Proc. of Second World Avocado Congress 1992 pp. 545-550

A Study of Avocado Germplasm Resources, 1988-1990. III. Ribosomal DNA Repeat Unit Polymorphism in Avocado

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Abstract. **Nuclear ribosomal DNA of advanced and primitive cultivars of avocado *(Persea americana* Mill.) collected in Mexico was surveyed for repeat length and restriction site variation. The ribosomal repeat encoding the 18S to 25S ribosomal RNA yields informative fragments after digestion with the restriction enzymes Pstl, Eco Rl and Sstl. Digestion with either Pstl and Sstl or Eco Rl and Sstl are sufficient to identify the botanical variety of an avocado cultivar. Moreover, cultivars representing *Persea americana* var. *guatemalensis* Williams and var. *americana* Mill, reveal a similar fragment pattern with either Pstl or Eco Rl, while the fragment patterns of cultivars of var. *drymifolia* (Schlecht. and Cham.) Blake are different from the fragment patterns of cultivars of the other two varieties. Assay of 5S ribosomal DNA, which is unlinked to the 18S to 25S ribosomal DNA, support these observations; these preliminary results suggest a closer phylogenetic relationship of var. *guatemalensis* and var. *americana,* compared to a more distant lineage of var. *drymifolia.***

In a recent review on the origin and taxonomy of avocado *(Persea americana* Mill.), Scora and Bergh (1990) described *P. americana* as a polymorphic species consisting of the wild varieties *floccosa* Mez., *steyermarckii* Allen, *nubigena* (Williams) Kopp, and the cultivated varieties *americana* Mill., *drymifolia* (Schlecht. and Cham.) Blake and *guatemalensis* Williams. Furnier *et al.* (1990) examined the phylogenetic relationships among these taxa by analyzing DNA restriction fragment length polymorphisms (RFLPs) in the chloroplast DNA and nuclear genes coding for cellulase and ribosomal RNA (rDNA). The assay of the variation of rDNA has proven to be a useful tool in plant systematic studies (Hemleben *et al.,* 1988). In the present study we examined the variation of 18S to 25S rDNA and 5S rDNA of advanced and primitive cvs. of avocado collected in Mexico in order to check the identification of the collected material and to gain further insight into the phylogenetic relationships in the subgenus *Persea.* The results of a selection of representative cvs. are presented.

Materials and Methods

Plant material. Avocado samples collected in Mexico are listed in Table 1. The presumed status of a sample was derived from morphological characters and from the collection source. The tentative identification of the botanical variety in the field was based on morphological characters as described, for example, by Bergh and Ellstrand (1987). Samples of advanced cultivars were provided from a collection of The Volcani Center in Israel. The presumed botanical variety of the advanced cultivars is based on a report of Malo *et al.* (1977).

DNA preparation. Total cellular DNA was isolated from freeze-dried leaves of individual trees by a modification of the method of Murray and Thompson (1980).

Source and labeling of probes. Variation of 18S to 25S rDNA was assayed using the rDNA clones pRZ52, pRZx, and pRZ7, from *Cucurbita pepo* L, covering a complete repeat unit (cf. Torres *et al.,* 1990). Variation of 5S rDNA was assayed using the clone pHWH 5a from *Petunia hybrids* (Frasch *et al.,* 1989). The probes were labelled with biotinyl 2-deoxyuri-dine 5-triphosphate (biotin-11-dUTP) by nick translation (Rigby *et al.,* 1977).

Southern blots and detection procedure. Digestion of DNA, elec-trophoresis, vacuum-blotting, hybridization and washing of filters was performed according to standard methods (Maniatis *et al.,* 1982). Detection of the rDNA fragments by a streptavidine-alkaline phosphatase-conjugate and a color producing substrate was according to the protocol of the manufacturer (GIBCO BRL, Gaithersburg, Maryland, USA). Fragment sizes were estimated by linear regression analysis. Standard markers were co-electrophoresed on each gel.

Results

Ten different rDNA fragment sizes could be detected in Pstl-digests of avocado DNA, ranging from 8.15 to 10.4 kb (Table 2). With the exception of 'Criollo 42'*, a* 9.05-kb fragment was found to be present only in accessions of var. *drymifolia.* Similarly, fragment sizes greater than 9.6 kb were restricted to accessions of var. *drymifolia,* again with the exception of 'Criollo 42'. It is noteworthy that leaves of 'Tochimilco 5' lack the anise odor of var. *drymifolia,* but reveal a typical rDNA fragment pattern of that variety. Accessions of var. *guatemalensis* and var. *americana* always contained a 9.3 kb fragment not found in accessions of var. *drymifolia.* 'Criollo 42', a presumed member of var. *guatemalensis* lacked the 9.3-kb fragment.

