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PROGRESS OF THE STUDY ON THE GENETIC RESOURCES OF AVOCADO IV. USE OF THE SPACER SIZE VARIATION OF RIBOSOMAL 5S GENES TO IDENTIFY THE HORTICULTURAL RACE OF AVOCADO GENOTYPES

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Abstract

'Universal' primers have been used to amplify the nontranscribed spacer (NTS) of the ribosomal 5S gene of avocado genotypes by the polymerase chain reaction (PCR). A rich size heterogeneity of NTSs of individual avocado genotypes was detected after high resolution polyacrylamide gel electrophoresis and silver staining. Specific sizes of NTS seem to be common for members of each horticultural race of avocado and may be used as a diagnostic tool to identify the horticultural race of avocado genotypes.

1. Introduction

Three horticultural or ecological races of avocado *(Persea americana* Mill.) can e distinguished, namely Mexican, Guatemalan, and West Indian; Bergh and Ellstrand (1987) gave a detailed typification of these groups. It is assumed, that the majority of avocado cvs. are 'pure' to each race, but that some of them may be actually hybrids of yet unknown lineage (Lahav and Gazit, 1994).

It is widely acknowledged that the heterogeneity of 5S ribosomal DNA (rDNA) an provide a useful tool in plant systematic studies (Sastri et al., 1992). In higher plants the genes that code for 5S rRNA are organized into clusters of tandem repeats. Every repeat unit consists of a transcribed region of approximately 120 base air (bp) and a NTS region varying in size between 100 bp and 700 bp (Sastri et al., 1992). It is this variation in size (and sequence) which makes 5S rDNA so suitable or plant systematic studies. The purpose of this study was to test the variation of NTS sizes between genotype representing the three horticultural races of avocado, and to determine whether it could be used as a diagnostic tool to identify the horticultural race of an avocado genotype. NTSs of avocado cvs. were selectively amplified using the PCR and 'universal' primers as applied previously to 5S genes of barley (Kolchinsky et al., 1991) and wheat (Cox et al., 1992).

2. Material and methods

2. 1. Plant material

Avocado leaf samples of cultivars (cvs.) or germplasm bank accessions were collected in Israel (The VoIcani Center, Bet-Dagan) or in Mexico (CICTAMEX, Coatepec Harinas, Mexico), lyophilized or oven-dried and sent to the University of Hohenheim for analysis.

2.2. DNA extraction

DNA was extracted according to Guillemaut et al. (1992), except for RPC-5 column chromatography which was replaced by phenol-chloroform extraction.

2.3. PCR

The PCR assay of the NTS of the 5S rDNA was adopted from Kolchinsky et al. (1991) and Cox et al. (1992).

2.4. Polyacrylamide gel electrophoresis

The amplified fragments were separated under denaturing conditions on a horizontal 10% polyacrylamide gel (Mini CleanGel; Pharmacia Biotech, Freiburg, Germany) with an amphoteric buffer system (DELECT forte buffer, pH 7.3; ETC- Electrophorese Technik, Kirchentellinsfurt, Germany). The DNA fragments were visualized by silver staining as described by Bassani et al. (1991).

3. Results

PCR amplification of NTS of avocado generated a variety of DNA fragments as revealed by polyacrylamide gel electrophoresis with subsequent silver staining (figure 1). The size of the PCR products varied from 200 bp to over 1000 bp. Inferences from Southern hybridizations (Bufler and Ben-Ya'acov, 1992, and unpublished results) suggest that fragments bigger than 800 bp are not representing true NTSs. Thus, in the present study NTS sizes of avocado generated by PCR ranged from 210 bp to 470 bp (figures 1 and 2).

A comparison of NTS sizes of 47 accessions of the subgenus *Persea* collected in Mexico and Central America included cultivated avocados, wild relatives of avocado and the semi-domesticated *P. schiedeana*. In this survey, NTS sizes common to each horticultural race of avocado could be identified. The results of 12 representative avocado genotypes are summarized in figure 2. It shows, for example, that Guatemalan and West Indian genotypes have a 470 bp and a 345 bp NTS which is not found in Mexican genotypes. Some Guatemalan genotypes exhibit NTSs of 245 bp and 250 bp in addition (or instead of, not shown) to the 345 bp NTS. The NTSs at 406 bp and (in most cases) also at 412 bp are common to Mexican race genotypes. The presence of the 432 bp NTS in genotypes of the Mexican or West Indian race allows to distinguish between the West Indian and Guatemalan race. Thus, the horticultural race of each genotype could be identified by the presence (or absence) of certain NTS variants. Some cvs., namely 'Topa Topa', 'Gainesville', 'Hass', 'Waldin', 'Simmonds' and 'Day, contain NTS variants common to at least two races and, therefore, are not considered as being 'pure' to a certain race (figure 2; cf. Lahav and Gazit, 1994). Moreover, the cvs. 'Hass', 'Day' and 'Simmonds' exhibit NTS variants rarely found in other genotypes of the cultivated avocado (figure 2).

