DNA POLYMORPHISMS IN CUBAN VARIETIES OF AVOCADO (*Persea americana* Mill.) AS DETECTED BY INVERSE SEQUENCE TAGGED REPEAT (ISTR) ANALYSIS

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ABSTRACT. A survey of the genetic diversity among Cuban commercial avocado varieties was initiated using ISTR analysis. Dice's dissimilarities between genotype pairs were calculated. A cluster analysis was performed based on dissimilarity using UPGMA as the clustering method. ISTR markers were efficient in detecting polymorphisms among the genotypes. The dissimilarity values ranged from 0.24 between var. "Suardía" and "Hass" to 1.00 between "Lula" and "Los Moros" or "CHI-3" with an average dissimilarity of 0.78. The efficiency of UPGMA in estimating genetic relationships between varieties was corroborated by the cophenetic correlation coefficient, which indicated that the distortion degree in the estimated dissimilarities was minimal. Ecological groups were not adequately represented in the dendrogram. Thus, West Indians, Guatemalan and Mexican genotypes were positioned across the dendrogram. The utility of ISTR for genotype identification and assessment of genetic diversity in commercial avocado varieties is discussed.

Key words: avocado, genetic variation, genetic markers

RESUMEN. Se utilizaron marcadores moleculares del tipo *ISTR* para desarrollar un análisis de diversidad genética en variedades comerciales del germoplasma cubano de aguacate. Los valores de disimilitud entre pares de genotipos fueron calculados según el complemento de Dice. Se realizó un análisis de conglomerado basado en los valores de disimilitud utilizando el método de Ligamiento o Unión Media (*UPGMA*). Los marcadores *ISTR* demostraron su eficiencia en la detección del polimorfismo existente entre los diferentes genotipos. Los valores de disimilitud obtenidos oscilaron desde 0.24 entre las variedades "Suardía" y "Hass" hasta 1.00 entre las variedades "Lula" y "Los Moros" o "CHI-3" con un promedio de disimilitud de 0.78. La eficiencia del *UPGMA* para estimar las relaciones genéticas entre las variedades fue corroborada mediante el coeficiente de correlación cofenética, el cual indicó que el grado de distorsión en los estimados de disimilitud fue mínimo. Los grupos ecológicos no estuvieron representados adecuadamente en el dendrograma, de forma tal que los genotipos antillanos, guatemaltecos y mexicanos, se distribuyeron indistintamente en él. Se discute la utilidad de los marcadores *ISTR* para la identificación de genotipos y estimación de la diversidad genética entre las variedades comerciales de aguacate.

Palabras clave: aguacate, variación genética, marcadores genéticos

INTRODUCTION

Breeding in avocado is quite difficult and only few genetic studies have been reported (1, 2, 3). The main limiting problems are the long juvenile period and the large area required for cultivation. Therefore, breeding programs of this crop are expensive (4). In Cuba, breeding efforts have been limited to traditional methods such as selection of varieties and its propagation (5). The principal commercial varieties planted have been introduced from different American regions and include accessions of the three ecological groups: Guatemalan, West Indian and Mexican.

The knowledge about genetic diversity between genotypes that may be used in crosses is very useful for plant breeding purposes. Current breeding efforts in fruit trees request for modern and effective tools. To this extent, molecular DNA marker techniques have been developed, which can be used for the identification, characterization and evaluation of genetic diversity in plants (6, 7).

Restriction fragment length polymorphism (RFLP) has been used for evolutionary and phylogenetic analyses within the *Persea genus* (8). Similar evolutionary and phylogenetic inference was obtained from mini- and microsatellite data (9). Some authors (10), studying *Persea americana* cultivars, reported that RFLP patterns distinguished the cultivars in the three ecological groups. These authors also used RFLP and RAPD techniques to

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measure the frequency of cross-pollination in avocado, concluding that these markers are useful for identifying effective pollen sources in breeding programs. More recently, race-specific RAPD markers have been identified (11). Additionally, genetic association between DNA fingerprint fragments and loci controlling agriculturally important traits has been demonstrated (4, 12).

