South African Avocado Growers' Association Yearbook 1985. 8:72-74

EVIDENCE OF DS RNA IN SOUTH AFRICAN AVOCADOS

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OPSOMMING

Die ekstraksie en PAGE skeiding van dubbel-band RNA is gebruik om die teenwoordigheid van avokado virusse 1, 2 en 3 in 'n klein monster Suid-Afrikaanse borne te onderskei. Die verwantskap tussen sekere virusse en sekere kultivars toon ooreenstemming met resultate wat in Kalifornia verkry is. Sommige verskille tussen Suid-Afrikaanse en Kaliforniese resultate word bespreek. Voorlopige aanwendings van die tegniek, naamlik ondersoek na die teenwoordigheid van virusse in kwynende kommersiele borne op Duke 6, en gesonde moederbome in die Avokadoverbeteringskema, word beskryf.

SUMMARY

Double-stranded (ds) RNA extraction and separation on polyacrylamide gels was used to infer the presence of avocado viruses 1, 2 and 3 in a small number of South African trees. The association of particular viruses with particular cultivars showed general agreement with results obtained in California. Some differences between local and Californian findings are discussed. Preliminary applications of the technique in screening declining field trees on Duke 6 and apparently healthy Avocado Improvement Programme mother trees for viruses are described.

INTRODUCTION

The extraction of double-stranded (ds) RNA associated with viruses several fungi and plants led to the use of ds RNA profiles as a means of identification of RNA-containing plant viruses (Morris & Dodds, 1979). Dodds *et al.* (1984) expressed their rationale as follows: "The ds RNA approach is based on the premise that plants not infected with RNA viruses or virus-like agents do not contain readily detectable amounts of homogenous segments of high molecular weight (>0.1 x 10^6) ds RNA." As these authors point out, the detection of reproducible, high molecular weight ds RNA profiles in plant extracts implies the presence of (1) a *ds* RNA virus; or (2) replicative forms of a ss RNA virus; or (3) an as yet unidentified entity.

Characterization of reproducibly produced ds RNAs has been carried out on only a few plant viruses (Dodds *et al.,* 1984) and the technique is probably not yet fully developed as a diagnostic tool. Dodds *et al.* (1984) have reviewed methods of extraction of ds RNA, and the limitations of the technique, which include: (1) ds RNA extraction is of no

use in identifying DNA-containing plant viruses, (2 (there have been some reports of finding high molecular weight ds RNA in healthy plants, (3) cryptic viruses characterized thus far have been found to contain ds RNA genomes but are not known to be associated with clearly defined plant disease. Healthy controls are therefore essential if the association between a particular ds RNA and a particular disease is to be established.

Jordan *et al.* (1983) found three distinct and reproducible ds RNA patterns in avocados, and proposed the existence of three avocado viruses to explain them. The status of a fourth ds RNA pattern is uncertain (Jordan *et al.* 1983; Ohr *et al.* 1983). Known information about AV1, 2, 3, and 4 is summarized in Table 1.

The research in this paper was undertaken simply to determine the virus status of a haphazard sample of South African avocado cultivars, mostly ¡n the University of Natal collection. Although initially there was no reason to suspect that viruses were involved in any disease syndrome in South Africa, it was decided to use the ds RNA method to screen some Hass on Duke 6 trees suffering from a serious decline and trunk pitting problem in the Transvaal (Moll, 1984).

METHODS AND MATERIALS

The techniques used for isolating ds RNA viruses from avocados under South African conditions were based on the method of Jordan *et al.* (1983), and its modifications (Dodds, pers. comm., 1983). All centrifugations were at 8000g in the JA-20 rotor of a Beckman J2-21 Centrifuge. Between 7 and 12 g of mature leaf tissue was rinsed, dried and ground in liquid nitrogen in a mortar and pestle. The resultant powder was transferred to a beaker at room temperature and allowed to thaw slightly, before adding 14 ml double-strength STE buffer, pH 6.8, 2ml 10% sodium dodecyl sulphate, 20 ml water-saturated phenol and 14 mg of bentonite. The mixture was shaken in a cold room at O°C for 30 min. After centrifugation for 1 5 min, the aqueous phase was pipetted off, the volume adjusted to 20 ml with STE buffer, pH 6.8, and 3.8 ml 95% ethanol added ("sample"). A column was prepared by mixing 2.5g of Whatman CF-11 Cellulose powder with approximately 30 ml of STE-1 5 buffer and allowing drainage, after which the sample was passed through the column. The column was washed with 80 ml STE-1 5 in two stages, and ds RNA eluted with 14ml STE in stages of 1, 6, 6, and 1 ml (Bar-Joseph, pers. comm., 1983). After the addition of 28 ml 95% ethanol and 0.7 ml 3.0M sodium acetate, pH 5.5, the sample was frozen overnight. Nucleic acid was precipitated by centrifugation for 30 min. The invisible pellet was resuspended in 1 ml STE, 2 ml 95% ethanol, and 0.05 ml 3.0 M sodium acetate, pH 5.5, and the sample frozen for 4 h. After centrifugation for 30 min, the in visible pellet was resuspended in 0.2 ml reservoir buffer (da Graca, 1981). One or two drops of glycerol were added to each sample, and 100to200µl layered onto 5% polyacrylamide gels. Gels were run at 3.5 mA per gel for 15 min, followed by 6 mA per gel for 5.5 to 6 h. Gels were prepared and stained with 3,5µg/ml ethidium bromide for 15 min, as described by da Graca (1981).

