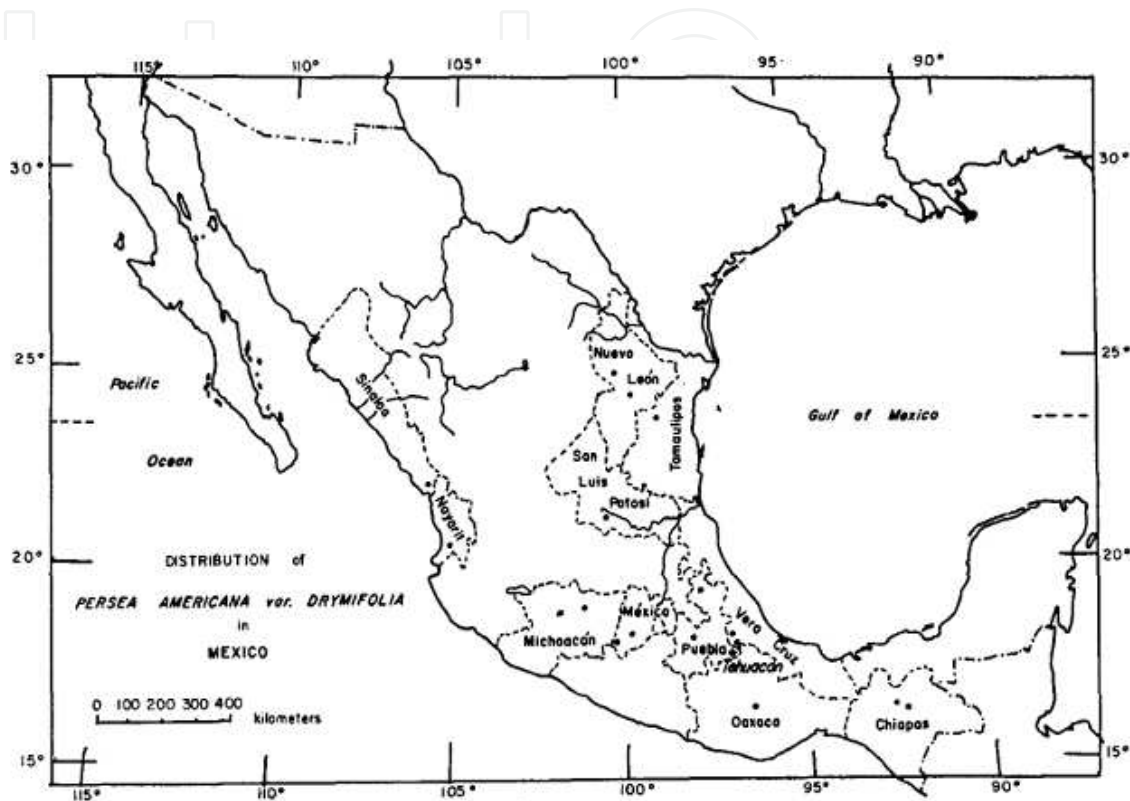


Figure 2. Geographical distribution of *Persea americana* var. *drymifolia* in Mexico [5].

The Mexican avocado is grown in different intensive agricultural systems and both in orchards and backyard gardens, these forms of production are important centres of experimentation, plant introduction, empirical improvement and shelters of unique genetic diversity that contain genes that have not been studied and could have potential use in breeding programmes. *Persea americana* var. *drymifolia* trees are characterized by their resistance to cold and high oil content, a very distinctive characteristic is its strongly aromatic leaves (anise scented) in almost all individuals; usually, they grow at altitudes greater than 2,000 m. The leaves are dark green with light green or reddish young shoots; the fruit has a thin, smooth and soft skin, the seed can be adhered or loose, the cotyledons are smooth or slightly rough, and it is common to find fibre in the flesh, although this is not found in most cultivated species [1].

Given that the avocado is an open-pollinated species, it contains great genetic variability with almost unlimited possibilities for utilization [7]; a wide diversity of germplasm allows the advancement of botanical and agronomic knowledge and the development of new cultivars [6]. The germplasm of Mexico has been the basis of breeding programmes in other countries, so it is necessary to focus on the exploration, classification and preservation of the germplasm

of the genus *Persea* to implement breeding programs. The generation of improvement varieties involves searching for genes that may exist in some cultivated varieties or wild plants; if these sources of genes do not exist, the possibility of the transferring of its characteristics will be lost forever.

In Nuevo Leon, Mexico, it is still possible to find different wild plants of the Mexican avocado (wild creoles) growing among natural vegetation, and their morphological traits are contrasting with cultivated varieties. Cultivated varieties consist of local selections of plants that have been grown for several years and which the growers have selected based on their production and quality (fruit size, mainly); these cultivars are grafted trees with genotypes of interest and are called 'improved creoles'. Native plants are those from avocado seeds that have not been grafted (seedlings) and are called 'creoles'. The genetic diversity of wild plants, improved creoles and creoles, represents a valuable source of genes and genetic combinations that can be used in *Persea* breeding programmes.

The main problems in identifying the genetic variability of Mexican avocados in Nuevo Leon, Mexico, are, among other factors, the diversity of local names that are used for the improved creoles, and the use of the same rootstock for grafting different varieties, resulting in a tree with branches belonging to different cultivars (Figure 3). Since 2005, in order to study this genetic diversity, we performed studies of the morphological and molecular characterization of Mexican avocado varieties.

