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APPLICATION OF SYNTHETIC OLIGONCLEOTIDE PROBES FOR DETECTION OF AVOCADO SUNBLOTCH VIROID

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OPSOMMING

Verskillende enkeldraad DNA fragmente (17 tot 23 nukleotiede lank) komplementer aan dele van die avokadosonvlek viroied (ASBV) RNA is gesintetiseer. Hierdie oligonukleotiede was aan die 5' kant gemerk met ^{32}P en gebruik as hibridisasie peilers om ASBV RNA monsterkolle op nitrosellulosefilters op te spoor. Die sensitiwiteit en spesifisiteit van hierdie sintetiese peilers is met die RNA van verskeie ASBV isolate ondersoek. Die voordele van sintetiese peilers vir die diagnose van viroiede en plantviroide word bespreek.

SUMMARY

Several single stranded DNA fragments (17 23 nucleotide long) complementary to Avocado Sunblotch Viroid (ASBV) RNA were synthesized chemically in vitro. The oligonucleotides were 5' end labelled with ^{32}P and used as hybridization probes for detection of ASBVRNA samples, dot blotted on nitrocellulose filters. The sensitivity and specificity of these synthetic probes for hybridization with RNA of several ASBV isolates was demonstrated and the advantage of applying synthetic probes for viroid diagnosis is discussed.

INTRODUCTION

Sunblotch, a graft and pollen transmitted disease is probably the most important bud wood sanitation problem of the avocado industry. The disease affects both the performance of the infected trees and the quality of their fruits. Recently a low molecular weight circular RNA has been associated with the disease. (Thomas & Mohamed 1979; Dale & Allen 1979; Palukaitis *et al.,* 1979; Semancik & Desjardins, 1980). Infectivity studies with highly purified RNA preparations have confirmed the viroid nature of ASBV (Allen *et al.,* 1981, Utermohlen *et al.,* 1981). The sequence of 247 nucleotide residues of the single-stranded circular RNA from ASBV was determined and a secondary structure model in which 67% of the ASBV residues are paired, was proposed (Symons, 1981).

The routine procedure for the diagnosis of sunblotch, using graft transmission to indicator seedlings is laborious and time consuming as 1½— 2 years may be required for characteristic symptoms to be developed. Recently, several rapid diagnostic methods have been developed for ASBV including (a) electrophoretic fractionation of

RNA from infected plants in polyacrylamide gels (De Graca & Mason, 1983; Utermohlen & Ohr, 1981) (b) liquid hybridization with an ASBV complementary DNA (cDNA) (Allen & Dale, 1981; Palukaitis *et al.,* 1981) and (c) dot spot hybridization of *in vitro* ^{32}P labelled ASBV-RNA with viroid samples potted on nitrocellulose paper (Rosner *et al.,* 1983). Recent methodological developments in oligonucleotides synthesis (Crocket, 1983) enable the construction of DNA fragments complementary to previously sequenced RNA molecules. In the present paper we demonstrate the application of several synthetic probes for ASBV diagnosis.

PROCEDURE AND RESULTS

Leaf extracts from healthy and ASBV infected avocado plants were spotted on nitrocellulose paper and hybridized with ^{32}P labelled synthetic probes. A detailed description of the experimental protocols was published recently (M. Bar-Joseph *et al.,* J. Virol Methods, In press) Only the spots of ASBV infected samples gave positive hybridization (Fig. 1A). Sap samples from infected plants were serially diluted and found to give positive hybridization at sample dilutions of 1:256 (Fig. 1 B).

DISCUSSION

Separation of ASBV or other viroid infected propagation material from healthy ones was based on bioassay on indicator plants and by PAGE electrophoresis. These assays are slow and laborious (Diener, 1983). Hybridization with the synthetic ASBV probes to samples bound to a solid support was at least 128 256 times more sensitive than PAGE (not shown). The higher sensitivity of dot spot hybridization and its simplicity (Owens & Diener, 1981 (appear to be extremely important for ASBV diagnosis as previous studies showed variations of over 1 000 -10 000 fold variation in ASBV concentration in different trees and branches (Dale & Allen, 1979; Palukaitis, *et al.* 1979). Dilution experiments carried out in a previous study reference clearly indicated that even among the leaves of the same branch 100-fold variations in viroid content are quite common. Interestingly the symptomless ASBV isolate with a genome size smaller than that of the symptom expressing isolates (Spiegel *et al.,* 1984) showed clear hybridization homology with the presently used probes.

