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INVESTIGATION OF THE OCCURRENCE OF VIRUSES AND VIRUS-ASSOCIATED NUCLEIC ACIDS IN AVOCADO

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UITTREKSEL

'n Komplementere DNA-fragment van beweerde avokadovirus 1 was gekloneer en as 'n nukleiensuurpeiler gebruik.

ABSTRACT

A complementary DNA fragment of putative avocado virus 1 was cloned and used as a nucleic acid probe.

INTRODUCTION

There has been an increasing concern about the possible presence of viruses in avocado, ever since the presence of double-stranded (ds) RNA has been indicated (Jordan *et al.,* 1983). Viruses are *a* great threat to the industry, especially where budwood and rootstocks are used constantly, a practice that spreads viruses very rapidly. The fear is well-founded and the possible occurrence of viruses should be investigated. The presence of large molecules of dsRNA (>0,1 x 10^6D) have always in the past been seen as an indication of viruses, either by the presence of dsRNA viruses or replicative forms of single-stranded RNA viruses (Jordan *et al.,* 1983; Morris & Dodds, 1979). Recently there have been reports in the literature of the presence of dsRNA in other crops, which have been shown not to be associated with viral infection, but which are a product of the plant itself (Wakarchuk & Hamilton, 1985).

To date no viral particles have been isolated from avocado, but the presence of dsRNA is very real.

Mainly three different dsRNA patterns have been identified and have been called putative avocado virus 1, 2 and 3 (Jordan *et al.,* 1983). Avocado virus (AV) 1 and AV3 are readily detectable in our South African cultivars, but AV2 has not yet been detected in any field-grown trees.

The question remains as to what the significance of the dsRNA is. The authors found it necessary to characterise these bands and develop reliable indexing tests in case any are shown to be responsible for any disease symptoms.

Conventional techniques for isolating, identifying and characterising viruses did not provide any answers. The authors chose the recombinant DNA route, which provided

(a) an alternate means to identify and study the viruses and their effects through hybridisation studies and sequence data analysis and (b) a probe to identify "virus free" rootstocks and budwood.

This report deals with the production of a DNA probe for AV1.

MATERIALS AND METHODS

DsRNA was isolated from avocado leaves according to the method of Morris and Dodds (1979).

First and second-strand complementary DNA was synthesised to methyl mercuric hydroxide-denatured RNA, using random primers according to the instructions of the Boehringer Mannheim cDNA synthesis kit.

The resulting cDNA was blunt-end ligated to S*ma*l cut and dephosphorylated pUC18 plasmid. Resulting plasmids were transformed into competent *Escherichia coli* JM105 cells. Transformants were screened for the size of cDNA inserts by isolating small-scale bacterial cultures followed by restriction endonuclease digestion with Pstl and EcoRI and agarose gel electrophoresis. Clones containing inserts of approximately 1 000 bp were selected.

Northern blot hybridisations were done to verify the specificity of the probes. The dsRNA was electrophoresed in a 5% polyacrylamide slab gel at 60 V and 30 mA for 18 h in Loening buffer [0,4M Tris, 0,2M Na-acetate, 0,02M EDTA, 1%SDS (ph 7,4) = 10X Loenin buffer]. The relative distances of the bands were measured. The gel was denatured in a 0,1 M NaOH solution for 20 min and then neutralised twice in a 5X TAE solution (1X TAE = 0,04M Tris-acetate,1mM EDTA pH7,5) for 10 min and once for 10 min in 0,5X TAE. The RNA was then electroblotted onto nylon hybridisation membranes in 0,5X TAE at 0,8V for 1 — 2h in the Biorad electroblotting system.

The membranes were baked under UV-light for 3 — 5 min and prehybridised in 50% formamide, 5X SSPE [5X SSPE = 0,6M NaCI, 0,0,75M trisodium citrate, 0.065M NaH_2PO_4 (pH 6,5), 0,01 M EDTA], 0,1% SDS and 0,1% fat-free milk powder.

Hybridisation was at 42°C for 16 h. Membranes were then washed twice for 10 min in a 2X SSC buffer (1X SSC= 150mM NaCI and 15mM sodium citrate, pH7), once in 2X SSC containing 0,1% SDS at 65°C for 15 min and finally in 0,2X SSC containing 0,1% SDS for 15 min at 65°C. After washing, the membranes were autoradiographed at 70°C.

Radioactive labelling of the plasmid probes were performed by nick translation using a BRL-nick translation kit.