3. First review of *Persea americana* var. *drymifolia* in Nuevo Leon, Mexico

In order to identify the genotypes of Mexican avocados (improved creoles, creoles and wild creoles), from June 2005 to November 2006, surveys were conducted in the municipalities of Dr. Gonzalez and Sabinas Hidalgo, located north of Nuevo Leon, Mexico, and in the municipalities of Aramberri, General Zaragoza and Rayones, located south of the state. A total of 30 improved creoles and 19 creoles and wild creoles, were identified. We collected five fruit of each of the trees that had fruit at the time of the visit (26 improved creoles and 12 creoles). The improved creoles collected in Dr. Gonzalez were Huevo de Toro Blanco, Larralde, Verde Perez and Perales, whereby the fruits of these cultivars mature in green color; and Anita, Floreño, Huevo de Toro, El Cuervo, Negro Santos Normal, Negro Santos Especial, Rodriguez and Rosita, the fruits of these improved creoles mature in black color (Figure 4), also were collected four creoles (Figure 5). In Aramberri, we collected the improved creoles: Mantequilla, Pagua, Platano Delgado, Maria Elena, Fuerte, Leonor, Pato and De la Peluqueria (Figure 6), as well as five creoles (Figure 5)-in the case of Mantequilla, the skin of the ripe fruit is yellow. In the General Zaragoza municipality, only two creoles were identified (Figure 5 and Figure 8). In Sabinas Hidalgo, improved creoles were identified, such as Anita, Floreño, Negro Santos and Pepe (Figure 7). In Rayones, the improved creoles were Verde Fuerte and Pagua (Figure 7), as well as two creoles (Figure 5). Four improved creoles had no fruits: Lampazos, Blanco and Sanjuanero of Dr. Gonzalez municipality, and Israel of Aramberri municipality.



Figure 3. Mexican avocado tree, A) rootstock, B) Platano Grueso improved creole.

Fourteen improved creoles were reported in Sabinas Hidalgo. In the study of evaluation of native avocados in the northern region of Nuevo Leon [8], six genotypes corresponded with those we found. In this same report, 10 improved creoles that were not documented by us were reported in Sabinas Hidalgo: Blanquito, Fosa, Cuervo, Pera, Sabroso, Chapeño, Pecoso, Pila, Salazareño, Especial. Negro Santos and Especial genotypes were reported in Bustamante [8]-this area was not considered in our study.

El Salto is a wild creole collected in the municipality of General Zaragoza and it was named this way because the trees were located in Parque Recreativo El Salto, located in this municipality. The fruit has a length of 2 cm and, according to researchers at Red Aguacate-SNICS-SAGARPA in Mexico, it could be a primitive Mexican avocado genotype-this fruit's characteristics are similar to those reported by Storey, who mentioned the discovery of a primitive form of avocado fruit which is about 2 cm length, in the pine and oak forests of Nuevo Leon, Mexico [4]; this vegetation type coincides with the area where we located this genotype (Figure 8).

The harvest of Mexican avocados in Nuevo Leon, Mexico, is performed during the period from June to December, although there are genotypes with fruit production throughout the year.

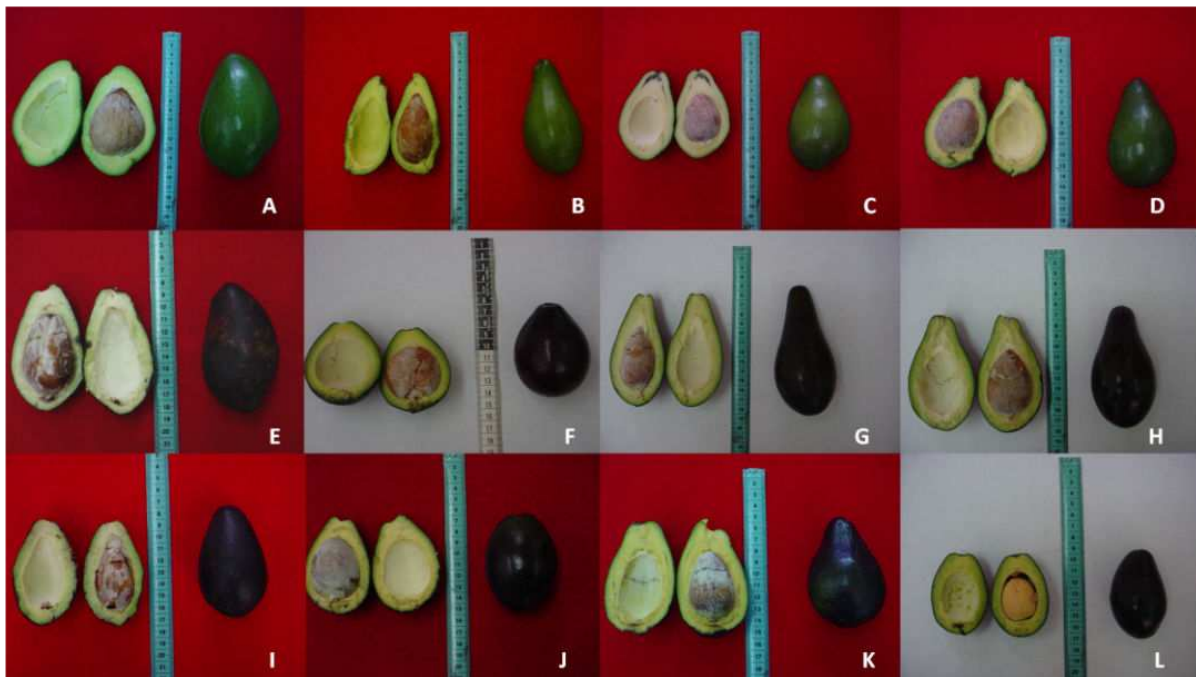


Figure 4. Mexican avocado improved creoles of Dr. Gonzalez, Nuevo Leon, Mexico. A) Huevo de Toro Blanco, B) Laralde, C) Verde Perez, D) Perales, E) Anita, F) Floreño, G) Huevo de Toro, H) El Cuervo, I) Negro Santos, J) Negro Santos Especial, K) Rodriguez, L) Rosita.