The main advantages of applying synthetic probes for hybridization are (a) the availability of large quantities of single stranded synthetic probes and long stability (b) the ease of diagnosis by ^{32}P end labeling of synthetic oligonucleotides(c) the possibility of constructing a battery of oligonucleotides useful for virus and viroid strain typing (d) the possibility of constructing probes to selected genomic sequences with low internal nucleotide base pairing (e)the possibility of constructing probes without the need of propagating the suspected viroid pathogen. This seams to be extremely important when considering the need to prepare probes for the diagnosis of foreign viral pathogens.

In conclusion and as a practical consideration the avocado propagator and grower is reminded that while remedies are now available for most of the destructive fungal problems sometimes even of a post-infection stage, virus and viroid problems, cannot be cured once they become established in the infected avocado tree. Thus varietal

improvement schemes should include both in the interim and in their long term phase facilities for rapid and accurate ASBV diagnosis.

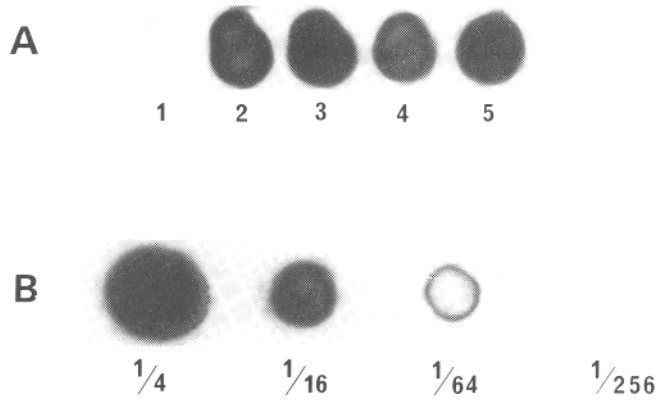

FIG. 1.
Detection of ASBV in plant material by dot spot hybridization. A ^{32}P labelled synthetic cDNA probe was used for hybridization. Leaf extracts were spotted on nitrocellulose paper. (A) Samples from uninfected avocado leaves (spots 2-5) and four Hass cv. plants infected with avocado sunblotch viroid (spots 2-5). (B) The sap sample from an infected plant was serially diluted 1/4; 1/6; 1/64 and 1/256.

REFERENCES

ALLEN, RN & DALE, JL 1 981. Ann. Appl. Biol, 98, 451.
ALLEN, RN, PALUKAITIS RH & SYMONS, RH. 1981. Aust. Plant Pathol. 10, 31.
CROCKET, GC. 1983. Aldrich mica Acta. 16, 47
DALE, J. & ALLEN, RN. 1979, Aust. PI. Path., 8,3
DIENER, TO. 1983, Viroids Adv. Virus Res., 28,241
DE GRACA, JV & MASON, TE. 1983, S. Afr. Avo. Gr. Ass., Yb. 6, 83
PALUKAITIS, P, HATTA, T, ALEXANDER D-Mc E & SYMONS, RH. 1981, Virology, 99, 145.
PALUKAITIS, P, RAKOWSKI, AG, ALEXANDER D-Mc E & SYMONS, RH. 1979, Ann. Appl. Biol., 98, 439
ROSNER, A, SPIEGEL, S, ALPER, M & BAR-JOSEPH, M. 1983, PI. Mol. Biol., 2,15.
SEMANCIK, JS & DESJARDINS, PR. 1980. Virology, 104, 117
SPIEGEL, S, ALPER, M, & ALLEN, RN. 1984, Phytoparasitica, 1 2, in press.
SYMONS, RH, 1981, Nucleic Acid Res., 9, 6527
THOMAS, W, & MOHAMED, NA, 1979, Aust. Plant. Pathol. 8,1
UTERMOHLEN, JG, & OHR, HD, 1981, Plant Dis., 65,800.
UTERMOHLEN.JG, DRAKE, RJ, DESJARDINS, PR, AND SEMANCIK, JS, 1981, Phytopathology, 71, 909.