

Genetic and chemical characterization of avocado commercial cultivars avocado of Risaralda Colombia

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Abstract- This research aimed at performing the molecular characterization of commercial Papelillo avocado (*Persea americana* cv Lorena) cultivars from the municipality of Marsella (Risaralda, Colombia), as well as the physicochemical analysis and antioxidant activity assessment of the pulp and seed. An evaluation of 50 individuals among commercial varieties and possible patterns was performed using 17 microsatellite markers. Proximate analysis of the pulp was performed, and the fatty acid profile of oils, the antioxidant activity by the DPPH and FRAP methods, and the total phenolic content were evaluated. From the cluster analysis, Dice index, and Principal Coordinates Analysis, it became evident that all the individuals showed a tendency to group by populations. In addition, the pulp revealed high fiber contents (4.96–20.64%) and moisture (80.75–82.96%); however, it showed low oil content (5.97–6.56%). The fatty acid found in the highest proportion in seed oil is linoleic acid and that in pulp oil is oleic acid. The antioxidant activity by the DPPH method for seed oil (87.87 to 91.04%) presented a greater inhibition concerning to the pulp oil (20.34% and 24.43%), this same trend was observed by the FRAP method. Concerning the content of total phenols, the seed oil (31.94–76.30 mg GAE g⁻¹) has a higher value than the pulp (30.18–54.30 mg GAE g⁻¹). The set of samples was characterized as a significant source of genetic variability; thanks to the excellent alternatives they provide as rootstocks for commercial varieties such as the 'Lorena' cultivars. The chemical classification carried out in this study is of great importance, due to the lack of information about the oil of the 'Papelillo' avocado cultivated in different regions of Colombia.

Index terms: Antioxidants, DNA, fatty acids, microsatellites, *Persea americana* Mill., total phenols

Caracterização genética, físico-química e avaliação da atividade antioxidante de cultivares comerciais de abacate de Risaralda, Colômbia

Resumo - Esta pesquisa teve como objetivo realizar a caracterização molecular de cultivares comerciais de abacate Papelillo (*Persea americana* cv. Lorena) do município de Marsella (Risaralda, Colômbia); bem como a caracterização físico-química e avaliação da atividade antioxidante da polpa e da semente. Realizou-se uma avaliação de 50 indivíduos entre variedades comerciais e possíveis padrões utilizando 17 marcadores microsatélites. Analisou-se a composição centesimal da polpa e avaliou-se o perfil de ácidos graxos dos óleos, o conteúdo fenólico total e a atividade antioxidante pelos métodos DPPH e FRAP. A partir da análise de cluster, índice de dados e análise de coordenadas principais, tornou-se evidente que todos os indivíduos mostraram uma tendência de agrupar por populações. Além disso, a polpa revelou altos teores de fibras (4.96 a 20.64%) e umidade (80.75 a 82.96%); no entanto, mostrou baixo teor de óleo (5.97-6.56%). O ácido graxo encontrado na maior proporção no óleo de semente é o ácido linoléico e o do óleo de polpa é o ácido oleico. A atividade antioxidante pelo método DPPH que o óleo de semente (87.87 a 91.04%) apresentou maior inibição em relação ao óleo de polpa (20.34% e 24.43%); essa mesma tendência foi observada pelo método FRAP. Com relação ao conteúdo de fenóis totais, o óleo de semente (31.94 a 76.30 mg de GAE g⁻¹) tem um valor mais alto que a polpa (30.18 a 54.30 mg de GAE g⁻¹). O conjunto de amostras foi caracterizado como uma fonte significativa de variabilidade genética, graças às excelentes alternativas que eles fornecem como porta-enxertos para variedades comerciais, como as cultivares 'Lorena'. A classificação química realizada neste estudo é de grande importância, devido à falta de informações sobre o óleo do abacate 'Papelillo' cultivado em diferentes regiões da Colômbia.

Termos de indexação: Ácidos graxos, antioxidantes, DNA, fenóis totais, microsatélites, *Persea americana* Mill.

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Introduction

By the time of this study, the avocado (*Persea americana* Mill) was being grown in five continents, particularly in tropical and subtropical countries, although South America cultivates most of these crops (FAO, 2012). Three horticultural races have been considered in this study for the avocado, viz., the Mexican (*P. americana* var. *drymifolia*), Guatemalan (*P. americana* var. *guatemalensis*) and Antillean or West Indian (*P. americana* var. *americana*) (PÉREZ et al., 2015). The avocado has great hybridization potential because it is highly cross-pollinated. Hybrids have been obtained between the Mexican and Guatemalan races and between this and the Antillana race, with varieties produced such as ‘Stand 8’, ‘Choquette’, ‘Collinred’, ‘Fuerte’, ‘Gwen’, ‘Hass’, ‘Lorena’, ‘Reed’, ‘Trapica’ and ‘Trinidad’, showing a better adaptation and a higher yield of these commercial fruits (BERNAL et al., 2008).

The production has arisen from different sources of native trees, and the selected cultivars were reproduced asexually (CAÑAS et al., 2015). The success of a commercial avocado crop depends upon the proper selection of the varieties to be planted, as well as the grafting methods to be applied, to ensure the other advantages such as continuity in production, the extension of the harvest periods, greater production yield, reduced risk of pest- and disease-related issues, improved crop development and higher fruit quality (CAÑAS et al., 2015).

Avocados are valuable to obtain food products and pharmaceuticals. The formation of secondary metabolites and nutritional composition of avocados are highly variable and are influenced by factors such as climate, soil, temperature, humidity, amount of rainfall during fruit development, and genotypic differences across varieties (HOWARD et al. 2003, THOMAS et al. 2005). Due to the high amount of nutrients in avocados, their consumption has increased worldwide, resulting in massive harvests (CEBALLOS; MONTOYA, 2013).