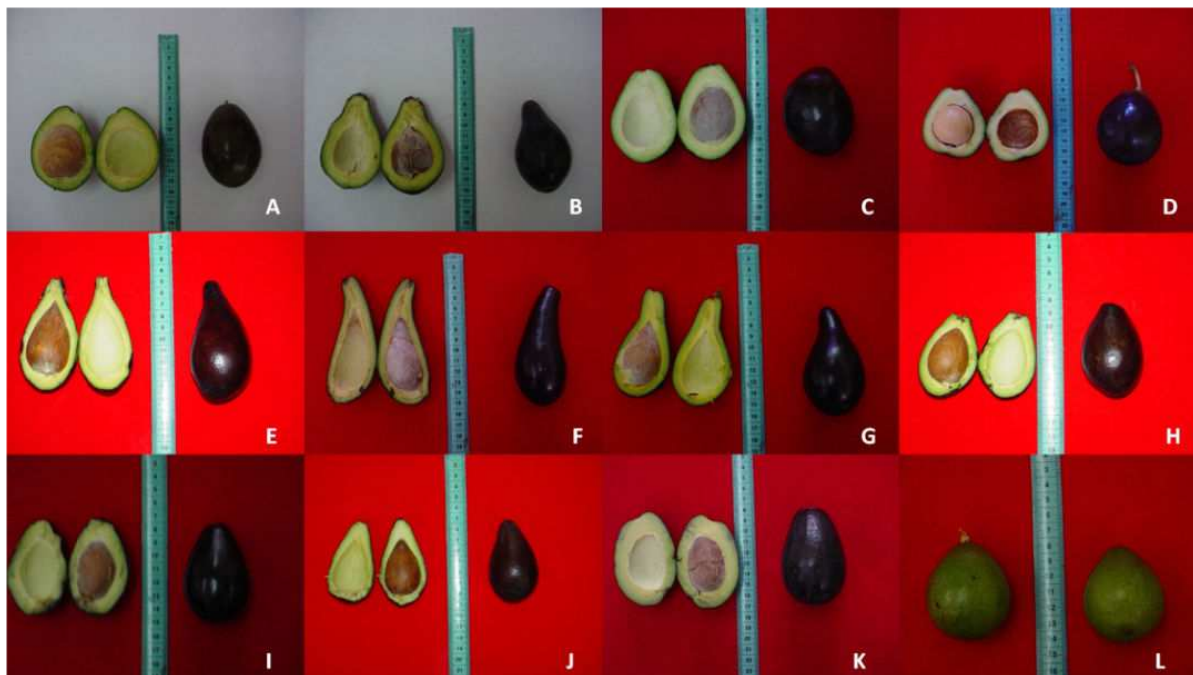


Figure 5. Creoles (seedlings) of *P. americana* var. *drymifolia*. A), B), C) and D) Dr. González; E), F), G), H) and I) Aramberry; J) General Zaragoza; K) Sabinas Hidalgo; L) Rayones.

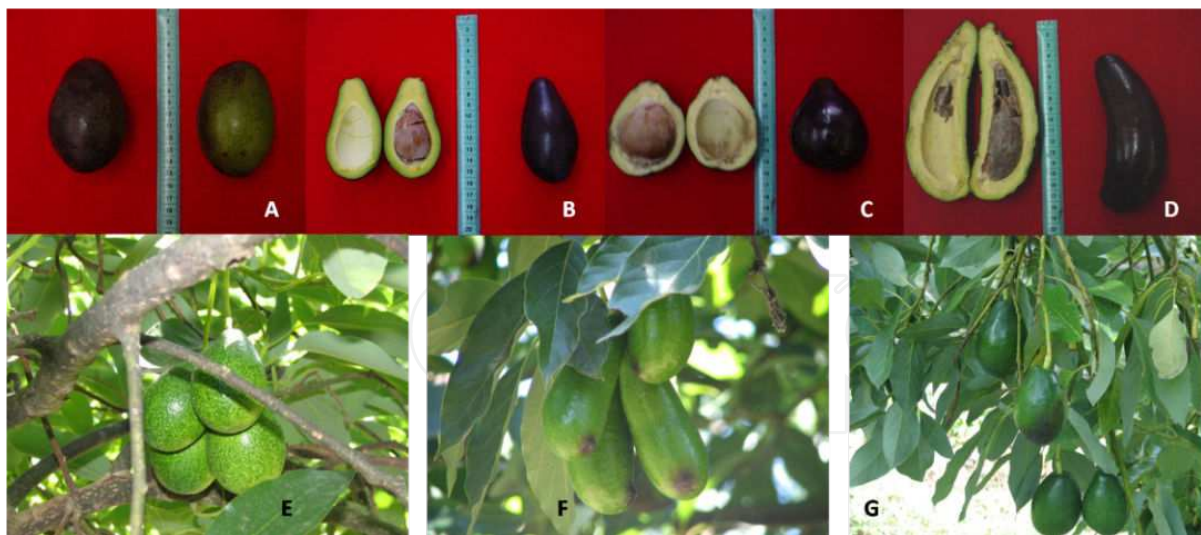


Figure 6. Mexican avocado improved creoles of Aramberri, Nuevo Leon, Mexico. A) Pagua, B) Maria Elena, C) Leonor, D) Pato, E) Mantequilla, F) Platano Delgado, G) De la Peluqueria.



Figure 7. Mexican avocado improved creoles of Sabinas Hidalgo and Rayones, Nuevo Leon, Mexico. A) Pepe (Sabinas Hidalgo); B) Verde Fuerte (Rayones).



Figure 8. Wild creole El Salto. A), B) fruits; C), D) trees.

4. Molecular and morphological characterization

In order to estimate the genetic diversity through morphological and molecular characteristics, in 2007 we worked with 42 trees of *P. americana* var. *drymifolia* of Aramberri and General Zaragoza, in Nuevo Leon, Mexico (Table 1).

Creole improved/creole	ID
Huevo de Paloma	P1
Campeon	P2, P6
Pagua	P3, P24
Platano Grueso	P4, P26
Platano Delgado	P7, P11, P12, P13, P14, P15, P21
Verde	P9
Maria Elena	P19, P33
Platano	P20, P27, P28, P29, P30, P38
Calabo	P23
Leonor	P25
Chino	P32
Creole	P5, P16, P18, P39, P44, P59, P60
Wild creole	P45, P46, P47, P48, P49, P50, P53, P56, P57

Table 1. Identification of Mexican avocados samples used to determine morphological and molecular diversity.

