Diagnosis of Citrus Exocortis and Hop Stunt-Homologous Citrus Viroids by Oligonucleotide Probes

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ABSTRACT. Three CEVd and two HSVd oligonucleotides have been evaluated as probes to diagnose citrus viroids in electroblot hybridization analyses in comparison with full-length CEVd and HSVd cDNA probes. CEVd-3 probe was the only oligo probe specifically reacting with different CEVd isolates in citrus or tomato and with CSVd (chrysanthemum stunt viroid) in tomato, but not with healthy controls. HSVd-5 oligo recognized only CVd-II infected samples. HSVd-3 oligo probe recognized CVd-IIa RNA, but not CVd-IIb, and allowed to discriminate CVd-IIa.

Bidirectional and sequential polyacrylamide gel electrophoresis (PAGE) have introduced a real innovation for the diagnosis of viroids (10,12). Since 1983 we have tested by PAGE samples collected directly from field plants by using young bark extracts and collecting samples during summer months (6). More recently, cloned or cDNA probes have been valuable in diagnosing citrus viroids and showing the lack of homology among viroids belonging to different groups (1, 2, 3, 5). We have also devised an oligo probe that enabled us to detect several CEVd isolates (7). In this paper, we confirm the possibility of detecting CEVd and CSVd with the probe CEVd-3 and have assayed other oligo probes to recognize different viroid-RNAs belonging to CVd-II group. Our probes were also compared to those devised by other researchers (11).

CEVd oligonucleotides were devised on a Visvader and Symons sequence of CEVd-A(13). HSVd oligonucleotides were devised on a hop stunt viroid isolate from grapefruit in Israel (9). Moreover, two HSVd oligonucleotides, devised by Sano *et al.* (11), have been synthetized (Tab 1). The oligos were prepared by phosphoramidite chemical synthesis (7).

As routine, 5-10 g of green bark collected from 2-3-month- old flushes of field citrus plants were used as starting material. Healthy and viroid-infected plants, previously PAGE analyzed for viroid pattern, were sampled. In total, six CEVd and eight CVd-multi infected samples from different sources were analyzed at least twice. Nucleic acids from citrus plants were phenol-extracted and partially purified by cellulose CF-11 chromatography (4). Nucleic acids from young CEVd- or CSVd-stem inoculated Rutgers tomato were extracted as suggested by Macquaire *et al.* (8). After denaturing PAGE low molecular weight RNAs were directly

TABLE 1
OLIGONUCLEOTIDES DEVISED ON CITRUS EXOCORTIS (CEVd) AND HOP STUNT (HSVd) VIROID SEQUENCES AND TESTED AS PROBES IN HYBRIDIZATION ANALYSIS

Name	Sequence	Nucleotide No.
CEV-1	5'CGAAAGGAAGGAGACGAGCTCCTG 3'	175-198
CEV-3	5'TTCCTCCAGGTTTCCCCGGGGATCCCTGAA 3'	84-113
CEV-4	5'TTGAAGCTTCAGTTGTTTCCACCG 3'	271-294
HSV-1[z]	5'GTTGCCCCGGGGCTCCT 3'	72- 88
HSV-2[z]	5'GGTAAGTACCTCCCT 3'	51- 65
HSV-3	5'CTCCTACGCCTCTCGCTGGATTCT 3'	95-118
HSV-5	5'ACCGAGAGGTGATGCCACCGGTCG 3'	215-239

[z]The sequence was devised by Sano, *et al.* (11).

electroblotted from gels to nylon Hybond N membranes (1). CEVd and HSVd DNA full-length probes were prepared, amplified and P^{32}-labeled by nick-translation as described previously (1). Oligonucleotides were labeled with Y-P^{32} ATP and the T4 polynucleotide kinase method (11). CEVd-1 and CEVd-4 oligo probes recognized CEVd but they gave nonspecific signals with healthy extracts. CEVd-3 probe hybridized with all CEVd isolates tested and did not with healthy citrus or tomato samples, or citrus samples infected with CVd-IIa and CVd-IIb, confirming that it is specific for CEVd in citrus. CEVd-3 also recognized CSVd (a different member of the PSTVd group) in extracts from inoculated tomato plants. With HSVd-oligo probes, best results were obtained with HSVd-3. By hybridizing electroblotted membranes, this probe specifically recognized CVd-IIa, but did not hybridize with CEVd, CVd-IIb, CCCVd-RNA_1 (marker) or healthy samples. HSVd-5 probe recognized CVd-IIa and CVd-IIb, but not CVd-I, CVd-III or CVd-IV. Hybridization analysis accomplished by probes devised by Sano, *et al.* (11) and viroid-RNAs of citrus samples showed that HSVd-1 recognized CVd-IIa and CVd-IIb RNAs, but did not recognize CEVd or healthy samples. The same results were obtained when companion membranes were hybridized with a full-lengh cDNA HSVd-probe. HSVd-2, selected specific for HSVd-grapevine, did not recognize CVd-IIa, CVd-IIb or healthy samples.

Our results showed that oligo probes were suitable to diagnose citrus exocortis viroid, citrus viroids group II and citrus viroid IIa confirming their capability to detect CVd group II and CEVd, the most frequent in citrus, even in mixed infections with other viroids. As expected, our probes behaved similarly to those prepared by Sano, *et al.* (11) devised from the same region. According to the result obtained, CVd-II RNAs infecting our plants differed from HSVd-grapevine strain since no hybridization signal was detected with HSVd-2. From a practical viewpoint it must be stressed that the samples processed in this study were collected from field plants, as it is currently done in our laboratory for PAGE tests. Compared to other tests, hybridization with oligo probes appears as sensitive as cloned full-length probes, and more sensitive than electrophoretic analysis. Moreover, mixing probes CEVd-3 and HSVd-5 could allow simultaneous detection of CEVd and CVd-II. CEVd-3 is valuable to detect also some PSTVd members as CSVd, whereas HSVd-5 would very likely recognize all the strains of the HSVd family.

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