South African Avocado Growers' Association Yearbook 1992. 15:29-31

Characterisation of dsRNA in avocados

G Cook, L H Nel and J M Kotzé
Department of Microbiology & Plant Pathology, University of Pretoria, Pretoria 0002

ABSTRACT

The hypothesis that the double-stranded (ds) RNA isolated from avocado is an indication of infection by a RNA plant virus has never been proven. On the other hand, it has not been disproven either, and the different dsRNA 's are still known as avocado virus (A V) 1, 2 and 3.

The authors have probed restriction fragments of avocado cellular DNA with cloned ^{32}P-labelled A V cDNA and discovered that the dsRNA nucleotide sequences are also present on plant DNA. Their evidence contradicts the hypothesis that viruses are responsible for the formation of the dsRNA species.

UITTREKSEL

Die hipotese dat die dubbeldraad (dd) RNA in avokado 'n aanduiding van infeksie deur 'n virus is, is nog nooit bewys nie. Nogtans is die teendeel ook nog nooit bewys nie, en die verskillende ddRNA-patrone is bekend as avokado virus (A V) 1, 2 en 3.

Die outeurs het restriksie fragmente van avokado sellulére DNA met gekloneerde ^{32}Pgemerkte AV cDNAS gepeil en ontdek dat die ddRNA nukkotied volgordes ook op die plant DNA teenwoordig is. Die hipotese dat virusse verantwoordelik is vir dieformasie van die ddRNA word deur hierdie resultate weerspreek.

The current understanding of virus infections in avocado is still limited to the knowledge of unexplained virus-like diseases and the existence of double stranded (ds) RNA species in some avocado trees. The symptoms of the virus-like diseases include low, unpredictable or irregular fruit yields, stem-pitting, leaf dwarfing, dwarfing and black streak (still not found in South Africa). The dsRNA species that can be isolated from avocado trees have been grouped into three compilations, which are currently known as putative avocado virus (AV) 1, 2 and 3.

More than 90% of all known plant viruses have single-strand (ss) RNA genomes, 85% of which are (+) RNA (Goldbach *et al,* 1990). When these RNA genomes are replicated during the course of the infection, replication intermediates (RI) and genome replicative form (RF) can be isolated from the host as stable dsRNA products of specific size. Using this rationale, the appearance of dsRNA in plants is considered useful for the diagnosis of viruses which are difficult to isolate. Although not foolproof, the relevance of dsRNA species as indicative of virus infection is underlined by numerous examples.

Some of the different plant viruses which are known to produce dsRNA in their hosts are listed in Table 1.

Efforts to isolate or identify virus particles from avocado have never been successful, even though the presences of dsRNA patterns were described as early as 1982 (Jordan *et al,* 1983). The investigation by the authors is therefore aimed at the characterisation of the dsRNA species found in avocado. They previously reported the cloning of dsRNA (AV_1) material and the use of the cloned cDNA as a radiolabelled probe (Cook & Nel, 1991). This probe was used to screen a great number of samples (>250). In control experiments dsRNA PAGE was also carried out.

Shown in Figure 1 is the polyacrylamideelectrophoretic pattern of the dsRNA species present in Fuerte trees. The large doublet of approximately 10 000 bp and the smaller RNA fragment of 900 bp comprise putative AV_1 whereas putative AV_3 is comprised by 3 dsRNA fragments in the 3 000-3 800 bp range as indicated. Hass, the other very important cultivar in South Africa, was found to contain only the AV_3 dsRNA species, as established by PAGE analyses.

The use of the AVVspecific cDNA probe on crude nucleic acid extracts from trees, yielded irregular results. An example of the autoradiograph from such an RNA dot blot is shown in Figure 2. The probe, being AV_1 -specific, hybridised to extracts from Fuerte trees. The authors occasionally obtained positive hybridisation results with the nucleic acid extracts from, for example, Edranol and Hass trees, as indicated in Figure 2. Since only AV_3 in Hass could be isolated and no dsRNA obtained from Edranol, this was an interesting result. However, AVj dsRNA could not be isolated from either positive tree.