In Colombia, while avocados are produced from low altitudes up to 2,200 m above sea level, mainly for the local market, Colombia has immense potential for export, both in the form of fresh and processed fruit, depending upon the traits of the varieties cultivated and the agroclimatic conditions in the producing region in which they are grown (BERNAL et al., 2014). The avocado cultivars which are distributed throughout Colombia correspond to different races and hybrids, with roughly 49% local varieties avocados of the total planted area, 26% correspond to the ‘Hass’ variety and the remaining 25% to the ‘Papelillo’ avocado varieties, such as ‘Lorena’, ‘Santana’ and others, which mostly belong to the Antillana race. The Tolima department and the Coffee Region from Colombia (Risaralda, Quindío, and Caldas departments)

supply 65% of the ‘Lorena’ variety of avocado fruit (BERNAL et al., 2014).

Among the commercially outstanding varieties of *Persea americana* Mill, is the ‘Lorena’ variety, known as “Papelillo” for having a low shell thickness. The ‘Lorena’ variety originated in the year 1957 in Palmira (Valle del Cauca, Colombia). It can be grown at low and medium heights, to 1500 meters above sea level. The fruits are large (400–600 g in weight), elongated, slightly oblique with lustrous peel and a long peduncle, and the seed is medium in size, ovoid, and symmetrical, with medium adherence to the pulp (ROMERO, 2012).

The Marsella municipality from the Risaralda department, Colombia, has a high concentration of agricultural enterprise and a variety of climatic ranges suitable to avocado cultivation, ‘Lorena’ cultivars especially. This fruit is well known for its quality, profitability, and largely contributes to the economic occupation of this sector. However, in the literature, the usage of the term ‘Papelillo’ is often confused. This is the correct name for the ‘Lorena’ cultivars, however, other Antillean and hybrid breeds can also be referred to as Papelillos, as well as those which do not belong to this race, such as the ‘Trapp’ and ‘Santana’ cultivars (BERNAL et al., 2014). Besides, Colombia experiences several obstacles, the lack of certification regarding their genetic identity the main one among them (CAÑAS et al., 2015). The use of tools like molecular pointers facilitates the identification, classification, and analysis of the genetic variability of the plant material (GUTIÉRREZ-DÍEZ et al., 2009).

Whereas avocado, depending on the variety, shows differences in chemical composition (CASTAÑEDA et al., 2015) and bearing in mind that the reported information on the ‘Lorena’ variety is scarce, the present study was conducted. In this work, the objective was to use the microsatellite markers and study the genetic distance of the avocados versus the ‘Lorena’ cultivars to determine the variability and genetic diversity in the avocado crops.

The ‘Lorena’ variety was grown and the genetic diversity of the cultivated ‘Patterns’ in the same locality was estimated, which could find use as rootstocks. Also, the pulp and seed of *Persea americana* Mill ‘Lorena’ variety were characterized, thereby increasing on this natural resource, contributing to its comprehensive use, and generating added value and new market opportunities.

Materials and methods

The study was carried out in Marsella municipality of Risaralda department, from 2015 to 2016. Three zones were selected according to the geographical distribution of the cultivated area in the municipality, and a sampling total of 19 avocado farms that produce the ‘Papelillo’ variety were selected, which represents the total coverage of the

municipality georeferencing of 04° 57' 34.1" N and 75° 44' 45.0" W and an altitude between 1,331 and 1,555 ± 5 meters above sea level.

Regarding the genetic analysis, the selected avocado trees included the 'Lorena' cultivars, species from the cultivars 'Santana' and 'Trapp' of the Antillean race and samples from the plant 'Patterns' were selected (a variety of tree that is often used as rootstocks for avocado grafts), preferably with good morpho-agronomic quality, characteristics and a high degree of acceptance by the farmer. From each of the plant materials, young and healthy leaves were collected from the trees in their production stages; then, they were stored in pre-labeled plastic bags for later analysis. Altogether 50 leaf tissue samples were collected, where 38 samples apparently corresponded to the 'Lorena' cultivars (Population "Lorena"), possibly, seven to 'Patterns' cultivars (Population "Patterns"), four from the 'Santana' cultivars and finally, one sample corresponding to 'Trapp' cultivars.

For the chemical analysis, fruits from 'Lorena' avocado cultivars were used. Likewise, a random sampling was carried out in a representative area of the municipality's crop, which covered 19 farms by selecting 3 sampling zones.

Microsatellite markers were used for the genetic analysis (SSR analysis). The DNA extraction was performed by using the commercial kit DNeasy plant mini kit (QIAGEN). The DNA visualization was carried out on 1% agarose gels and HyperLadder IV marker. The amplification reactions were done in a final volume of 12.5 µL with 300 nM of each of the primers, 0.25 mM of dNTP's, 1X of the reaction buffer, 1 U of Taq polymerase, 1.5 mM of MgCl₂ and 10 ng µL⁻¹ of DNA.

The SSRs developed by ASHWORTH *et al.*, (2004) (AV microsatellites) were selected. For this, a first amplification was performed, and finally, 17 SSRs with polymorphic amplification patterns were selected (Table 1). The conditions of the DNA amplification were those suggested by ASHWORTH *et al.*, (2004). The amplification profile was as follows: 95 °C for two minutes of initial denaturation, thirty cycles of 95 °C for one minute, 54 °C at 68 °C for one minute (depending on each primer) and 72 °C for one minute and a final extension of 72 °C for ten minutes. The amplified products were separated via electrophoresis in 6% polyacrylamide denaturing gels. The gels were stained with silver nitrate, using the protocol of BENBOUZA *et al.*, (2016).

A thorough analysis of the pulp was conducted, in which total ash, moisture, protein, crude fiber, and fat were determined according to the AOAC standard (1995). The pulp was dried using a microwave, varying its power, while the seed was dried using a stove at 60 °C. Subsequently, oil was extracted from the pulp and seeds by Soxhlet extraction using n-hexane as a solvent for 6 and 24 h, respectively (MORENO *et al.* 2003, GALVÃO

et al. 2014). The solvent was evaporated under reduced pressure at 40 °C, and the oils obtained were stored at 4 °C until further analysis.