Inverse sequence-tagged repeat (ISTR) analysis is a PCR-based technique applicable to animal, plant and microbial genomes (13). It consists of PCR amplification of spacer sequences separating a subset of *copia*-like *Eco*RI repetitive elements (14, 15). ISTR polymorphisms have been useful for the study of genetic diversity and mapping in coconut (15, 16, 17) and barley (18) and the technique has been recommended as useful in the certification of cereal varieties (19).

Here our first efforts are presented to evaluate this DNA marker technique in a set of 18 Cuban avocado varieties for its potential in genetic diversity analysis and its application in future marker-assisted breeding of fruit trees.

MATERIALS AND METHODS

Plant material. The varieties studied constitute the Cuban avocado germplasm bank, located at "Güira de Melena" Station of the Citrus and Fruit Research Institute (Table I).

Table I. List of avocado varieties used in the study with indications of their ecological groups

Varieties	Ecological group
1- Pedro Luis	(Hybrid) Guatemalan x West Indian
2- Itzamná	Guatemalan
3- Lula	(Hybrid) Guatemalan x West Indian
4- Jaruco 1	West Indian
5- Sicilia	West Indian
6- Los Moros	West Indian
7- José Antonio	West Indian
8- Wilson	West Indian
9- Casimiro	West Indian
10- Amado Gómez	Guatemalan
11- Catalina	West Indian
12- CHI-3	West Indian
13- Choquette	(Hybrid) Guatemalan x West Indian
14- Monroe Estación	(Hybrid) Guatemalan x West Indian
15- California	Guatemalan
16- Suardía	Guatemalan
17- Hass	(Hybrid) Guatemalan x Mexican (10)
18- Duke	Mexican

Isolation of genomic DNAs. Young leaves from 18 varieties were used to isolate genomic DNA in a two-step CTAB method (20) modified (15).

Amplification and analysis of polymorphic DNA. Primers for forward (F_3, 5´-GTC GAC ATG CCA TCT TTC-3´) and backward reaction (B_2B, 5´-GGA TAT CCT ATG AAT CAA GC-3´) previously designed (13) were used in PCR amplification. Both primers were labeled using polynucleotide kinase and (g-^{33}P)ATP (Amersham-Pharmacia- Biotech). PCR reactions were performed according to standard protocols (15) in a final volume of 25 µL containing 25 ng of genomic DNA, 200 µM dNTPs, 2.5 mM $MgCl_2$, 1x PCR buffer (Gibco/BRL), 2.5 pmoles of each primer, and 1 unit of Taq DNA polymerase (Gibco/BRL). The amplification program consisted of the following steps: step 1, 95ºC/3min; step 2, 95ºC/ 30sec; step 3, 45ºC/30sec; step 4, 72ºC/2min; step 5, 72ºC/10min, with 40 cycles of steps 2 to 4. Amplified fragments were mixed with an equal volume of formamide dye and denatured at 95ºC for 5 min. Two µl-aliquots were separated on a 4 % polyacrylamide gel. Radioactive-labeled DNA bands were made visible by autoradiography.

Analysis of data. Autoradiograms were visually scored for the presence (1) or absence (0) of bands. The binary matrix was used to construct a distance matrix between all pairs of genotypes according to complement of Dice's coefficient (21) using the WinDist program (22). Based on this distance matrix, the unweighed pair group arithmetic mean analysis (UPGMA) in the SAHN program of the NTSYS-pc package (23) was used to produce a cluster phenogram of genotypes.

RESULTS AND DISCUSSION

In the present study, ISTR analysis was used as the DNA marker technique in genetic diversity analysis of avocado. Using a single ISTR primer combination, a total of 157 PCR amplification products were revealed with good scorability (Figure 1). All of them represented polymorphic DNA fragments. The polymorphism index (polymorphic bands/total of bands) for ISTR in coconut ranged between 48-100 % depending on the primer combination used (14). Our results agree with those reported by these authors. Additionally, the degree of polymorphism detected here is comparable to that obtained using minisatellite sequences (4).