In working towards an explanation for these results, the following observations were considered:

-dsRNA patterns are mostly cultivar-specific;

-specific cultivars are always positive for the same dsRNA's;

-dsRNA's do not necessarily correlate with disease symptoms, but are found in normal plants; and

-the dsRNA concentration did not show much seasonal variation.

It therefore seemed reasonable to postulate that the dsRNA's may originate from the plant genome. If so, the identification of a plant genomic DNA sequence, identical to the AV_1 RNA sequence, would explain the positive hibridisation of AV_1 with the crude nucleic acid extracts (probably also containing some DNA fragments) of trees which do not contain AV_1 dsRNA (Figure 2).

TABLE 1 A summary of different viruses which induce dsRNA in infected plants

Virus family	Virus	Reference
Tobamovirus	TMV	Bar-Joseph *et al* (1983) Zelcer *et al* (1981)
Cucumovirus	CMV	Bar-Joseph *et al* (1983) Kaper and Diaz-Ruiz (1977)
Potyvirus	TEV	Gadh and Hari (1986)
Tombusvirus	TBSV	Henriques and Morris (1979)
	TCV	Henriques and Morris (1979)
	CRSV	Henriques and Morris (1979)
Closterovirus	ACLSV	Dodds and Bar-Joseph (1983)
	CNFV	Dodds and Bar-Joseph (1983)
	BYV	Dodds and Bar-Joseph (1983)
	CTV	Dodds and Bar-Joseph (1983) Bar-Joseph *et al* (1983) Lee (1984)
Luteovirus	BWYV	Falk and Duffus (1984)
	BYDV	Gildow *et al* (1983)
Carlavirus	PVM	Tavantzis (1984)

To investigate, the authors performed restriction fragment length-type experiments. Total cellular DNA was purified from avocado trees known to be AV_1 positive, as well as from trees known to be AV_1 negative, as determined by dsRNA PAGE analyses. Briefly, leaf tissue (10 g) was ground to powder in liquid nitrogen. Extraction buffer (100 mM Tris.Cl, pH8; 100 mM EDTA; 250 mM CaCl; 100 µg/mℓ protein ase K) (50 mℓ) was added and the extract adjusted to 1% sarcosyl with a 10% sarcosyl solution. After 60 min incubation at 55°C, the lysate was centrifuged (10 min, 6 000 rpm, 4°C) and the supernatant extracted twice with chloroform: isoamyl alcohol (24:1). Nucleic acids were alcohol-precipitated, treated with RNA ase and purified by CSCℓ density gradient centrifugation according to standard protocols (Ausubel *et al,* 1987).

The purified cellular DNA's were digested with different restriction enzymes (Boehringer Mannheim), according to the manufacturer's instructions. Agarose gel electrophoresis in a Tris-acetate-EDTA buffer system, followed by Southern blotting of the DNA fragments to a nylon membrane, was carried out as described by Maniatis *et al* (1989). AV_1 specific cDNA was radioactively labelled and hybridised to the Southern blots as previously described (Cook & Nel, 1991).