The physical characterization of the oil was performed by determining the density and refractive index at 25 °C and 40 °C according to the IUPAC standard (PAQUOT, 1979). Chemical characterization was conducted by measuring the acidity index, peroxide value, iodine content, and saponification value, which were determined according to the procedures prescribed by IUPAC (PAQUOT, 1979). For the determination of fatty acids present in avocado pulp and seed oils, the methyl ester profile (FAME) was obtained according to the ISO 5509 method (INSTITUTO PORTUGUÊS DA QUALIDADE, 2000). The chromatographic analysis of the sample was performed on an AT 6890N gas chromatograph (GC) equipped with a 60 m × 0.25 mm × 0.25 µm DB-23 column and flame ionization detector (FID). The injection was done in Split mode (50:1) and the flow rate of helium carrier gas was 1 mL min⁻¹ as described in the ISO 5508 method (INSTITUTO PORTUGUÊS DA QUALIDADE, 1990).

Antioxidant activity was evaluated by two methods using polar extracts of avocado pulp and seed oil, using ethanol as the extraction solvent. For the 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) assay, the methodology described by BRAND-WILLIAMS *et al.* (1995) with some modifications was used. In brief, 30 µL of each extract was added to 2 mL of DPPH solution (20 ppm) in methanol. The solutions were then incubated for 30 min at room temperature and protected from light. The radical discoloration was measured at 517 nm using a Thermo Scientific Evolution 60S spectrophotometer (Thermo Fisher Scientific, Massachusetts, United States). The results were expressed as inhibition percentage. For the FRAP assay, the reagent was prepared by mixing 300 mM acetate buffer solution (pH 3.6), 10 mM TPTZ solution in 40 mM HCl, and 20 mM FeCl₃ solution (10:1:1). The sample (60 µL) was mixed with FRAP reagent (1,800 µL). The reaction mixture was incubated at 37 °C for 30 min, and the readings were taken at 593 nm (CALDERÓN-OLIVER *et al.* 2016). The results were expressed as mg ascorbic acid g⁻¹ of oil. To determine the total content of phenols in the extracts, the method described by CALDERÓN-OLIVER *et al.* (2016) was used with the following modifications: 50 µL of each extract was taken, after which 250 µL of Folin-Ciocalteu reagent (1:1) and 750 µL of 20 % Na₂CO₃ were added. After 30 min incubation at room temperature and protection from light, readings were performed at 760 nm using a Thermo Scientific Evolution 60S spectrophotometer. The results were expressed as mg Eq gallic acid g⁻¹ of extract.

Statistical analysis was conducted where the bands obtained from each gel were evaluated and allelic genotyping was developed using the program GenAlex

6.7 (PEAKALL AND SMOUSE, 2007) of the amplified products. This enabled us to study the genetic variability between and within the populations, apart from the Molecular Variance Analysis (AMOVA). Thereafter, the genetic distances (NEI, 1978) among the cultivars were calculated. Principal coordinates analysis (PCA) was applied to identify the patterns of the genetic relationships present among the individuals assessed. The efficiency of the markers in the varietal identification was observed by estimating the allelic diversity of the microsatellites in determining the PIC value (polymorphism information content). Through cluster analysis, diversity was evaluated by applying the UPGMA method (unweighted pair-group method with arithmetic average) and the Dice index, generated by the statistical package PAST (Paleontological Statistics Software Package for Education and Data Analysis) (HAMMER et al., 2001).

Finally, in the physicochemical characterization, all the results were expressed as the average \pm standard deviation. The statistical treatment of the data obtained was carried out through an analysis of variance (ANOVA) of a factor with the Tukey test and a level of significance of 95 %. The analyses were performed using the InfoStat program, to compare whether there were significant differences in the parameters evaluated.

Results and discussion

With the applied methodology, high-quality nuclear DNA was obtained and characterized using the SSR molecular technique. Generally, one locus was amplified by each primer pair; however, only two loci were amplified with the primers AVD.021, AVT.448, and AVT.005b. Several amplifications revealed a diploid nature, and these findings concurred with the evidence from the analysis of the collected germplasm population and avocado seedlings using the SSR markers, where diploid inheritance has been shown for most markers (BORRONE et al., 2009). In fact, 13 microsatellite markers were used to generate the differentiation among the varieties to enable each to be distinguished from the other. The number of alleles per locus ranged from 2 (AVT.038) to 13 (AVT.158, AVT.191, AVT.448, AVT.386, AVT.143) with an average of 11.6 alleles per locus (Table 1). GALINDO-TOVAR et al., (2011) reported close to 10.75 alleles per locus using 4 microsatellite primers to evaluate the genetic diversity of 44 *P. americana* foliar samples. In this study, the locus AVT158 exhibited the lowest heterozygosity of 0.708 (Table 1), while the remaining gave values close to 1, highlighting the wide genetic distance between the populations studied.

A high polymorphic level was also observed with the ICP values being higher or close to 0.5, varying from 0.289 (AVT.158) to 0.652 for the AVT.020 GAT primer, which expressed the highest polymorphism. The results of this study coincide with those reported by GUZMÁN et al., (2017), who characterized the structure of a collection of 318 avocado accessions in Mexico using microsatellite markers, to evaluate the breed of origin of these accessions. The 17 SSR markers yielded 20 loci in all. Their genetic variability established that the 'Lorena' population showed many alleles (35.500) because it concentrated most of the individuals analyzed (Table 2). The population of 'Patterns', however, presented the highest number of frequent (3.700), informative (3.124), and exclusive alleles (0.900), with the 'Lorena' population being second and displaying the highest genetic variability observed in the 'Patterns' population. Utilizing standard trees as rootstocks are the best way to enable the avocado crops to have the success of a commercial crop, in which the varieties like the 'Lorena' cultivars which are well accepted in the market, are guaranteed, have strong adaptability to thrive in the same ecosystem, and can transfer not only the performance of the 'Patterns' population (development and productivity) but also all of their genetic variability (CAÑAS et al., 2015).