The avocado cultivars tested were Hass, Fuerte, Pinkerton, Edranol, G6/West Indian, Santana/Ettinger, Duke 7, Duke 7/Ettinger, Ettinger/Duke 7, Sharwil/Ettinger, Pinkerton/Ettinger, Bacon/Duke 7, Ferdyn/West Indian, G755, A11/6 and A3/7,

maintained in the phytotron and glasshouses of the University of Natal in Pietermaritzburg, and Fuerte, Edranol, and Hass, from a farm near Baynesfield. Four mother trees each of important rootstock, seed and scion cultivars, namely Edranol, Ryan, Duke 7, Hass and Fuerte, were also tested, to examine the feasibility of testing trees in the Avocado Improvement Programme for virus content using the ds RNA technique.

With respect to the Duke 6 related problem, leaves were sampled from Duke 6 trees from the University of Natal in Pietermaritzburg, the Citrus and Subtropical Fruit Research Institute and Hall & Sons in Nelspruit, and declining Hass/Duke 6 and Hass/Duke seedling from Westfalia Estate near Tzaneen. Two plants each of Duke 6, Duke 7 and G6, inoculated from a source of stem pitting (S.P. van Vuuren, pers. comm.), and a single control plant of each cultivar, were also tested.

In the present study, avocado viruses were identified only by comparison with the patterns published by Jordan ef *al.* (1983). No ribonuclease characterization and molecular weight determination were carried out, although ds RNA extracts of TMV and CMV-infected hosts were prepared in the early stages of the work to perfect the technique and give some idea of the positions to which ds RNA bands from avocado migrated.

RESULTS

Two ds RNA patterns were readily identified as those of AV2 and AV3 from their appearance and position (Fig. 1, a and b). A band corresponding to the major segments of AV1 was regularly observed; a single band (Fig. 1c) was common but a doublet as described by Jordan *et al.* (1983) was observed in the same position on a number of occasions (Fig. 1d). Although the minor, highly mobile segment associated with AV1 was never observed in the present work, the slowly-migrating band has been tentatively identified as the ds RNA of AV1. Other minor bands reported by Jordan *et at.* (1983) as being present below the doublet, were frequently, but not always, observed.

Of the avocado cultivars tested most appeared to be free of infection with viruses detectable by the ds RNA extraction method (Table 2). Of particular interest were the field and phytotron Duke 6 and Duke 7 plants, which were consistently free of ds RNA, in spite of the presence in Duke 6 of severe stem pitting. Almost all the Fuerte plants showed both AV1 and AV3. Four Hass tested carried AV3 and one field tree gave negative results in two tests. Edranol appeared to be free of viruses in most tests. The single Sharwil tested contained AV3. A Natal rootstock selection, A11/6, proved to be most reliable in providing clear bands of AV2 and AV3. The virus content of Edranol, Fuerte, Hass, and Duke 7 (Table 3) mother trees showed good agreement with that found in other sources. All four Ryan mother trees contained AV3.

Numerous attempts were made to detect avocado viruses in Duke 6 plants, either on their own roots or on rootstocks, but without success (Table 2). Four of these were field trees from Nelspruit and Pietermaritzburg, the latter showing stem pitting symptoms (Table 2). In addition, leaves from the scions of three Hass/Duke 6 trees showing strong symptoms of decline, and three Hass Duke seedling trees from Westfalia Estates

contained AV3, which was typical of the Hass trees from other sources (Table 2). Two seedlings each of Duke 6 and Duke 7, inoculated about 8 mo previously from a source of stem pitting, were also tested, as were control indicator plants. Two G6 indicators inoculated from the stem pitting source, as well as the control, contained AV1 (Table 2).

Reproducibility was fairly good. However the brightness of bands varied between batches and very occasionally, bands were obvious on gels run from one extraction but not from another performed on the same source material. It is clear that for critical work it will be necessary to carry out at least two, and preferably three, extractions on each tree under test.

Bands from some of the sources were consistently very faint. In attempts to concentrate any ds RNA in Duke 6, about 10g of leaf material were used initially and several extracts from the same tree were pooled after the second centrifugation to give an effective starting mass of as much as 60g. Two standard extractions were performed on leaves and bark of the two Duke 6 trees from Nelspruit.