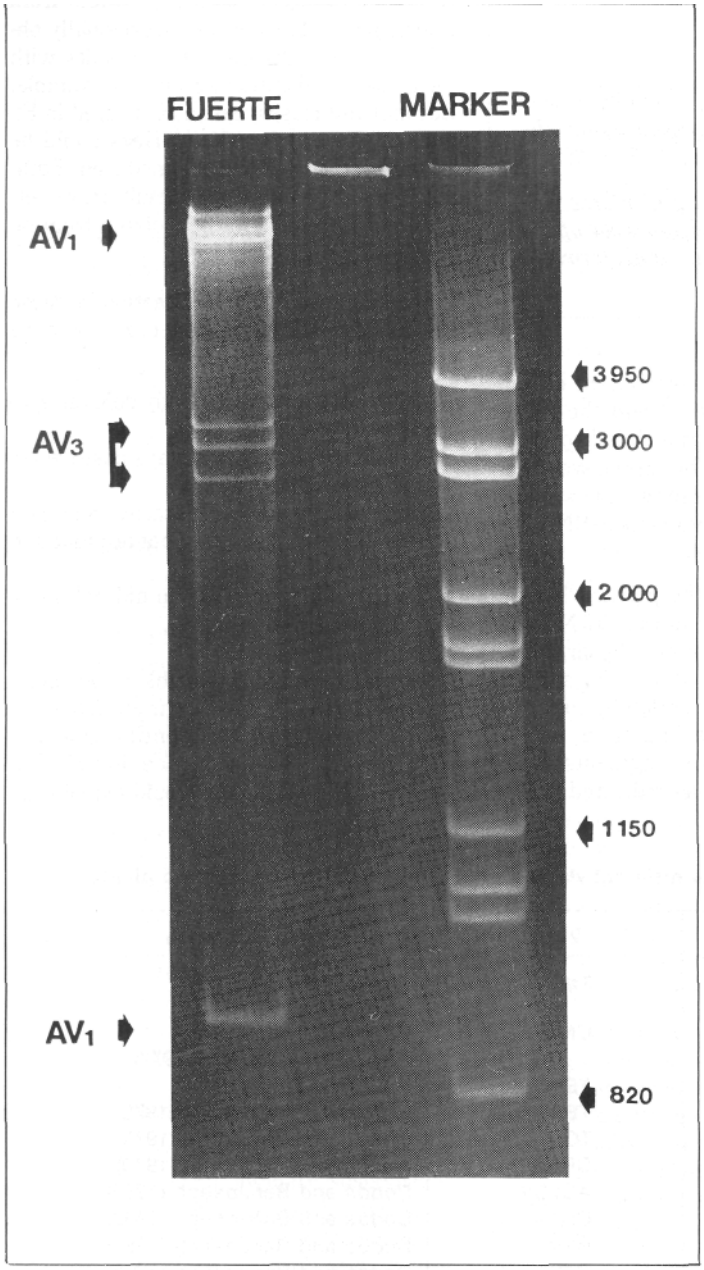

Fig 1 Electrophoretograph of dsRNA species isolated from a Fuerte tree. RNA was purified and PAGE carried out as previously described (Cook & Nel, 1991). The dsRNA's comprising AV_1 and AV_3 are indicated on the left. Purified Bluetongue virus dsRNA was used as molecular weight marker and the sizes of the dsRNA fragment are indicated on the right in base-pairs.

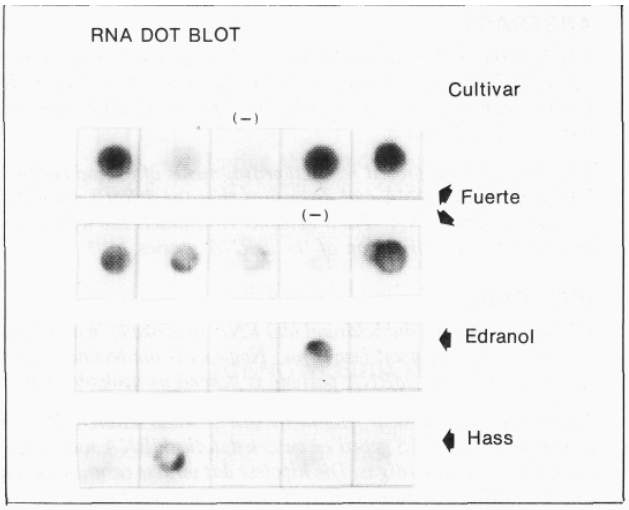

Fig 2 Autoradiograph of crude nucleic acid extracts from different avocados which was blotted to a nylon membrane, denatured and hybridised to a ^{32}P-labelled AV_1-specific cDNA probe. The probe was labelled in nick translation reaction (BRL) and the hybridisation was carried out at 42°C, followed by three high stringency washes as previously described (Cook & Nel, 1991). Negative controls were included as indicated. Refer to text for details.