The collections of the samples were conducted both in gardens and in areas of wild vegetation. The identification of the samples was performed according to the local name provided by the growers—those samples of non-grafted trees were identified as creoles and samples collected in areas of wild vegetation were identified as wild creoles. Eleven improved creoles corresponding to 26 samples, seven creoles and nine wild creoles were identified (Table 1). The Platano Grueso improved creole is more commercially accepted by the characteristics of size and appearance of the fruit; this cultivar characteristically presents an early harvest starting cycle, in mid-June, which limits its production from concentrating in a relatively short period of time [9]. The tendency of some producers is the replacement of treetops to grafts of this cultivar. Some other cultivars are preserved due to its adaptive characteristics, palatability and harvest out of season, allowing marketing for the best price. The study of the evaluation of creole avocados in the southern region of the state of Nuevo Leon reported 23 improved varieties [9], of which 13 coincide with those that have been identified in our studies.

Due to the lack of definition in the branching pattern of trees by grafting different varieties in the same plant (Figure 3), it was decided only to perform a morphological evaluation of the fruits. For the molecular analysis of the samples and the morphological analysis of their

fruits, 10 leaves and five ripe fruits per tree were collected. The characterization of the fruits was performed according to the morphological descriptors for avocado fruit given by the International Plant Genetic Resources Institute [10]. The characteristics were: fruit weight (g), fruit length (cm), fruit diameter (cm), seed weight (g), seed length (cm), seed diameter (cm), seed cavity length (cm), seed cavity diameter (cm), ratio of the fruit length/fruit diameter, and ratio of the seed weight/fruit weight. In order to determine the variables with greater weight on the morphological characterization and the behaviour of each variable, the mean, standard deviation and coefficient of variation were estimated. The data analysis was performed by principal components analysis and based on this; a cluster analysis was performed using the UPGMA method (un-weighted pair groups method with arithmetic averages) with the Gower distance (1-S) [11]. Statistical analyses were performed using the software InfoStat/Pv.2006p.3 [12].

In the fruit's morphological characterization, the traits with higher coefficients of variation were: seed weight (39.42%) and fruit weight (38.42%); the seed cavity length and fruit length showed coefficients of 24.82% and 20.34%, respectively. Values greater than 50% suggest that the characteristics have the highest variability within a species, while values below 20% indicate little variability in the species [11], so these characteristics were considered as classificatory.

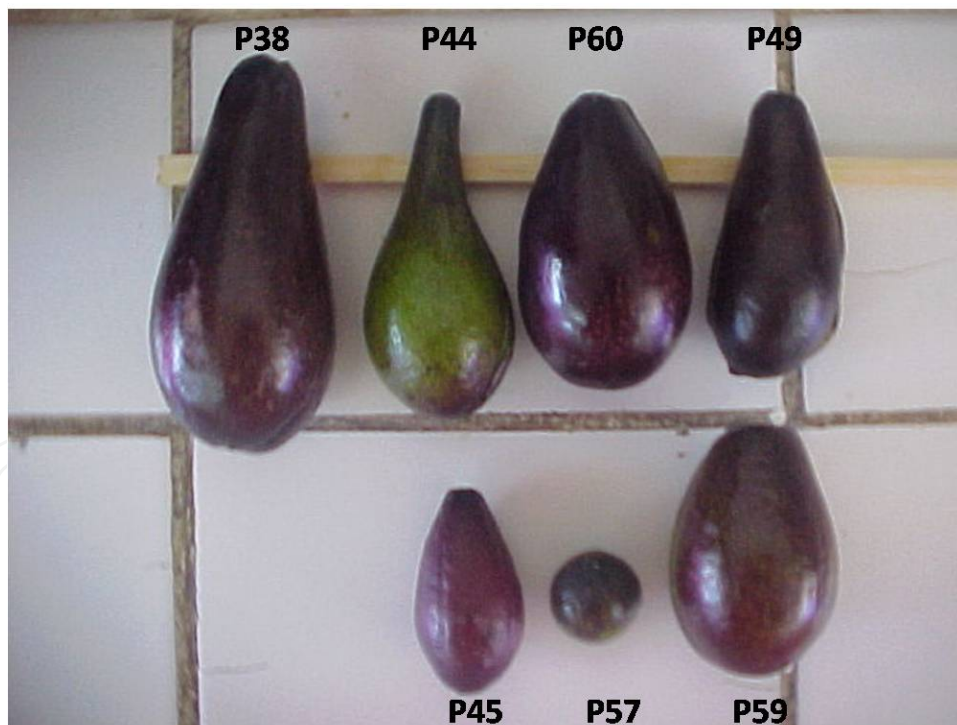


Figure 9. Comparison of the sizes and shapes of fruits of *Persea americana* Mill. var. *drymifolia* from Aramberri and General Zaragoza, Nuevo Leon, Mexico [13]. The sample identification is according to Table 1.

As to the seed weight and fruit weight, the highest averages were 69.76 g and 251.40 g, respectively, corresponding to P26 (Platano Grueso); the lowest values for this variable were

7.08 g and 10.53 g, respectively, corresponding to P57 (El Salto). In the case of seed cavity length, the highest average corresponded to P21 (Platano Delgado) with 11.46 cm, the lowest average was 2.60 cm and corresponded to P57 (El Salto). For fruit length, the highest average value was 12.76 cm and corresponded to P4 (Platano Grueso), the lowest value corresponded to El Salto and was 2.88 cm; the fruit traits of this wild creole contrast sharply with the others samples collected (Figure 9).

The relationship between the traits evaluated and the similarity between the samples was performed by principal component (PC) analysis with standardized data. The PC1 and PC2 represented 75.8% of the total variance, values greater than 60-70% explaining a reasonable percentage of the total variability of the samples [14]. The traits with the greatest influence in PC1 (59.7%) were fruit weight, seed weight and fruit length, while in PC2 (16.1%) they were seed diameter and seed cavity length. The cophenetic correlation was 0.984; PC analysis results coincided with the results of analysis of variation coefficients; of the samples organized in six clusters, P57 had the highest distance, and P45 and P48 (wild creoles) formed a cluster. P53, P56 and P11 formed another cluster-the first two corresponding to wild creoles while P11 corresponds to Platano Delgado. The samples P25 (Leonor) and P21 (Platano Delgado) were separated from the rest of the samples, while the other samples were grouped into a cluster.