All the genotypes were distinguished using the primer combination mentioned above. Each genotype was characterized by specific band patterns (Figure 1). These results suggest that ISTR can be useful for genotype identification in avocado. Different kinds of molecular markers such as RAPD (24), STMS (25) and AFLP (26) have been recommended for this purpose. Techniques showing complex DNA profiles are the best candidates (19, 27). In this sense, the usefulness of ISTR for variety certification could be demonstrated.

Genetic distances were calculated for each genotype based on 157 polymorphic bands (Table II). In order to investigate the genetic variability and relationships among the different avocado genotypes, a cluster analysis was performed. Dissimilarity coefficients between the 18 genotypes were calculated according to Dice´s complement. The dissimilarities obtained ranged from 0.24 between Suardía and Hass to 1.00 between Lula and Los Moros or CHI-3 with an average dissimilarity of 0.78. This value indicates that ISTR analysis could be an effective tool to explore genetic diversity in avocado. The genetic diversity revealed contrasts to that obtained in coconut by the identical technique. These differences could be explained because of the allogamy phenomenon, which is well known in avocado, while natural coconut populations of dwarf ecotypes tend to be autogamous (15).

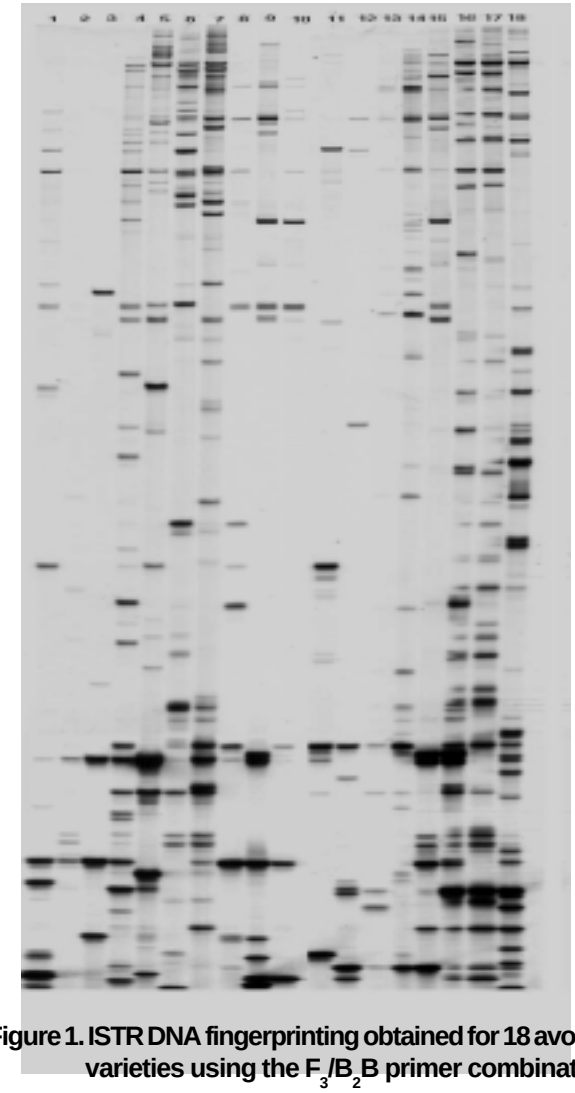

Figure 1. ISTR DNA fingerprinting obtained for 18 avocado varieties using the F_3/B_2B primer combination

The cluster analysis was based on dissimilarity and using UPGMA as the clustering method. A cophenetic matrix was computed from the tree matrix and compared to the original dissimilarity matrix in order to measure goodness of fit (28). The efficiency of UPGMA in estimating genetic relationships between varieties was corroborated by the cophenetic correlation coefficients ($r=0.77$, $p< 0.001$), which indicated that the distortion degree in the relationship of the estimated dissimilarity was minimal. Furthermore, the results supported the idea that the dendrogram depicted in Figure 2 showed a proper representation of associated dissimilarity matrixes.