Table 1. The microsatellite markers selected for the molecular characterization of the 'Papelillo' avocado and its respective polymorphisms by locus* N: Number of alleles per population. *He: Expected heterozygosity, *Ho: Observed heterozygosity, *F: Wright's fixation index. *Polymorphism Information Content

Primer	Mating temperature (°C)	Accession number GenBank	Motif	Primer sequences (5'-3')	Size (pb)	*N	*He	*F	*PIC	
AVT.005b	62.0	KC795703	(CAT) ₅	F:TTAGCAGCAGATAGAGGGAGAG R:GGACCTGCCTTGTGGATTAG	186	11	0.75	0.4	-0.909	0.567
AVT.020GAT	54.0	KC795704	(GAT) ₉	F:CTACATAGATCGAAAATAAGG R:ATCTGGCTAATGAAAATGTTGG	164	12	0.75	0.464	-0.615	0.652
AVT.021	65.0	KC795705	(ATC) ₈	F:ACTCTCGCCTCTGCGTTGAT R:GACTCAACATGGTTAGAACAAAGGC	136	12	1	0.582	-0.721	0.486
AVT.038	56.0	KC795706	(TCA) ₈	F:GATTAAGATGACCCCTGAAG R:GATTTGGCTCAAGATAGATC	190	2	0.75	0.433	-0.733	0.615
AVT.106	66.0	KC795707	(TCA) ₆	F:CCAAATCAAAAGGCAACGAAAGAAC R:GCAAAGGAGGCGGTTTCGAGAT	309	12	1	0.576	-0.737	0.48
AVT.143	66.0	KC795714	(GAA) ₈ (GAT) ₆	F:CCCAACATCTACTTAGCGCAATAG R:ATCATATCGTCTTCCACCGTCGTT	211	13	1	0.506	-0.975	0.385
AVT.158	62.0	KC795715	(GAT) ₇	F:ACGAAATTACGGGTTATTCACA R:TCCACAACTTCTACAGGGTCGT	267	13	0.71	0.379	-0.868	0.289
AVT.191	68.0	KC795708	(ATG) ₇ (TGG)	F:TCCACAACTTCTACAGGGTCGT R:GGAAAGATAACGCACCTTGAGTTC	170	13	1	0.5	-1	0.375
AVT.226	62.0	KC795709	(TCA) ₆ (CTT) ₄	F:GGCTGACTTTTATAGTCCGATGT R:TCCGATTGACAGTGGATTGTT	298	12	1	0.589	-0.697	0.501
AVT.386	60.0	KC795710	(TGA) ₈	F:ACAAACCCAAACATAAATGCT R:AATAGAAGTGACATCCGACC	229	13	1	0.619	-0.616	0.541
AVT.448	60.0	KC795712	(GAT) ₈	F:ACGGTGTGGAAAGAAAGATG R:GCACTTCAATCAATGCTTAC	448	13	1	0.586	-0.707	0.49
AVT.517	59.0	KC795713	(GAT) ₆	F:AATCCTTCCACTCAGAAACT R:TACACAAAACGACAAAGAATGG	229	12	1	0.621	-0.61	0.536
AVO.102	58.0	KC795702	(GA) ₁₂	F:TTCGCCCTTATCAGCGTTAG R:TCTTGGAAAAGCCCTACTCC	153	12	1	0.59	-0.695	0.494
AVD.002	66.0	KC795692	(CT) ₁₅ (CA) ₁₃	F:TGCATACTTTAGCCCCATATGT R:GGATCAATGGTTTGATAACAGG	327	12	0.75	0.423	-0.775	0.6
AVD.003II	63.0	KC795697	(TC) ₁₉	F:TCCCTTCAAGTCTAAGATTAGCC R:GACCAACAATAATTTGCCCCAC	192	12	1	0.578	-0.73	0.479
AVD.006	58.6	KC795698	(TC) ₉ (AC) ₁₉	F:GGGAGAGATGTAITGAGCA R:ACTTGGTCGTFAGATTGTAAAT	314	12	1	0.541	-0.849	0.429
AUCR.418	57.0	KC795695	(GT) ₁₂ (GA) ₁₃	F:AGATGGCTTTCTCCTCTGA R:TTTGACACACAATCCAACTAIG	379	12	1	0.575	-0.738	0.478
						11.6	0.92	0.526	-0.765	0.497
						Average				

Table 2. Genetic variability in 50 individuals from the ‘Lorena’, ‘Santana’, ‘Trapp’ (Papelillo), and ‘Patterns’ populations.

Population	*N	*Na	*Ne	*No	*Ho	*He	*F
‘Lorena’	35.500	2.500	2.365	0.500	0.997	0.562	-0.804
‘Patterns’	6.550	3.700	3.124	0.900	0.993	0.649	-0.564
‘Santana’	3.650	2.250	2.128	0.200	0.900	0.495	-0.850
‘Trapp’	0.800	1.600	1.600	0.100	0.800	0.400	-1.000
Total average	11.625	2.725	2.304	0.425	0.923	0.526	-0.793

* N: Number of alleles per population. *Na: Number of alleles with frequencies greater than or equal to 5%, *Ne: Number of Informative alleles, *No: Number of Exclusive alleles, *He: Expected heterozygosity, *Ho: Observed heterozygosity, *F: Wright’s fixation index.

Although the ‘Trapp’, ‘Lorena’, and ‘Santana’ varieties are commonly called Papelillos, it is necessary to clarify that the ‘Trapp’ and ‘Lorena’ varieties are of the Antillean race, meanwhile, the ‘Santana’ variety is the cross between a Guatemalan race tree with an Antillean race tree. The ‘Trapp’ variety was developed in California and introduced to Colombia in the 1960s. The ‘Lorena’ variety was released in Colombia by the Colombian Agricultural Institute (ICA) in 1957.