Eco Rl digests of accessions of var. *drymifolia* yielded less uniform fragment patterns compared with var. *guatemalensis* and var. *americana* (Table 3). A 3.5-kb fragment was restricted to accessions of var. *drymifolia.* A 4.6-kb fragment was found in some accessions of var. *drymifolia,* but was not detected in accessions of both var. *guatemalensis* and var. *americana.* Instead, accessions of the latter two varieties always contained a 3.1-kb fragment not found in accessions of var. *drymifolia.*

Exceptions are 'Aquila 4' and 'Criollo 42', which possess or lack, respectively, a 3.1-kb fragment. The occurrence of a 2.9-kb fragment in ‘Tochimilco 1' also represents a remarkable exception.

Only accessions of var. *guatemalensis* yielded a 0.9-kb fragment after Sstl-digestion and probing with the 18S to 25S rDNA (Table 4). Furnier *et al.* (1990) also reported that this fragment is unique to var. *guatemalensis.*

The hybridization patterns in Southern blots of Bam Hl-digests probed with 5S rDNA indicated two repeat length variants of 5S rDNA for avocado; a 560-bp repeat unit for var. *guatemalensis* and var. *americana,* and a 540-bp repeat unit for var. *drymifolia* (Table 4).

Discussion

The repeat lengths of 18S to 25S rDNA found in higher plants extend from ca. 8 kb to ca. 14 kb (Hemleben *et al.,* 1988). It seems reasonable, therefore, to assume that the fragments generated by Pstl represent different repeat lengths of avocado 18S to 25S rDNA. Thus two types of polymorphism could be detected: (1) a repeat length polymorphism detectable after digestion with Pstl, and (2) a restriction site polymorphism detectable after digestion with Eco Rl or Sstl. It is expected from these results that digestion of avocado DNA with either Pstl and Sst I, or Eco Rl and Sstl and probing with 18S to 25S rDNA yields enough information to identify the botanical variety of an avocado cultivar. However, digestion with either Pstl or Eco Rl alone, will only allow the positive identification of var. *drymifolia* and will leave var. *guatemalensis* and var. *americana* un-separated as one group with similar fragment patterns (Tables 2 and 3). Likewise, digestion with Bam HI and probing with 5S rDNA reveals similar repeat lengths for var. *guatemalensis* and var. *americana,* but a different repeat length for var. *drymifolia* (Table 4). These preliminary results, therefore, suggest a relatively close phylogenetic relationship between var. *guatemalensis* and var. *americana,* compared to a more distant lineage of var. *drymifolia.* This is in contrast to Scora and Bergh (1990), who stated that the three varieties are about equally distinct from each other. It is in agreement, however, with earlier classifications, for example by Kopp (1966), who did not separate var. *guatemalensis* and var. *americana* in two distinct varieties.

The foregoing discussion fits likewise to advanced and primitive cultivars, with the exception of 'Criollo 42'. 'Criollo 42' has been identified in the field as var. *guatemalensis,* but it did not reveal typical fragment patterns of that variety in the rDNA assay (Tables 2 and 3), except for the presence of a 0.9-kb fragment common to members of var. *guatemalensis* (Table 4). It may be suggested, therefore, that 'Criollo 42' is not a member of var. *guatemalensis,* but may belong to the complex group of the ancestors of 'Guatemalan criollos' (cf. Schieber and Zentmyer, 1977). Moreover, 'Aquila 4', which has been identified in the field as var. *drymifolia,* revealed a 3.1-kb fragment typical for var. *guatemalensis* and var. americana (Table 3). Probably, 'Aquila 4' is not 'pure' var. *drymifolia,* but of hybrid origin. Tochimilco 1', an other presumed accession of var. *drymifolia,* showed an unusual 2.9-kb fragment in the Eco Rl digest (Table 3). This

fragment has also been detected in one accession of *P. schiedeana* (unpublished results). In fact, some of the relatively rare fragments, as 8.15-kb, 9.2-kb, and 4.5-kb, were detectable in various accessions of *P. schiedeana* and other *Perseas* (unpublished results). It may therefore be speculated that the relatively high variability observed in rDNA fragment patterns of primitive cultivars reflects remote events of hybridizations.

In conclusion, the assay of rDNA proved to be useful in the identification of the variety of primitive and advanced cultivars of avocado. The variation of rDNA data may suggest a relatively close phylogenetic relationship between var. *guatemalensis* and var. *americana,* leaving var. *drymifolia* somewhat distinct.

We thank R. Torres and W. Wenzel for gifts of rDNA clones and helpful advice, and F. Bangerth for supporting this research. We gratefully acknowledge the cooperation of A. Barrientos Priego, E. de la Cruz Torres, and L. Lopez Lopez. We appreciate the financial support provided by the German-Israel Agricultural Research Agreement (GIARA).

