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Monitoring avocado sunblotch disease in South Africa

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SYNOPSIS
The dot-blot hybridisation technique was used to test 3 125 avocado nursery trees on a commercial scale in South Africa. Each tree was tested twice with a synthetic probe of 23 nucleotides. Only 2 per cent of the trees tested were positively infected and are therefore symptomless carriers of the disease.

INTRODUCTION

Sunblotch is a widespread disease, which affects the performance and fruit quality of avocado (*Persea americana* Mill) in many avocado producing countries (Mohammed & Thomas, 1979).

The disease was first described by Colt (1928), who attributed it to physiological stress caused by sunburn. Palukaitis, Hatta, Alexander & Symons (1979) reported a low molecular weight circular RNA associated with the disease. Symons (1981) determined a molecular weight (mw) of about 6-7 x 104 daltons and a sequence of 247 nucleotide residues for the avocado sunblotch viroid (ASBV) RNA, while Semancik & Desjardins (1980) detected five low mw RNAs in extracts of infected tissue, ranging from 61 000 to 185 000 daltons.

ASBV can be transmitted through seed, grafting, pollen and from diseased scions to healthy rootstocks (Desjardins, Drake & Swiecki, 1980; Palukaitis el. al, 1979). Since sunblotch is probably the most important budwood sanitation problem for nurserymen and also is of economic importance to the avocado farmer, effective control of this disease was urgently required. One approach of controlling the disease was by screening mother trees for infection, with the aim of supplying the industry with ASBV-free nursery trees. This paper reports on the indexing of 13 South African nurseries for ASBV by means of the dot-blot hybridisation technique developed by Bar-Joseph, Segev, Twizer & Rosner (1985).

MATERIALS AND METHODS

A single stranded DNA of 23 nucleotides, synthesised in Israel was used as the probe. Thirteen nurseries took part in the scheme and a total of 3 125 trees were involved. Each tree was tested twice during a 15-month period. Ten young leaves and, if present,

flush or flowers were collected from each tree, refrigerated and transported to the laboratory. Sunblotch positive and negative leaf samples were included in each batch. Sunblotch positive leaf samples were also kept at 4°C for up to six months and were extracted at monthly intervals.

Nucleic acid extraction method

The protocol for ASBV extraction used in this study was essentially similar to that described by Bar-Joseph, Segev, Blickle, Yesodi, Franck & Rosner (1986). Leaves were wiped with cotton wool, cut into strips and 2,5g samples used for extraction. Leaf samples extracted during the first round were homogenised in an Ultra Turrax, while samples from the second round were ground in a mortar and pestle in liquid nitrogen. The powder obtained with the second method was transferred to a centrifuge tube and 10 ml 4 per cent p-Aminosalicylic acid (PASA) (Sigma), as well as 8 ml water-saturated phenol were added. After centrifugation for 10 minutes at 8 000 g in a Sorval SS 34 rotor, the aqueous phase was removed and 1 ml 3M sodium acetate (pH 5,2) plus 20 ml 75 per cent ethanol added to it before being frozen overnight at -20°C. Nucleic acids were precipitated by centrifugation for 30 minutes. The transparent pellet was resuspended in 400 µl double distilled water (ddw) and transferred to an Eppendorf tube supplemented with 100 µl 3M sodium acetate and 1 ml ethanol (95 per cent) and frozen for three hours. After centrifugation for five minutes, the transparent pellet was resuspended in 100 µl ddw and stored at -20°C until hybridisation.

Hybridisation and autoradiography

Plant extracts, extracted during the first round were spotted (3 µl) onto nitrocellulose BA 85 (Schleiger & Schull) filter paper, while extracts from the second round were spotted (3 µl) onto Hybond N nylon filter paper (Amersham). Sunblotch positive and negative samples were included throughout as controls. Filters were hybridised with the 32 P-5-end-labelled probe and autoradiographed on X-ray film (3M).

RESULTS

Results obtained during the first round were difficult to interpret due to non-specific binding and background staining on the nitrocellulose paper. This problem was overcome during the second round by substituting the nitrocellulose paper with nylon filters. ASBV was detected in 10 of the 13 nurseries examined. In total, 2 per cent of the 3 125 trees involved, tested positively (Table 1). Not one of these trees showed any sign of sunblotch, and they were therefore symptomless carriers of the viroid.

The reliability of the indexing technique was confirmed by positive results obtained with sunblotch positive leaf samples after storage for up to four months at 4°C.

TABLE 1 Occurrence of avocado sunblotch viroid in South African nursery trees.

Nursery	Number of trees indexed	% Trees testing positive
A	1621	1

B	200	2,5
C	76	9,2
D	200	3,5
E	200	6,0
F	42	2,3
G	286	1,4
H	100	0
1	50	0
J	100	0
K	100	2
L	100	4
M	50	4
Total	3125	2%*

Percentage based on total number of trees positive out of total examined.

DISCUSSION

The small percentage of positive tests indicates that the majority of mother trees in South Africa are free from ASBV. However, the fact that the positive trees were symptomless, suggests that they can be important sources of contamination. It is known that 86-100 per cent of fruit from symptomless carrier trees transmit ASBV, compared to only 2-5 per cent of fruit from symptomatic trees (Mohammed & Thomas, 1979). Furthermore, seedlings from symptomless mother trees usually never show any signs of disease, but scions grafted on to them may develop symptoms (Desjardins et al, 1979). Elimination of ASBV positive trees identified in this investigation should thus greatly contribute to the reduction of avocado sunblotch in South Africa.

Previously detection of ASBV infected propagation material was based on bio-assays with indicator plants and on PAGE, both slow and laborious techniques (Diener, 1983). Hybridisation of samples with a synthetic probe, as used in the present investigation, proved to be a quick, simple, sensitive and reliable means of indexing plants on a commercial scale for ASBV. The 23 mer oligonucleotide length and specifically selected base pairs of the present probe ensured a high specificity towards ASBV. Bar-Joseph et al (1985) reported that the symptomless ASBV isolate, which has a smaller genome size than the symptom expressing isolate (Spiegel, Alper & Allen, 1984), showed clear hybridisation with this probe.

Lastly, testing of each tree twice reduced the possibility of missing ASBV due to seasonal variations in viroid concentration or to inherent differences in the incident of the viroid. Allen & Dale (1981), for instance, found that the concentration of ASBV varied from 5 to 5 000 dry mass equivalent between leaves and young blossoms on a single infected tree.

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