In this study, the expected and observed heterozygosity recorded values of 0.526 and 0.923 on average, respectively (Table 2). According to FERES et al. (2008) differences observed in the heterozygosity are attributed to the presence of null alleles, which mask the heterozygous individuals under a band. If heterozygosity is a measure of genetic diversity, these indices suggest high genetic diversity. The index of fixation (F) was on average -0.793, registering negative values in all the populations (Table 3), which indicated that the observed heterozygosity was higher than expected. These results emphasize low homozygosity, meaning, the higher prevalence of heterozygous individuals.

Table 3. Nei’s genetic distances, between the populations of the ‘Lorena’, ‘Patterns’, ‘Santana’, and ‘Trapp’ varieties.

Population	‘Lorena’	‘Patterns’	‘Santana’	‘Trapp’
‘Lorena’	0.000			
‘Patterns’	0.336	0.000		
‘Santana’	0.922	0.674	0.000	
‘Trapp’	0.747	0.669	0.806	0.000

Molecular variance analysis (AMOVA) showed a high degree of genetic differentiation (Phi PT) of 0.626, indicating the presence of a greater difference between the populations, with a 63% variation. However, within each population, the individuals did not display as high a degree of diversity (37%). Some studies, like the one conducted by CHEN et al. 2008, have reported genetic differentiation in avocado attributed to the effect of latitude and the distances in the sampling sites.

The Nei genetic distance implied a high level of similarity among the cultivars analyzed, indicating the presence of less genetic distance between the ‘Lorena’ and ‘Patterns’ populations (0.336), offering evidence for the genetic compatibility between the materials and the possible use of the ‘Patterns’ individuals as rootstocks. The greatest genetic distance was identified between the ‘Lorena’ and ‘Santana’ population of nearly 1.0 (0.922), attributed to the differences between the races from which each population arises. On the contrary, the

‘Lorena’ and ‘Trapp’ populations presented a genetic distance of 0.747; although these two cultivars came from the same Antillean race. Each variety reveals specific phenotypic characteristics. The ‘Trapp’ variety showed close similarity with the ‘Lorena’ variety, although they are genetically distant.

The principal coordinates analysis (PCA) tended that all the individuals investigated appeared to group by variety (Figure 1). All the ‘Lorena’ cultivars were grouped in the lower and upper right quadrants revealing a strong genetic relationship, except for one cultivar that displayed closer similarity to the individuals of the ‘Patterns’ population. In the upper left quadrant, all the possible ‘Patterns’ appeared a little more scattered, because of their high genetic variability. Finally, in the lower-left quadrant were all the individuals of the ‘Santana’ and ‘Trapp’ cultivars, revealing great genetic distances from the remaining populations. These results correspond to the Genetic Distances (NEI, 1978).

After subjecting the data to cluster analysis, a dendrogram was obtained which showed the formation

of four groups produced by the individuals collected based upon their variety, revealing distances between the cultivars belonging to the same botanical race and the interracial hybrids (Figure 2). Group I, which included the cultivars of ‘Santana’ avocado, a hybrid produced from the Guatemalan x Antillean races, is the one which

showed the greatest genetic distance concerning the others. Group II involves the largest number of individuals all corresponding to the ‘Lorena’ cultivars (the subject of this study) (Antillean breed). Group III identifies a genetic difference in the ‘Trapp’ individuals from the rest of the cultivars and Group IV. This refers mostly to the individual ‘Patterns’ (all close to the Antillean races).

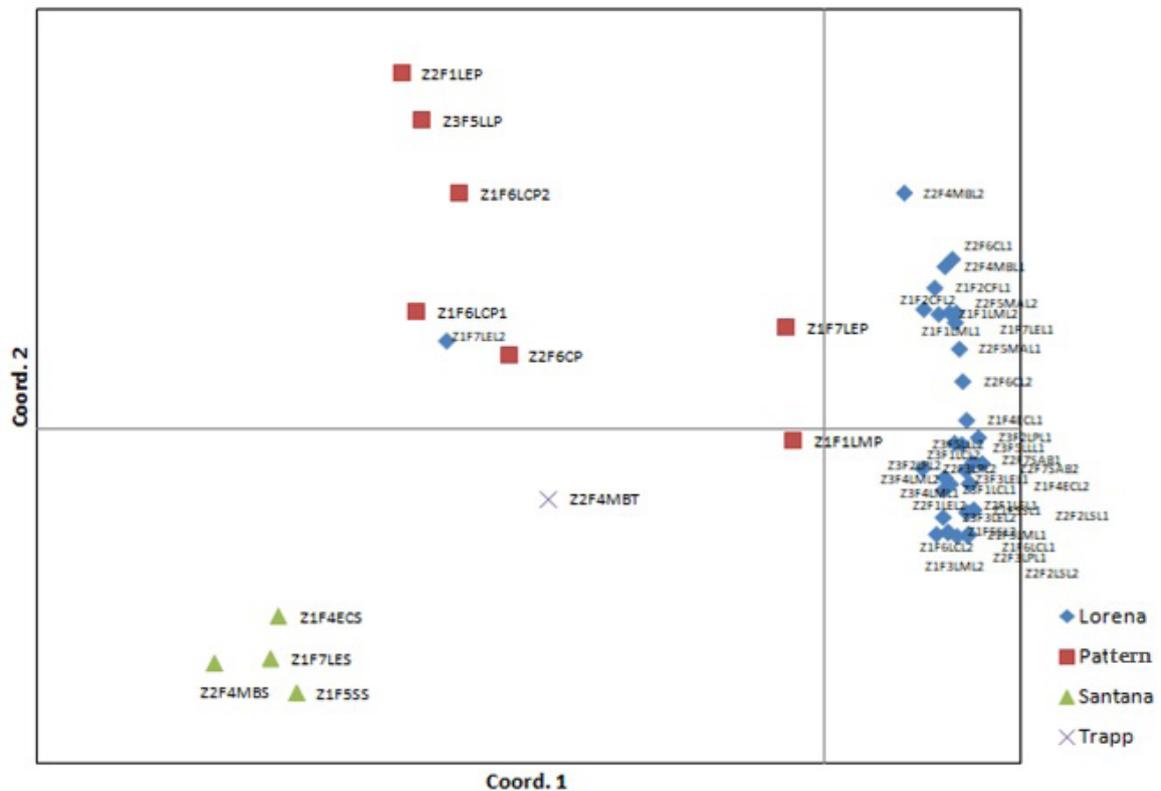


Figure 1. Analysis of the principal coordinates among the avocado cultivars.