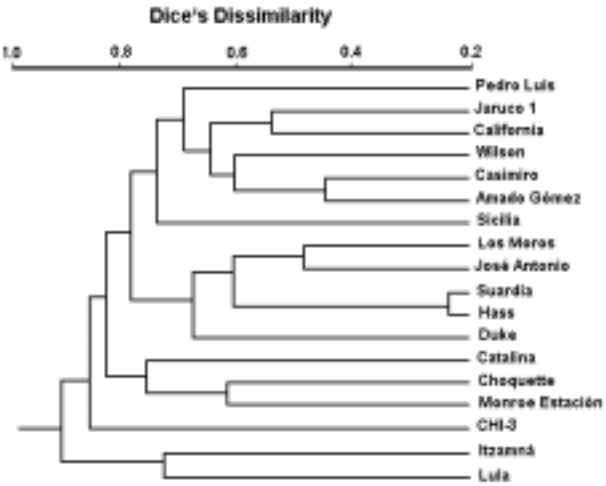

Figure 2. UPGMA dendrogram of 18 avocado varieties based on ISTR dissimilarities

Ecological groups were not adequately represented in the dendrogram according to their putative origin, since West Indian, Guatemalan and Mexican genotypes were positioned across the dendrogram. These results agree with those reported by other authors (9), but contrast with some others (10, 11). Several possible explanations can be considered in support of our results. Firstly, the classification of races is based on morphological characters of the tree and fruit. Efficiency of these descriptors for phylogenetic classification is limited by their low number, poor polymorphism and by the difficulty of defining and quantifying them. Additionally, these characters are influenced by environmental conditions. Therefore, the environmentally affected descriptors could contribute to misclassification. The race definition in avocado has been strongly questioned (9). Secondly, the ISTR analysis explored polymorphisms of non-coding

Table II. Genetic dissimilarities between the avocado varieties expressed as percentage of shared ISTR bands

Varieties	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1- Pedro Luis																		
2- Itzamná	87																	
3- Lula	82	71																
4- Jaruco 1	72	80	87															
5- Sicilia	79	95	80	61														
6- Los Moros	78	91	100	77	78													
7-José A.	87	86	90	63	64	49												
8- Wilson	71	82	67	60	71	82	82											
9- Casimiro	57	86	73	57	63	84	76	58										
10- A. Gómez	72	88	89	64	91	84	87	63	45									
11- Catalina	71	74	92	78	80	78	83	76	74	86								
12- CHI-3	78	100	100	80	95	92	87	86	82	74	79							
13- Choquette	94	100	89	88	91	92	90	85	100	91	78	83						
14- Monroe E.	91	100	96	67	73	75	69	71	68	80	75	85	65					
15- California	73	76	77	57	73	70	59	67	59	74	86	90	84	70				
16- Suardía	76	86	86	70	74	63	52	83	82	90	83	82	82	72	62			
17- Hass	78	92	96	68	76	64	58	88	94	93	91	81	77	75	69	24		
18-Duke	69	91	97	67	74	71	66	87	85	91	87	80	88	82	70	67	60	

regions of DNA. Therefore, it is not surprising to find differences between morphological and DNA marker results. However, future studies could be conducted in order to support this affirmation. Thirdly, the use of unique primer combination could arise in an inadequate representation of genetic diversity of this germplasm.

In the present study, the potential of ISTR for genotype identification and genetic diversity studies in commercial avocado varieties was assessed. This work constitutes the first effort to characterize the commercial avocado germplasm in Cuba. It should be extended using a major number of primer combinations, in order to obtain a better estimate of genetic diversity of Cuban avocado germplasm.

In addition, our results show that a single ISTR primer pair was sufficient to distinguish all 18 genotypes investigated in this study. It points out to the usefulness of this DNA marker for variety certification, for the identification of duplications in germplasm collections and for marker-assisted breeding of avocado.

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