The dendrogram was performed with the following variables: seed weight, fruit weight, seed cavity length and fruit length; with the addition of the ratio of fruit length/fruit diameter and the ratio of seed weight/fruit weight, the cophenetic correlation increased to 0.888. Four clusters were formed at a distance of 0.23; two clusters coincide with those formed by the principal components analysis. Cluster I was formed with P57, cluster II with P21. Cluster IV was formed by five samples identified as wild creoles (P45, P47, P48, P53 and P56), sample P16 (creole), P1 (Huevo de Paloma), P25 (Leonor) and P32 (Chino). Cluster III was formed with the remaining samples (Figure 10).

Although the morphological differences between fruits are evident, only the sample P57 was identified. Linkage distances within the dendrogram reflected the differences and inconsistencies between presumably identical genotypes; we expected that the samples identified with the same name would be grouped together, which did not happen. These results agree with those reported by [15], who assert that fruit size is a trait that does not help in the differentiation of wild and cultivated avocado plants, because trees produce fruits of different sizes. Avocado groups are not well represented when the morphological characteristics of fruits are used [16].

Traditional plant identification is performed by phenotypic characterization-this is slow and limited, and the expression of the quantitative characteristics is subject to environmental influences. Molecular markers allow the identification, classification and use of genetic diversity present in the genomes of plants; differences or similarities at the DNA level in the individuals are observed directly. The AFLP (amplified fragment length polymorphism) is a highly efficient molecular marker [17]-in avocados they have been used to study the genetic diversity of germplasm [16, 18, 19].

The molecular characterization of the samples was performed using AFLP molecular markers; AFLP generation was performed using the IRDye Fluorescent AFLP Kit for Large Plant

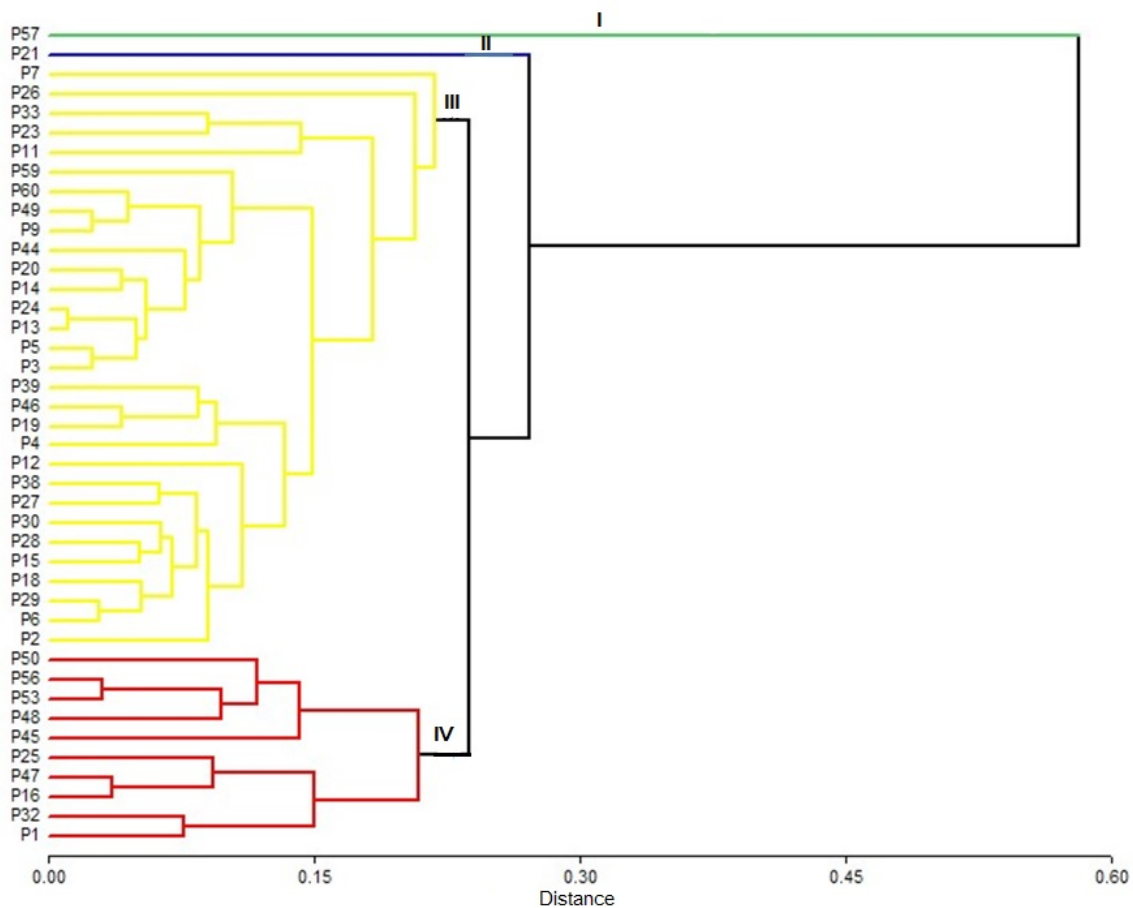


Figure 10. Dendrogram of the fruit's morphological characteristics of 42 *Persea americana* Mill. var. *drymifolia*.

Genome Analysis (LI-COR® Biosciences); for the construction of the binary data matrix, it was assumed that bands with the same molecular weight are identical, assigning the number 1 to the presence of bands and the number 0 to the absence of bands [13]. Similarity indices were determined by the Gower coefficient (1-5). A cluster analysis of the molecular data was performed using the UPGMA method using standardized variables through the Info/Gen 2006p.1v software [20]; the cophenetic correlation coefficient was calculated.

With the cluster analysis of 683 AFLP markers (Figure 11), two clusters were defined (distance 0.14). The samples P9 (Verde), P44 and P39 (Creoles), P38 and P29 (Platano), P4 (Platano Grueso), P3 (Pagua) and P23 (Calabo), were not clustered. The separation of these samples shows that they are different genotypes, but in those samples that were not clustered with samples with the same local name, the question is whether there is genetic variation among the improved creoles. Cluster "I" was composed of samples P48, P46 and P45, identified as wild creoles, while cluster "II" was composed of 32 samples. By decreasing the distance to 0.09 in the dendrogram, samples identified as Platano (P30, P15, P13, P12) and Platano Delgado (P11), were grouped; while P56, P50 (both wild creoles) and P32 (Chino) samples were separated. The clustering at very short distances (0.015) is between P6 (Campeon), P7 (Platano Delgado) and P1 (Huevo de Paloma), and between P53 (wild creole) and P33 (Maria Elena),

which shows that they are closely related. The clustering at distances greater than samples identified with the same name calls into question the genetic identity of improved creoles.