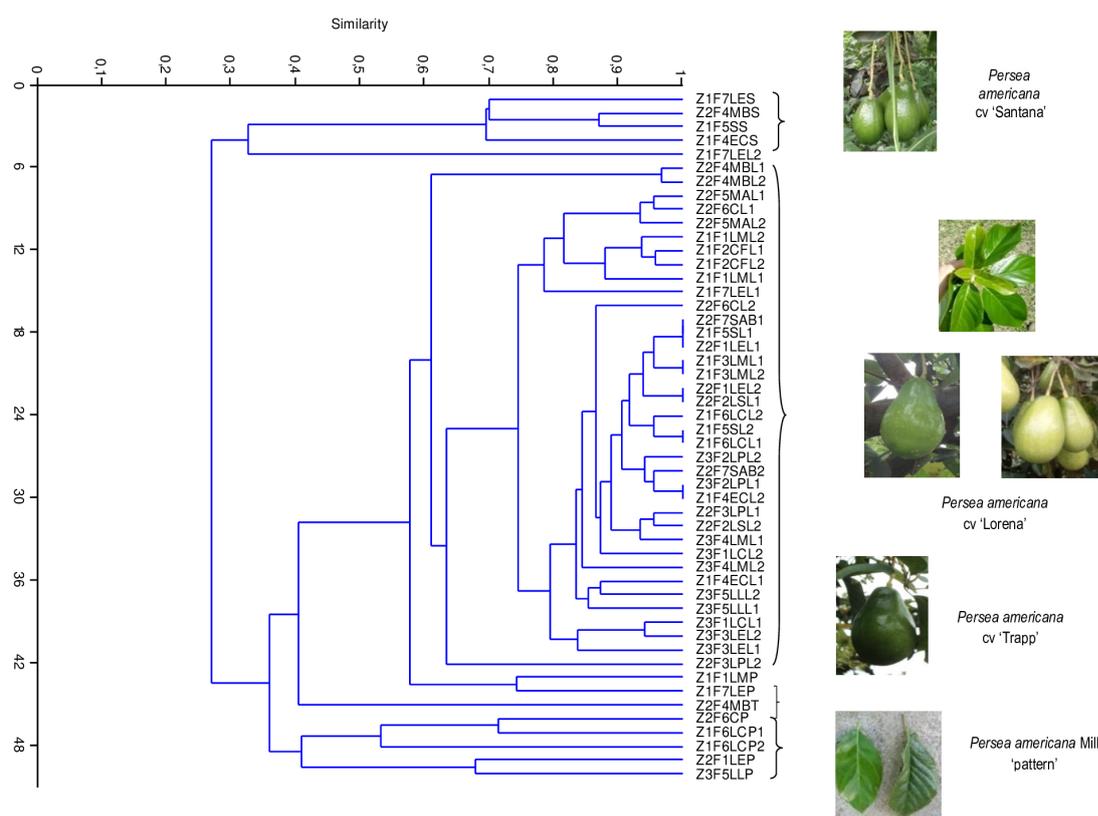


Figure 2. Cluster analysis using the Dice indices of the avocado populations.

The genetic analysis showed a high genetic fidelity in the individuals collected, as well as information following the genetic and phenotypic relationships among the commercial cultivars investigated. It enables the selection of the local standard avocados with suitable traits for use as rootstocks. This encourages the local agriculture, contributes towards the commercialization of the fruit, and thus boosts the economic activity of the region.

After genetically corroborating which were the cultures corresponding to 'Papelillo' avocado 'Lorena' variety, the chemical analysis was continued. Table 4 presents the results obtained from the proximate analysis of pulp. According to the statistical analysis, no significant

differences ($p > 0.05$) were found between the areas evaluated for lipid content, crude fiber, moisture, and proteins. The lipid content obtained for the Colombian 'Lorena' variety avocado pulp (6.23%) was lower than other avocado varieties of the Guatemalan race such as 'Fortuna', 'Collison', 'Barker', and 'Breda', which were in the range of 11–16% as reported by KRUMREICH et al. (2018) and GALVÃO et al. (2014). Furthermore, the fiber content (14.96 a 20.64 %) is higher than that reported by MAITERA et al. (2014) and OLIVEIRA et al. (2013). This high fiber content is of substantial importance considering human nutrition, thereby conferring an additional nutritional value to 'Papelillo' avocados grown in Risaralda.

Table 4. Proximate analysis of the pulp of Papelillo avocados

Parameter	SAMPLING ZONES		
	1	2	3
Ash (%)	0.43 ± 0.10 ^A	0.71 ± 0.22 ^B	0.60 ± 0.09 ^{A^B}
Fiber (%)	20.64 ± 4.28 ^A	14.96 ± 10.91 ^A	15.21 ± 6.98 ^A
Moisture (%)	82.96 ± 2.25 ^A	80.75 ± 4.21 ^A	82.77 ± 2.87 ^A
Lipid (%)	6.17 ± 1.73 ^A	6.56 ± 2.32 ^A	5.97 ± 2.37 ^A
Protein (%)	1.46 ± 0.05 ^A	1.64 ± 0.41 ^A	1.62 ± 0.22 ^A

Values are mean ± standard deviation. Data for the same type of sample in the same row with different superscripts differed significantly ($p \leq 0.05$); results expressed on a wet basis.