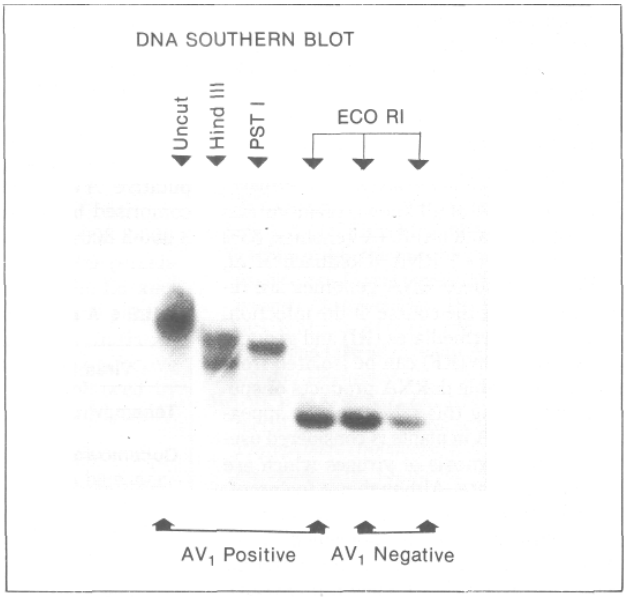

Fig 3 Autoradiograph of an avocado DNA Southern blot which was probed with ^{32}P-labelled AV_1-specific cDNA was extracted, digested with restriction enzymes, electrophoresed and blotted to a nylon membrane as described in the text. The different restriction enzymes used in the analysis are indicated at the top of the autoradiograph, whereas the AV_1 dsRNA status of the plants from which the DNA was prepared, is indicated at the bottom of the autoradiograph. Refer to the text for discussion.

The results are presented in Figure 3. It is evident that the AV_1 -specific cDNA hybridises to avocado cellular DNA isolated from trees which are AV_1 positive. When the DNA was digested with Hind III, Pst 1 or Eco R1, different fragment(s) were found to hybridise with the 900 bp cDNA probe.

In addition, it can be seen that the labelled AV_1specific cDNA hybridises to the DNA of AV_1free trees. These trees do not contain the AV_1 dsRNA, yet the AV_1 probe hybridises to the same fragment of DNA (as indicated by the Eco R1 fragmentation) as in the case of trees that do not contain AV_1 dsRNA (Figure 3).

DISCUSSION

The true nature, origin and significance of the dsRNA's AV_1 2 or 3 in avocado trees and their possible role as replicating virus genomes have never been clarified. Using cloned cDNA derived from AV_1 dsRNA, the authors probed crude nucleic acid extracts from a large number of avocado trees, including low and high producers and different cultivars. Based on the finding that the hybridisation results did not always correspond with the dsRNA PAGE analyses, and that the occurrence of .neither $AV_{1;}$ 2 or 3 could be correlated with disease symptoms, the plant genome was investigated as the possible donor of the dsRNA's.

By probing Southern blots of restriction enzyme fragments of plant DNA with AV_1 specific cDNA, the authors could prove that the dsRNA's originate from the plant genome. Significantly, the DNA sequence for AV_1 dsRNA is also present on the genome of trees that do not contain AV_1 dsRNA. This sequence has a specific location on the plant DNA, as indicated by the precise patterns obtained with different restriction enzymes.

It is not known why these dsRNA species are produced at a constant level in healthy plants. A fascinating question also surrounds the finding that cultivars which do not express dsRNA, may have the genetic potential, by posessing the same DNA fragment that gives rise to dsRNA production in other cultivars.