To perform the comparative analysis between cluster groups formed in the morphological and molecular analysis, a mixed data matrix was constructed with morphological data and AFLP binary data; a cluster analysis was performed by UPGMA with the Gower distance (1 S) through the software Info/Gen 2006p.1v [20]. As in previous cases, the cophenetic correlation coefficient was calculated.

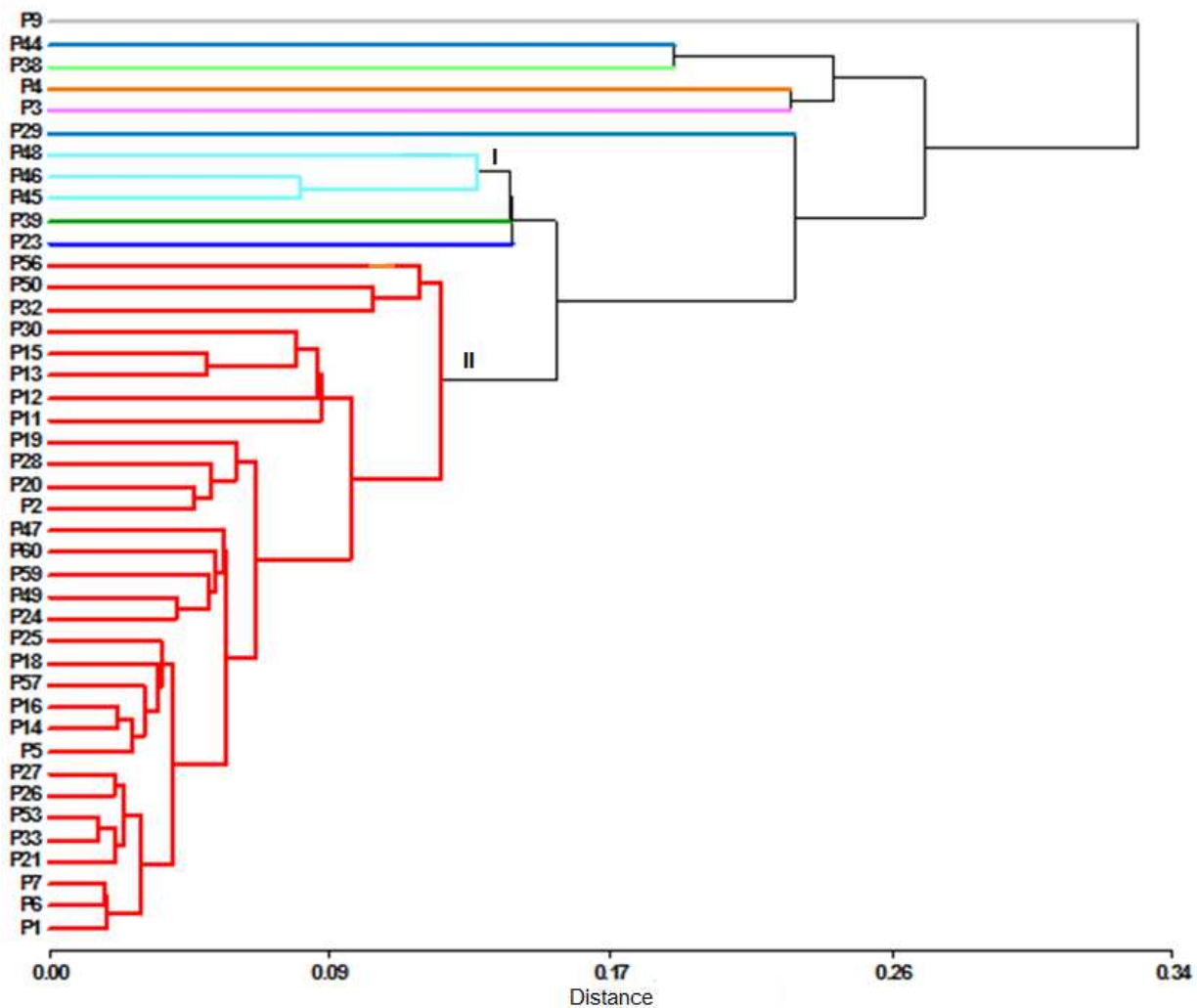


Figure 11. Dendrogram of molecular data (AFLP markers) of 42 *Persea americana* Mill. var. *drymifolia*.

The cluster analysis of the molecular and morphological data showed one cluster (I) formed by 31 samples (distance of 0.13), while 11 samples showed no clustering pattern: P48 (wild creole), P57 (wild creole), P45 (wild creole), P9 (Verde), P44 (creole), P4 (Platano Grueso), P3 (Pagua), P56 (creole), P50 (wild creole), P38 (creole) and P29 (Platano) (Figure 12). The separation of P9, P44, P3 and P4 coincided with that obtained in the molecular data analysis. The separation of P48, P57, P45, P56 and P50 identified as wild creole is a difference between

the analysis of mixed data and analysis of molecular data. With increasing distance in the dendrogram, the P57 and P45 (wild creoles) samples were clustered; these samples are characterized by the lowest values of fruit length and seed length. When the cluster distance was increased in the dendrogram, the influence of the morphological variables in the formation of the groups was evident, so the group consisting of P48, P57 and P45 was characterized by the lowest seed weight values. When analysing in detail the scheme of clustering, we observed that P26 and P27 were grouped at a shorter distance-an important feature of these samples is that they showed the highest fruit weight and that they correspond to the improved creoles Platano Grueso and Platano, respectively. As such, we can conclude that they present similarities.