Moisture content (80.75 % and 82.97 %) was within the range reported by TANGO et al. (2004) for varieties 'Winslowson', 'Vitória', and 'Waldin', which oscillated between 80.9 and 81.9 %, but was higher than that reported by GALVÃO et al. (2014) for varieties 'Fortuna', 'Collison', and 'Barker' with values of 72.2 %, 76.4 %, and 75 %. A protein content lower than that reported for the 'Breda' variety of 1.73 % (KRUMREICH et al, 2018), however, was found, the protein content obtained (1.57 %) is higher than that reported by OLIVEIRA et al. (2013) who reported protein content of 0.74–1.90 % for various avocado varieties.

Statistically significant differences ($p < 0.05$) were found between the three zones evaluated for the ash content of the 'Lorena' variety avocado pulp, contents similar to those established for the 'Breda' variety (0.6 %) (KRUMREICH et al, 2018), but lower than those reported for the 'Hass' and 'Fortuna' varieties (OLIVEIRA

et al, 2013). The variation of the parameters evaluated is possibly associated with environmental and genetic factors, cultivation practices, and climatic conditions that affect the development of the plant. Besides, knowing the characteristics of the fruits contributes to the selection of materials according to food or industrial applications (KEVERS et al., 2007; ÁLVAREZ et al., 2012).

The average values obtained for the physicochemical characterization parameters of 'Papelillo' pulp and seed oils are shown in Table 5. The peroxide index values for seed and pulp oils were 4.32 ± 1.63 and 2.12 ± 0.82 mEq $O_2 g^{-1}$, respectively, which are within the range established by the Mexican standard NMX-F052-SCFI-2008 for commercial avocado oils, indicating that the oils had not initiated oxidative deterioration through the formation of labile and volatile hydroperoxides (RENGIFO-GRATELLI, 2015).

Table 5. Physicochemical parameters of Papelillo pulp and seed oils.

Parameter	Pulp			Seed		
	1	2	3	1	2	3
Peroxide index (meq $O_2 Kg^{-1}$)	2.86 ± 0.71^A	3.64 ± 0.06^A	2.56 ± 0.37^A	2.72 ± 0.00^A	6.38 ± 0.35^B	3.86 ± 0.05^C
Acidity index (% Oleic acid)	0.50 ± 0.06^A	0.78 ± 0.06^B	0.65 ± 0.05^B	2.49 ± 0.01^A	1.38 ± 0.01^B	1.74 ± 0.05^C
Density 25°C (g mL⁻¹)	0.89 ± 0.00^A	0.89 ± 0.00^B	0.87 ± 0.00^B	0.93 ± 0.00^A	0.84 ± 0.00^B	0.90 ± 0.00^C
Refraction index 25°C	1.4545 ± 0.0004^A	1.4592 ± 0.0028^B	1.4513 ± 0.0072^C	1.4678 ± 0.0006^A	1.4508 ± 0.0006^B	1.4357 ± 0.0001^C

Values are mean \pm standard deviation. Data for the same type of sample in the same row with different superscripts differed significantly ($p \leq 0.05$).

The acidity index value (Table 5) found for pulp oil was lower in zone 1 than in zone 2 and 3. Likewise, they were lower than those reported in the standard NMX-F-052-SCFI -2008 of a maximum of 1.5% for avocado oil. While in the seed oil statistically significant differences were found between each zone, ($p < 0.05$), being higher in zone 1 which is as reported by BORA et al. (2001) for strong variety. This parameter represents the content of free fatty acids produced by the hydrolysis of triglycerides, by the action of lipase enzymes and factors such as heat and light (ADARAMOLA et al., 2016; JIANG et al., 2016).

The values found for density reflected statistically significant differences ($p < 0.05$) for the oil of both fruit tissues. Besides, it was observed that zone 1, for pulp oil, had the lowest value; contrary to what was observed in the seed oil, where the highest value was obtained from the same area. Regarding the refractive index, statistically significant differences were found between the three sampled areas of pulp and seed oils, finding that zone 3 (in seed oil) has similar values to those reported for the 'Barker' variety (GALVÃO et al., 2014).

Table 6 presents the results obtained from the fatty acid profile analysis for pulp and seed oil, finding that pulp oil was mainly composed of palmitic acid (C16: 0), palmitoleic (C16: 1), oleic (C18: 1) and linoleic (C18: 2) with oleic acid being the one that was in greater proportion,

with a range of 45–53%. The seed oil was composed of palmitic acid (C16: 0), oleic (C18: 1), and linoleic acid (C18: 2), with linoleic acid being the one that was found in the highest proportion with a range of 21–28%, followed by palmitic acid with a range of 11–16%.

Table 6. Fatty acid profile of pulp and seed oil of Papelillo avocados.