Findings by the authors contradict the hypothesis that the AV_1 dsRNA species are virus-like. Results not presented in this paper indicate a similar situation in the cases of AV_2 and AV_3. At least part of the sequence for the AV_1, 2 and 3 dsRNA can be found on the DNA of all avocado trees thus far investigated.

REFERENCES

AUSUBEL, F M, BRENT, R, KINGSTON, R E, MOORE, D D, EIDMAN, J G, SMITH, J A & STRUHL, K (eds) 1987. Current protocols in molecular biology. Vol 1. John Wiley & Sons, New York.

BAR-JOSEPH, M, ROSNER, A, MOSCOWITZ, M & HULL, R 1983. A simple procedure for the extraction of double-stranded RNA from virus infected plants. *J Virol Methos* **6**, 1 - 8.

DA GRACA, J V & TRENCHT, T N 1985. Evidence of dsRNA in South African avocados. *S A Avocado Growers' Assoc Yrb* **12,** 72 - 74.

DALE, J L, PHILLIPS, D A & PARRY, J N 1986. Double-stranded RNA in banana plants with bunchy top disease. *J Gen Virol* **67,** 371 - 375.

DODDS, J A & BAR-JOSEPH, M 1983. Doublestranded RNA from plants infected with closteroviruses. *Phytopathology* **73,** 419 - 413.

DODDS, J A, MORRIS, T J & JORDAN, R L 1984. Plant viral double-stranded RNA. *Ann Rev & Phytopathol* **22,** 151 - 168.

FALK, B W & DUFFUS, J E 1984. Identification of small single- and double-stranded RNAs associated with severe symptoms in beet western yellows virus-infected *Capsella bursa-pastoris. Phytopathology* **74,** 1224 - 1229.

GADH, I P S & HARI, V 1986. Association of tobacco etch virus related RNA with chloroplasts in extracts of infected plants. *Virology* **150,** 304 - 307.

GILDOW, F E, BALLINGER, M E & ROCHOW, W F 1983. Identification of double-stranded RNAs associated with barley yellow dwarf virus infections. *Phytopathology* **73,** 1570 - 1572.

HENRIQUES, M C & MORRIS, T J 1979. Evidence for different replicative strategies in the plant tombusviruses. *Virology* **99,** 66 - 74.

JORDAN, R L, DODDS, J A & OHR, H D 1983. Evidence of viruslike agents in avocado. *Phytopathology* **73,** 1130 - 1134.

KAPER, J M & DIAZ-RUIZ, J R 1977. Molecular weights of the double-stranded RNAs of cucumber mosaic virus strain S and its associated RNA 5. *Virology* **80,** 214 - 217.

LE CLERCQ, H Y H 1989. Observations of the quality of avocados on the overseas market. *S A Avocado Growers' Assoc Yrb* **12,** 14 - 16.

LEE, R F 1984. Use of double-stranded RNAs to diagnose Tristeza virus strains. *Proc Fla State Hort Soc* **97,** 53 - 56.

MANIATIS, T, FRITSCH, E F & SAMBROOK, J 1982. Molecular cloning: A laboratory manual. Vol 1. Cold Spring Harbor Laboratory, Gold Spring Harbor. New York.

SAMBROOK, J, FRITSCH, E F & MANIATIS, T 1989. Molecular cloning: A laboratory manual. 2nd Edition. Cold Spring Harbor Laboratory, Cold Spring Harbor. New York.

SISCO, P H, GARCIA-ARÉNAL, F, ZAITLIN, M, EARLE, E D & GRACEN, V E 1984. LBN, a male-sterile cytoplasm of maize, contains two double-stranded RNAs. *Plant Science Letters* **34,** 127 - 134.

TAVANTZIS, S M 1984. Physicochemical properties of potato virus M. *Virology* **133,** 427 - 430.

ZELCER, A, WEABER, K F, BALAZS, E & ZAITLIN, M 1981. The detection and characterization of viral-related double-stranded RNAs in tobacco mosaic virus-infected plants. *Virology* **113,** 417 - 427.