Once a positive clone had been obtained for AV1, the probe was hybridised against crude extractions of various avocado trees to determine the incidence of AV1 in different cultivars, as previous work showed a very specific correlation between the incidence of the "viruses" and the cultivar (Da Graca & Trench, 1985; Jordan *et al,* 1983). For the extractions 2,5 g leaves were ground up in liquid nitrogen and 10 mℓ of a 4% para-aminosalicylic acid solution in 1X STE and 8 mℓ phenol was added, which was shaken well and centrifuged at 5 854 g in the SS-34 rotor of a Sorvall RC-5B centrifuge. The supernatant was precipitated overnight at -20°C with two volumes ethanol and 1 mℓ

sodium acetate. The precipitate was centrifuged down and the pellet dissolved in 400 $\mu\ell$ H_2O. A second ethanol precipitation was done and the pellet was resuspended in 100 $\mu\ell$ H_2O.

Of each extract 10 $\mu\ell$ was denatured with 20 $\mu\ell$ 100% deionised formamide, 7 $\mu\ell$ 37% formaldehyde and 2 $\mu\ell$ 20X SSC at 65°C for 15 min. This was cooled on ice and spotted onto nylon hybridisation membranes. These were baked under UV-light for 3 — 5 min and prehybridised. The blots were then probed, washed and autoradiographed as described.

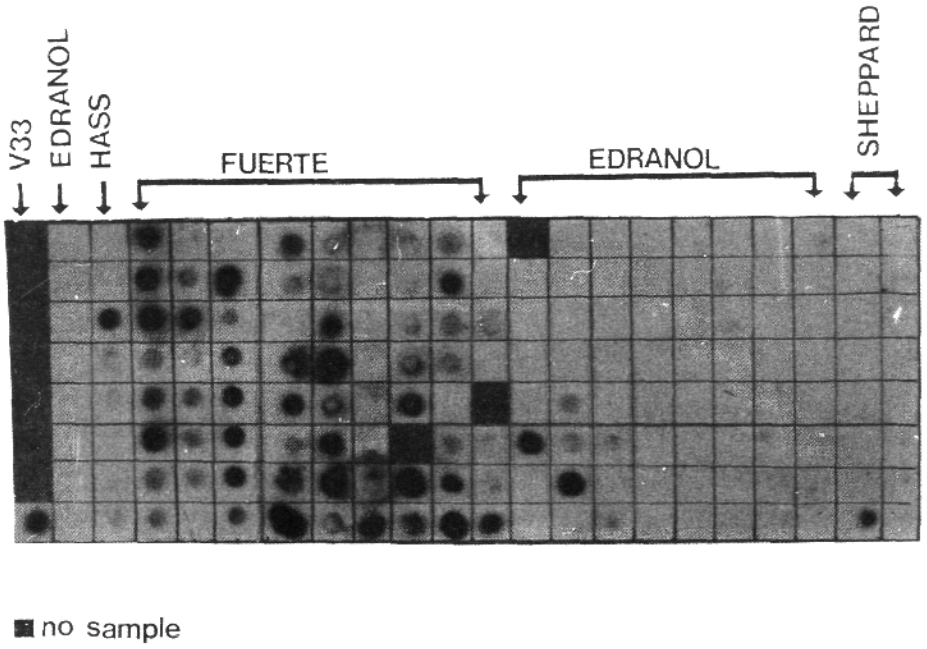

Fig 1 Autoradiograph of a dot blot hybridisation of crude extracts of avocado trees probed with the AV1 specific probe.

RESULTS AND DISCUSSION

A 931 bp complementary DNA fragment of AV1 was cloned and used as a nucleic acid probe. Figure 1 is an autoradiograph of a dot blot hybridisation. Extracts of various avocado trees were hybridised with the probe. AV1 was shown to be present in almost all Fuerte trees. The Edranol cultivar was mostly free of AV1 except for a few trees. The Hass cultivar is reported to be free of AV1 (Da Graca & Trench, 1985; Jordan *et al,* 1983), but here one tree positively hybridises with the probe.

The occurrences of the “viruses” seem to be closely related to cultivar. If indeed these bands prove to be viruses, one can assume that there is a resistance in certain cultivars to the viruses. Alternately if the RNA is proven to be of plant origin, it will then be a trait of each cultivar.

The same protocol is being followed with the other two putative avocado viruses. Once

probes for all three viruses have been obtained, more significant deductions can be made as to the correlation of disease with the incidence of the three "viruses".

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