Fatty acid	Pulp Zone			Seed Zone		
	1	2	3	1	2	3
Caprylic acid	< 0.1 ± 0	< 0.1 ± 0	< 0.1 ± 0	ND	ND	ND
Capric acid	< 0.1 ± 0	< 0.1 ± 0	< 0.1 ± 0	ND	ND	ND
Undecanoic acid	ND	ND	ND	0.1 ± 0	0.1 ± 0	0.1 ± 0
Lauric acid	< 0.1 ± 0	< 0.1 ± 0	< 0.1 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0
Tridecanoic acid	ND	ND	ND	0.1 ± 0	0.1 ± 0	0.1 ± 0
Myristic acid	0.1 ± 0	< 0.1 ± 0	0.1 ± 0	0.85 ± 0.1 ^A	0.85 ± 0.1 ^A	0.85 ± 0.1 ^B
Pentadecanoic acid	< 0.1 ± 0	< 0.1 ± 0	< 0.1 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0
Palmitic acid	27.05 ± 0.4 ^A	24.85 ± 0.5 ^B	26.2 ± 0.1 ^{AB}	16 ± 0.8 ^A	16 ± 0.3 ^A	16 ± 0.5 ^A
Palmitoleic acid	5.65 ± 0.1 ^A	5.45 ± 0.1 ^A	6.65 ± 0 ^B	1.15 ± 0.1 ^A	1.15 ± 0.1 ^A	1.15 ± 0 ^A
Heptadecanoic acid	< 0.1 ± 0	< 0.1 ± 0	< 0.1 ± 0	0.3 ± 0 ^A	0.3 ± 0 ^B	0.3 ± 0 ^B
Stearic acid	0.9 ± 0	1 ± 0	0.9 ± 0.3	1.2 ± 0	1.2 ± 0	1.2 ± 0.1
Oleic acid	46 ± 0.3 ^A	52.9 ± 0.6 ^B	45.2 ± 0 ^A	5.1 ± 0.1 ^A	5.1 ± 0.1 ^B	5.1 ± 0.1 ^A
Linoleic acid	14.6 ± 0 ^A	14.6 ± 0 ^A	14.6 ± 0 ^A	28.1 ± 0.7 ^A	28.1 ± 0.4 ^A	28.1 ± 0.8 ^A
gamma-Linolenic acid	ND	ND	ND	1.3 ± 0.1 ^A	1.3 ± 0 ^A	1.3 ± 0.1 ^B
Linolenic acid	1.8 ± 0 ^A	1.8 ± 0 ^B	1.8 ± 0 ^A	5.95 ± 0.1 ^A	5.95 ± 0.1 ^B	5.95 ± 0.1 ^C
Arachidic acid	0.2 ± 0	0.2 ± 0	0.2 ± 0	0.2 ± 0	0.2 ± 0	0.2 ± 0
Eicosanoic acid	0.2 ± 0	0.2 ± 0	0.2 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0
Eicosadienoic acid	ND	ND	ND	0.1 ± 0	0.1 ± 0	0.1 ± 0
Behenic acid	0.1 ± 0	0.1 ± 0	0.2 ± 0	0.65 ± 0.1 ^A	0.65 ± 0 ^A	0.65 ± 0.1 ^A
Tricosanoic acid	< 0.1 ± 0	< 0.1 ± 0	< 0.1 ± 0	0.3 ± 0	0.3 ± 0	0.3 ± 0
Lignoceric acid	0.1 ± 0	0.1 ± 0	0.1 ± 0	1.1 ± 0.1 ^A	1.1 ± 0.1 ^A	1.1 ± 0 ^A

ND: Not detected; Values are mean ± standard deviation. Data for the same type of sample in the same row with different superscripts differed significantly ($p \leq 0.05$).

Table 7 shows the results of total phenolic content and antioxidant activity for the seed and pulp oils measured by DPPH and FRAP assays. The values obtained from the antioxidant activity by DPPH showed statistically significant differences ($p < 0.05$) between the zones for

pulp and seed oil. For pulp, a range between 19.27% to 24.66% was found with values similar to that found by ABAIDE et al. (2017), while for seed a range of 87.31% to 91.64%, was found being highest than that reported by ADARAMOLA et al. (2016) of 51.54%.

Table 7. Total Phenols Content, DPPH and FRAP antioxidant activity for pulp and seed oil of Papelillo avocados

Material	Zone	DPPH (% Inhibition)	FRAP (mg AA g ⁻¹)	Total Phenols (mg GAE g ⁻¹)
Pulp	1	22.29 ± 1.0 ^{AB}	0.14 ± 0.013 ^A	1750.84 ± 54.29 ^A
	2	20.34 ± 1.1 ^A	0.12 ± 0.01 ^A	1581.54 ± 33.06 ^B
	3	24.43 ± 0.23 ^B	0.13 ± 0.02 ^A	1436.04 ± 30.18 ^C
Seed	1	89.84 ± 0.20 ^A	0.49 ± 0.02 ^A	12697.35 ± 31.94 ^A
	2	91.04 ± 0.60 ^B	0.57 ± 0.01 ^B	8522.36 ± 76.34 ^B
	3	87.72 ± 0.41 ^C	0.34 ± 0.02 ^C	8955.89 ± 46.79 ^B

Values are mean ± standard deviation. Data for the same type of sample in the same row with different superscripts differed significantly ($p \leq 0.05$).

The antioxidant activity by the FRAP method for pulp oil did not show significant differences ($p > 0.05$) between the zones, contrary to that found for the seed oil. On the other hand, the values obtained from the total phenolic content for the seed and pulp oil were statistically different between the zones. Besides, the values found in this study are higher than those reported by WANG et al. (2010) both for the pulp and for the seed of eight varieties of avocado, which ranged from 0.6 ± 0.1 to 4.9 ± 0.7 mg GAE g⁻¹ pulp and 19.2 ± 3.3 to 51.6 ± 1.6 mg GAE g⁻¹ seed. The values obtained were of great importance considering the cosmetic applications, giving added value to both the pulp and the seed of the ‘Lorena’ variety of avocado fruits.

Avocado fruits have been categorized as a significant source of different compounds with benefits for human health (DING et al., 2007). For this reason, many studies have confirmed that variety, geographic region, weather conditions, and ripening stage affect the synthesis of primary and secondary metabolites, among which fatty acids, phenolic compounds that have different biological activities and that confer different properties, stand out (KEVERS et al., 2007; WANG et al., 2010; ÁLVAREZ et al., 2012).

Conclusion

The population possessing the greater genetic variability was the one related to the ‘Patterns’ variety, followed by the ‘Lorena’ cultivars population, revealing the potential of the rootstocks and their significance in developing the avocado crop in the region, and considering the compatibility of the races. Sample grouping based on the breeds and standards indicated the strong degree of variability among the different cultivars as well as the conservation of the individual plants of the ‘Lorena’ cultivars.

Also, it was found that the ‘Lorena’ variety avocado pulp grown in Risaralda, Colombia had high fiber and moisture contents compared to other varieties. On the other hand, the characterization of pulp and seed oils was achieved, finding that they were non-drying oils, with physicochemical characteristics that did not exceed the limits established by international standards. Furthermore, the high content of phenolic compounds and unsaturated fatty acids gave added value to the fruits of *Persea americana* ‘Lorena’ variety grown in the department of Risaralda-Colombia and potential for possible industrial uses.

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