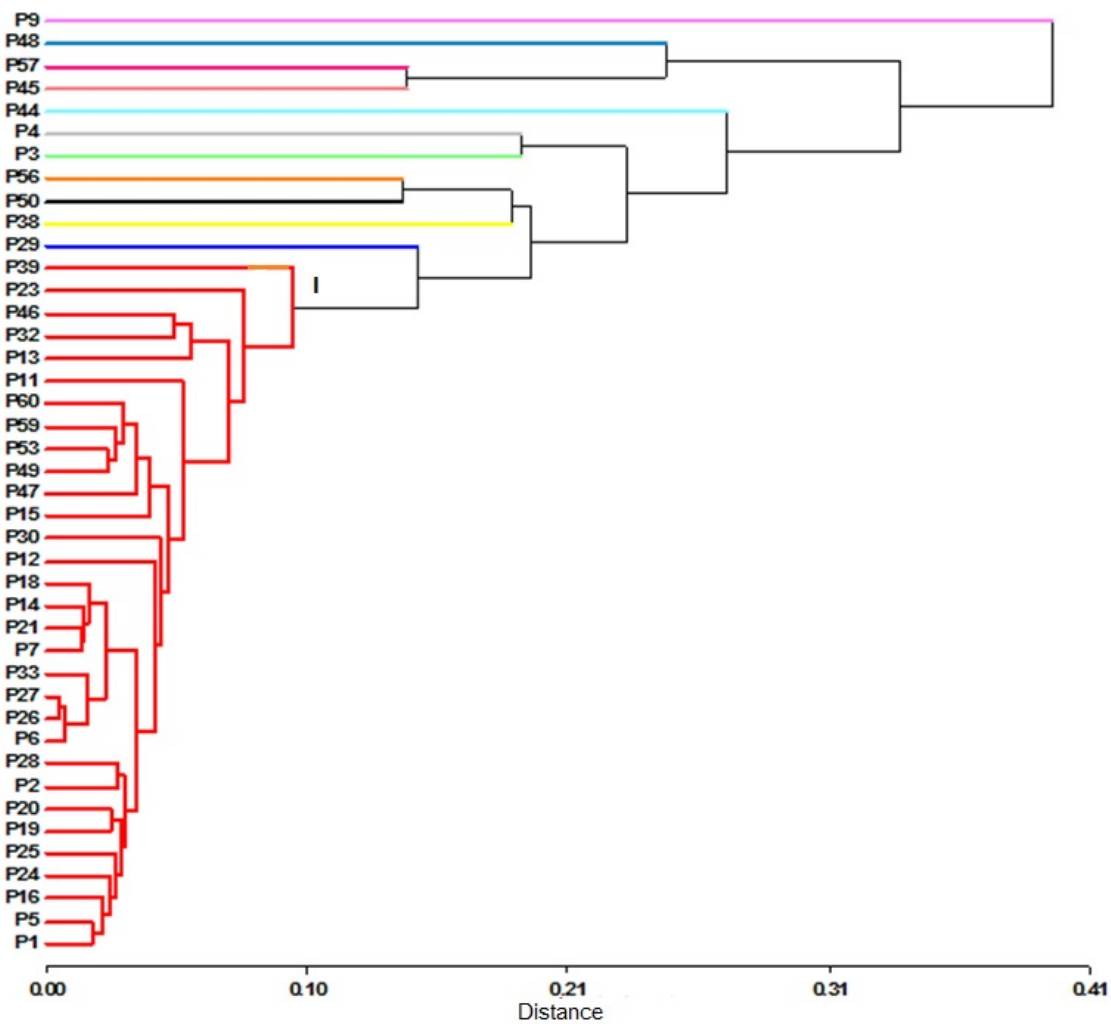


Figure 12. Dendrogram of morphological characteristics and molecular data (AFLP markers) of 42 *Persea americana* Mill. var. *drymifolia*.

The results of this study demonstrated that AFLP molecular markers allow the estimation of the genetic diversity of the Mexican race of *Persea americana* Mill. when molecular data are

combined with morphological data. When only molecular data generated by AFLP are used, the differentiation between genotypes was ambiguous; we demonstrated the effectiveness and facility of AFLP used to characterize avocado accessions based on the race origin [19]; however, there is no reference about its usefulness in differentiating between varieties.

5. *In vitro* culture callus

We have worked with *in vitro* culture techniques to conserve the germplasm of different avocado genotypes. The selection of the explant to be used in the conservation of plant tissue depends on the resources and the goal of the project; in avocados, different explants have been used to obtain different types of morphogenesis [21]. The avocado morphogenetic capacity under *in vitro* conditions is linked to the use of materials with juvenile characteristics [22]. The avocado cotyledon callus was kept for over 15 years without apparent differentiation conditions [23]-these tissues remained as callus divisions through a series of small segments and were transferred to fresh media at intervals of one-to-three months. The callus tissue is essential for obtaining somatic embryos by indirect embryogenesis [24, 25]. Avocado somatic embryos were regenerated from calluses [26-29]. We used the developed young leaves of a Mexican avocado tree (six years old) as explants to establish an *in vitro* culture callus.

The avocado leaves were washed with a commercial detergent and running water for 30 minutes. Subsequently, petiole were cut and the leaves were placed in an antioxidant solution, 400 mg L⁻¹ of ascorbic acid, 150 mg L⁻¹ of citric acid and 30 g L⁻¹ of sucrose with 1 g L⁻¹ systemic fungicide metalaxyl-M (Ridomil, Syngenta Agro), for 2 min at a vacuum pressure of -20 bar; they were placed in alcohol 70% v / v for 30 s followed by three rinses with sterile water, then placed in a solution of 2.4% NaOCl with two drops of Tween 20 for each 100 ml for 15 min, followed by three rinses with sterile distilled water; the leaves were kept in sterile antioxidant solution above without systemic fungicide, until their establishment *in vitro*. Explants of 1 cm² were obtained and placed in glass vials with 20 ml of DCR medium [30] pH 7.5, supplemented with vitamins and growth regulators, 200 mg L⁻¹ of inositol, 50 mg L⁻¹ of glutamine, 500 mg L⁻¹ of casein hydrolyzate, 0.3 mg L⁻¹ of 6-benzylaminopurine, 0.01 mg L⁻¹ of naphthaleneacetic acid, 0.1 mg L⁻¹ of thiamine, 0.5 mg L⁻¹ of nicotinic acid, 0.5 mg L⁻¹ of pyridoxine, 2 mg L⁻¹ of glycine, 10 mg L⁻¹ of ascorbic acid, 10 mg L⁻¹ of citric acid, 1.5 mg L⁻¹ of 2,4-D, 0.3 mg L⁻¹ of kinetin, 20 g L⁻¹ of sucrose and 4.5 g L⁻¹ of Phytigel. The glass vials with the explants were placed in a controlled temperature of 20 ° C ± 2 ° C in complete darkness for callus formation. Callus formation from the leaves was completed out in 30 days (Figure 13).