DISCUSSION

AV1, 2 and 3 have been readily detected in a number of South African trees, including some widely-grown cultivars, using techniques developed at Riverside. Although the researchers there have made progress towards characterizing AV1, 2 and 3, and there have been tentative suggestions that AV2 and AV3 are associated with black streak disease of avocados (Ohr ef *at.,* 1983), their importance in causing avocado disease is poorly understood.

The demonstration that AV1,2, and 3 are seed-transmitted (Ohr *et al.*, 1983) is a good reason for testing rootstock, seed-source and budwood mother trees destined for the improvement programme in South Africa. Since elimination of viruses in avocados has not been completely successful, the presence of viruses should not be considered a basis for elimination of mother trees at present, but simply used to interpret future problems which may be explained by virus content.

The failure to detect ds RNA in Duke 6 does not eliminate the possibility that the decline and pitting are caused by a virus. The causal virus might be present in levels too low to detect and the mass of starting material might need to be increased above the levels already tested or it might be a DNA virus. Another possibility is that the stem pitting is a morphological characteristic of Duke 6, as already suggested by Moll (1984), and that the decline is a bud-union disorder due to a virus or viruses contained in the Hass scion. AV3 is not graft-transmissible (Ohr *et al.,* 1983).

ACKNOWLEDGEMENTS

We acknowledge a grant from SAAGA which made it possible to employ one of the authors (T.N.T.). We also thank the following for avocado leaf samples: the Antel family, Professor B.N. Wolstenholme, Westfalia Estate, the Citrus and Subtropical Fruit Research Institute, and H.L. Hall & Sons.

TABLE 1 Summary of available information about avocado viruses 1, 2 and 3 (Jordan *et al.*, 1983; Ohr *et al.*, 1983).

Pattern no.	No. of dsRNA segments	Characteristics of ds RNA segments *1	Other properties of viruses
1	3	Minor, one, 0.55 x 10⁶	High rate of seed transmission
		Major, doublet, 6.0 - 6.5 x 10⁶	Graft transmissible.
		Minor bands always present below major bands	Possibly eliminated in hot water and hot air treatment
2	1	Major, one, 3.0 x 10⁶	High rate of seed transmission. Graft transmissible. Implicated in blackstreak disease?
3	3	Major, three, 2.0 x 10⁶	High rate of seed transmission.
		1.9 x 10⁶	Not graft-transmissible
		1.7 x 10⁶	Implicated in blackstreak disease? Not heat sensitive in hot water treatments?
4?	1	Major, one, 12-14 x 10⁶	No information

*** 1 Molecular weights given in daltons.**

TABLE 2. Avocado viruses found in field, glasshouse and phytotron grown plants of 15 avocado cultivars.

Cultivar	No. of trees tested *1	Avocado viruses found *2	Avocado viruses reported by Jordan *et al.*, 1983
Hass	2	3	3, 2-3
Hass	1	-	
Fuerte	5	1+3	1+3
Sharwil/Ettinger	1	3	
A11/6	1	2+3	
Edranol	4	-	
Duke 6	4	-	
Duke 7	4	-	
Pinkerton	2	-	
G6/West Indian	1	-	1,1+2
Santana/Ettinger	1	-	
Ettinger/Duke 7	1	-	
Bacon/Duke 7	1	-	-,2
Ferdyn/West Indian	1	-	
G755	1	-	
A3/7	1	-	

* 1 Most plants tested at least twice, and usually more frequently.
* 2 Identification of avocado viruses based on dsRNA patterns found, as explained under Methods and materials.

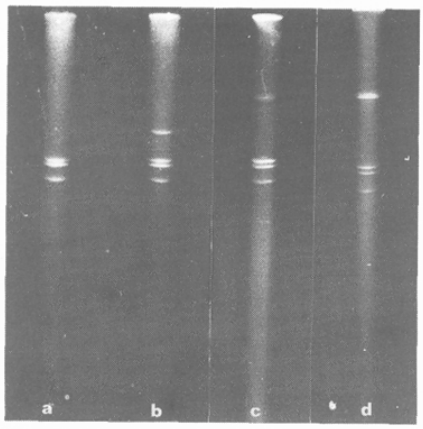

Fig. 1: Double-stranded RNA patterns obtained from avocado leaves: a – AV3; b – AV2 and AV3; c – AV1 major band and AV3; d – AV1 major band showing doublet and AV3.

TABLE 3. Virus content of mother trees of 5 cultivars destined for the Avocado Improvement Programme

Cultivar	No. of trees tested *1	No. of trees positive *2	Viruses identified
Edranol	4	0	
Fuerte	4	4	3+1
Hass	4	4	3
Ryan	4	4	3
Duke 7	4	0	

*1 Trees tested twice

*2 Positive in at least one out of two tests

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