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Table 1. Collection data of avocado accessions collected in 1990 in Mexico.

Name of sample	Location of collection site	Collection source	Presumed status of sample
Aquila 3	Aquila (State of Vera Cruz, 1750 m alt.)	farm land	primitive cv.
Aquila 4	Aquila (State of Vera Cruz, 1750 m alt.)	wild	primitive cv.
Tochimilco 1	Tochimilco (State of Puebla, 2020 m alt.)	backyard	primitive cv.
Tochimilco 5	Tochimilco (State of Puebla, 2020 m alt.)	orchard	primitive cv.
Tochimilco 7	Tochimilco (State of Puebla, 2020 m alt.)	orchard	primitive cv.
Amatenango 1	Amatenango (State of Chiapas, ca. 1850 m alt.)	backyard	primitive cv.
Criollo 42	Amatenango (State of Chiapas, ca. 1850 m alt.)	backyard	?
Tantima 2	Sierra Tantima (State of Vera Cruz, ca. 500 m alt.)	wild	wild
Tantima 4	Sierra Tantima (State of Vera Cruz, ca. 500 m alt.)	wild	primitive cv.

Table 2. PstI restriction fragments of 18S to 25S rDNA detected in advanced and primitive cvs. of avocado. (+) indicates presence of fragments; (-) indicates that fragment was not detected[z].

Cultivar	Kb						
	10.4	9.95	9.6	9.45	9.3	9.05	8.65
Var. *drymifolia*							
cv. Topa-Topa	+	+	-	-	-	+	-
cv. Gainesville	-	+	+	-	-	+	+
Aquila 3	+	+	-	-	-	+	-
Aquila 4	+	+	+	-	-	+	-
Tochimilco 1	-	+	+	-	-	+	+
Tochimilco 5	+	-	+	-	_	+	+
Var. *guatemalensis*							
cv. Nabal	-	-	-	-	+	-	+
cv. Benik	-	-	+	-	+	-	+
Amatenango 1	-	-	-	+	+	-	+
Criollo 42	-	+	-	+	-	+	+
Var. *americana*							
cv. Waldin	-	-	+	-	+	-	+
cv. Simmonds	-	-	+	-	+	-	+
Tantima 2	-	-		-	+	-	+
Tantima 4	-	-	+	-	+	-	+

[z] In addition, a 10.15-kb fragment and a 9.2-kb fragment have been detected in 'Aquila 3', and a 8.15-kb fragment has been detected in cv. Gainesville and 'Tochimilco 5'.

Table 3. Eco RI restriction fragments of 18S to 25S rDNA detected in advanced and primitive cvs. of avocado. (+) indicates presence of fragments; (-) indicates that fragment was not detected [z].

Cultivar	Kb						
	4.9	4.6	4.5	4.15	4.05	3.5	3.1
Var. *drymifolia*							
cv. Topa-Topa	+	+	-	-	+	+	-
cv. Gainesville	+	+	-	-	-	+	-
Aquila 3	+	-	+	+	+	+	-
Aquila 4	-	+	+	+	+	+	+
Tochimilco 1	+	+	-	+	+	+	-
Tochimilco 5	+	-	-	-	-	+	-
Var. *guatemalensis*							
cv. Nabal	+	-	-	-	-	-	+
cv. Benik	+	-	-	-	-	-	+
Amatenango 1	+	-	-	+	-	-	+
Criollo 42	+	-	+	+	-	-	-
Var. *americana*							
cv. Waldin	?	-	-	-	-	-	+
cv. Simmonds	?	-	-	-	-	-	+
Tantima 6	+			+			+

[z] In addition, three informative fragments, 3.6 kb, 3.05 kb, and 2.90 kb, have been detected in the accessions 'Aquila 4', 'Criollo 42', and 'Tochimilco V, respectively.

Table 4. The occurrence of a 0.9-kb fragment of 18S to 25S rDNA after digestion with SstI and repeat lengths of 5S rDNA detected in advanced and primitive cvs. of avocado. (+) indicates presence of fragment; (-) indicates that fragment was not detected.

Cultivar	0.9 kb fragment	Repeat length
Var. *drymifolia*		
cv. Topa-Topa	-	540 bp[z]
cv. Gainesville	-	540 bp
Aquila 3	-	540 bp
Aquila 4	-	540 bp
Tochimilco 1	-	540 bp
Tochimilco 5	-	540 bp
Var. *guatemalensis*		
cv. Nabal	+	560 bp
cv. Benik	+	560 bp
Amatenango 1	+	560 bp
Criollo 42	+	560 bp
Var. *americana*		
cv. Waldin	-	560 bp
cv. Simmonds	-	560 bp
Tantima 2	-	560 bp
Tantima 4	-	560 bp

[z] bp, base pair