Using the same culture medium, two subcultures of each callus were performed with a period of 30 to 45 days between them. AFLP markers were generated and 341 polymorphic bands were used to calculate the polymorphic information content (PIC) and the genetic distance to measure genetic stability of the avocado callus. We analysed molecularly 94 samples, the mother plant, 31 calluses formed from the mother plant explants, 31 calluses from the first subculture, and 31 calluses from the second subculture-the results of this research are in press.

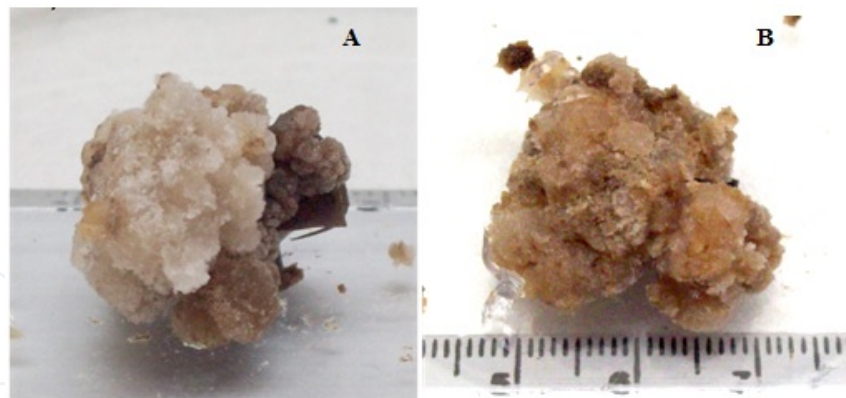


Figure 13. A) Avocado callus from leaves, B) avocado callus from the second subculture.

6. Present investigations

Actually, we are working with the fruit gene expression of genotypes with contrasting characteristics (Figure 14). Using the technique of differential display [31], we obtained differential fragments of the genome of the fruit (Figure 15); these amplified fragments were sequenced in order to identify regions of the genome to help us in the identification and differentiation of genotypes (improved creoles, creoles and wild creoles). We are analysing the sequences obtained with data from the NBCI and hope that these results can be used in the future in the genetic characterization of genotypes as well as in avocado breeding programs.



Figure 14. Fruits of *Persea americana* Mill. var. *drymifolia* of Aramberri and General Zaragoza, Nuevo Leon, Mexico. 1) Pato, 2) Amarillo, 3) Cuerno, 4) El Salto (wild creole), 5) Huevo de Paloma, 6) Mantequilla, 7) Criollo Bola (creole), 8) Pagua, 9) Platano, 10) and 14) Criollos (creoles), 11) Campeon, 12) Platano Delgado, 13) Platano Gueso, 15) Criollo Todo el Año (creole), 16) Maria Elena, 17) Leonor, 18) Salvador.

In order to preserve all the genetic variability of the Mexican avocado that has been detected in Nuevo Leon, Mexico, a local germplasm bank is being implemented together with growers where they can safeguard copies of improved creoles, creoles and wild creoles. The intention is to have plant material for them and to start in a short period with the creation of a breeding program for the Mexican avocado.

7. Conclusion

The genetic diversity of *Persea americana* var. *drymifolia*, found in Nuevo Leon, Mexico, is broad and can be used for incorporation into breeding programme cultivars, as well as for use as rootstocks and inter-stocks; wild varieties are a genetic patrimony that can provide innovative sources of advantageous use for producers. Identifying the characteristics of agronomic interest (genes) boosts the Mexican avocado crop through its alternate use (processed fruit, oil extraction, medicinal use, use as a condiment, animal feed, etc.). The classification and conservation of plant germplasm should be seen as an activity to preserve this heritage of diversity-it requires a major effort to preserve the genotypes of cultivated plants, but especially wild and native plants that are threatened with the destruction of their natural habitat and being replaced by improved varieties.

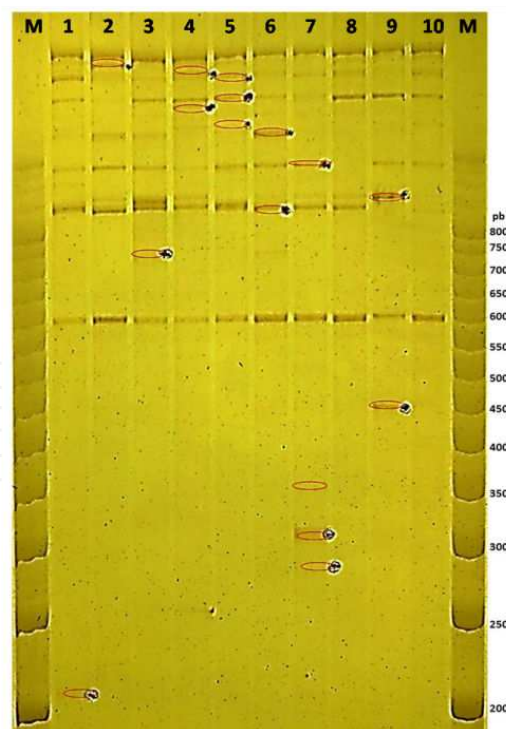


Figure 15. Differential fragments of *Persea americana* var. *drymifolia* fruits. Lanes: M, molecular weight marker, 1) Pato, 2) Cuerno, 3) El Salto, 4) Mantequilla, 5) Criollo Bola, 6) Criollo 2, 7) Platano Grueso, 8) Criollo Todo el Año, 9) María Elena, 10) Leonor. Polyacrylamide gel 6%.

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