4. Discussion

In a previous study, Bufler and Ben-Ya'acov (1992) reported a 5S rDNA repeat size based on Southern hybridization of 560 bp for Guatemalan and West Indian race avocado, and 540 bp for Mexican race avocado. These results correspond reasonably well with the NTS size of 470 bp for Guatemalan and West Indian race avocado and 460 bp for Mexican race avocado if 80 bp are added for the transcribed region (the remaining 40 bp represent binding sites for the 'Universal' primers, but become part of the NTS after amplification. Thus, to estimate the correct size of a

NTS, 40 bp should be subtracted). Other repeat sizes are hardly detectable by Southern hybridization.

Fumier et al. (1990) and Bufler and Ben-Ya'acov (1992) examined restriction fragment length polymorphisms (RFLPs) of 18S-25S rDNA of avocado, with the latter authors also reporting on the repeat size variation of 5S rDNA. In these studies enough variation of rDNA could be observed to distinguish the horticultural races of avocado. However, compared to the PCR-based assay of NTSs the Southern hybridization-based assays are more laborious and time consuming and less sensitive. Our results also demonstrate, that separation of PCR products on a highly resolving polyacrylamide gel and highly sensitive silver staining are prerequisites to fully exploit the variation of the NTSs of avocado. Although it seems unlikely that the variation of NTSs of 5S rDNA gives a good reflection of variation of the entire genome, it may provide a diagnostic tool to identify the horticultural races of avocado cvs. and germplasm collections. As a qualitative test, the NTS assay may also support classification in the subgenus *Persea* and provide clues for phylogenetic relationships.

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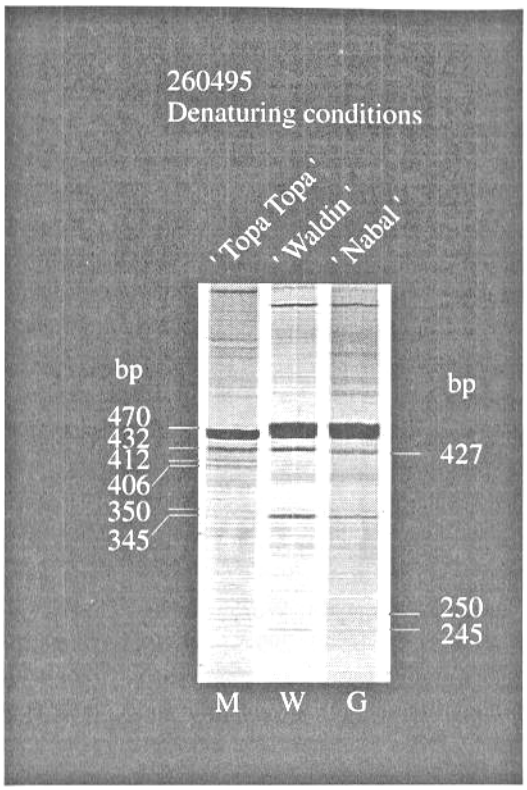

Figure 1 - PCR products of 5S gene NTS assay of three cvs. of avocado separated by polyacrylamide gel electrophoresis and silver staining

Size of NTS (bp)	M1	M2	M3	M4	G1	G2	G3	G4	W1	W2	W3	W4
470												
460												
450												
432												
427												
412												
406												
400												
367												
350												
345												
280												
275												
270												
262												
257												
250												
245												
210												
Horticult. race	Mexican				Guatemalan				West Indian			
	M1	Topa Topa			G1	Nabal			W1	Waldin		
	M2	Gainesville			G2	Hass			W2	Simmonds		
	M3	Aquila 1			G3	Amatenango			W3	Day		
	M4	Aquila 3			G4	Comitan 2			W4	Tantima 2		

Figure 2 - Sizes of NTS of 5S gene of avocado genotypes of the Mexican, Guatemalan, and West Indian horticultural race.