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BARD

FINAL REPORT

PROJECT NO. US-700-83

**Detection of Citrus and Avocado Virus Diseases
by Analysis of Viral Genomes**

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BARD Grant #US-700-83

Final Report

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Detection of citrus and avocado virus diseases by analysis of viral genomes.

ABSTRACT

The project was aimed at evaluating the use of certain nucleic acid analyses and methods for the detection of viruses of subtropical plants such as citrus and avocado. Such techniques can complement existing greenhouse and serological detection methods, especially as tools for isolate discrimination.

Most of the work centered on dsRNA analysis of citrus tristeza virus. The method was optimized and found adequate for testing field trees at times when environmental conditions were not extreme. Reliable and strain specific patterns of dsRNAs were detected in some hosts, including sweet orange, citron and Citrus excelsa. In other hosts such as grapefruit and lemon dsRNA profiles could be reproducibly changed, indicating that mixtures of strains may have been present in the isolates before they were passed through these hosts. Most isolates that were unable to cause seedling yellows or stem-pitting reactions in grapefruit, lemon and sour orange seemed to lack a Mr 0.5×10^6 dsRNA that was prominent in other CTV isolates.

Synthetic probes were made based on published sequence data for avocado sunblotch viroid and tobacco mosaic virus, and these were shown to be adequate for specific detection of these agents.

INTRODUCTION AND OBJECTIVES

The project funded by BARD grant #US-700-83 was aimed at evaluating the use of certain nucleic acid analyses and methods for the detection of viruses of subtropical plants. The need for appropriate laboratory assays to confirm, complement or replace greenhouse indexing for known viruses and their strains of crops such as citrus and avocado is generally well recognized, and has resulted in a considerable expenditure of research time by plant virologists in Israel, Florida and Riverside, as well as in other centers for research on such crops. Techniques which will detect previously unrecognized agents are also needed.

The stated objectives of the project were two-fold as originally proposed and are summarized as follows.

1. To diagnose viruses of subtropical fruit tree crops by detection of nucleic acids.

- a. analyze dsRNAs of citrus viruses in the collection at UCR
- b. make hybridization probes for detection of target viruses
- d. give special emphasis to detection of seedling yellows forms of CTV.
- e. use similar approaches to investigate avocado viruses.

2. To select and analyze cross protecting strains of CTV.

The second objective was requested to be minimized by the review panel, was not included in the revised objectives, and will not be further discussed other than to note two publications, Roistacher and Dodds, 1987 and Roistacher, Dodds and Bash, 1988.

MAIN REPORT

Several publications have resulted from the project and copies of them will form the majority of this section of the report. They are listed in the references section and copies or reprints are collected in the Appendix. A general summary of results for each of the objectives follows, and can be used as a guide to the pertinent publications.

Analysis of dsRNAs of citrus viruses in the collection at UCR with special emphasis given to detection of seedling yellows forms of CTV (Objectives 1a and 1d combined).

Emphasis was placed on the development of dsRNA analysis for CTV in various citrus host, and adequate methods were developed (Dodds, Tamaki and Roistacher, 1985, Dodds et al. 1985, Dodds et al., 1987a) whereby good results could be obtained from 2.0 g of

bark from field trees or greenhouse grown plants. The major dsRNA detected was twice the size of the genomic RNA, which was sized for the first time by ourselves (Bar-Joseph et al., 1985a). Additional dsRNAs of smaller size were the focus of much of the comparisons which follow. Recovery of major and minor dsRNAs by the methods developed was used to determine the effect of host on dsRNA for a given isolate (Dodds et al., 1987a). In general hosts that gave high yields of dsRNA of similar quality from experiment to experiment were sweet orange, citron, Citrus excelsa and Mexican lime. These host were reliable for all isolates tested. Other hosts were of lesser value for most isolates, and these included lemon, grapefruit and sour orange, although some isolates did well in these host also.

When sweet orange was used as the propagative host, variability of dsRNA profiles for a given isolate was at a minimum (Dodds et al., 1987b), and isolate differences were apparent. It was possible to group isolates by their ability to cause the accumulation of a specific dsRNA ($Mr\ 0.5 \times 10^6$). Isolates which appeared unable to do so were typically very mild in several indicator hosts such as lemon, sour orange, grapefruit and sweet orange. Actually very few CTV isolates have this quality, but this is the typical condition for isolates obtained from s. California, and so the method will be useful for discriminating mild from severe isolates in that location. Analysis for the specific dsRNA was not capable of distinguishing seedling yellow strains from non-seedling yellows strains which had other forms of aggressiveness such as stem-pitting in one or more hosts.

The time of the year in which dsRNA can most reliably be detected in field trees (Dodds et al., 1987a) was determined to be those months when environmental conditions were the least severe, which is February, March and April, and also November and December, in s. California.

The effects of host passage on dsRNA profiles for selected isolates was minimal when passage was to and from hosts such as sweet orange and citron. When grapefruit was used as a host, dsRNA profiles were altered, most noticeably by the loss of specific dsRNA components, which remained undetectable so long as grapefruit or lemon were used for subsequent propagations. Upon passage back to sweet orange however the lost components either returned to readily detectable levels at the first analysis, or remained undetectable, depending on the isolate. These results suggest that mixtures of strains are likely to be components of many isolates of CTV (Dodds et al. 1988a).

In addition to the original reports of research, the results of these studies has also been included in reviews (Bar-Joseph et al., 1986; Dodds, Valverde and Mathews, 1987; Dodds, 1986; Dodds et al., 1987c). These were aimed at pointing out the usefulness of dsRNA analysis for detection of RNA viruses, and other dsRNAs of less certain origin (Evenor, Bar-Joseph and Izhar, 1988), points which were also emphasized by testing of Nicotiana glauca plants for viral dsRNAs in Israel (Chevalier, Rosner and Bar-Joseph, in preparation).

Hybridization probes for detection of target viruses

Purified CTV dsRNA was readily melted, end-labelled and used as a CTV specific probe to detect virus in sap by dot-blot hybridization, and it could also be used to analyze dsRNAs in polyacrylamide gels (Dodds et al., 1987b). These results indicated that all of the CTV specific dsRNA segments described on stained gels had sequence homology to CTV, confirming their viral origin. Synthetic probes complementary to avocado sunblotch viroid were synthesized, and found to be useful for the detection of this agent (Bar-Joseph et al., 1985b). The advantages of making synthetic probes based on published sequences of genomes of plant viruses and viroids was reviewed and discussed (Bar-Joseph et al., 1986, Yesodi et al, in preparation). Other uses of synthetic probes have also been investigated (Beckmann and Bar-Joseph, 1986, Yesodi et al., in preparation)

Using similar approaches to investigate avocado viruses

The major emphasis was placed on citrus viruses, but it has been shown that dsRNA analysis can detect virus-like agents in a broad range of avocado varieties (Dodds et al, 1985). It still remains to be demonstrated if any of these putative agents are involved with or indeed cause diseases of avocado such as black streak (Bar-Joseph, Giband and Yesodi, 1987). Detection of sunblotch viroid was discussed in the previous section.

COOPERATION

The two laboratories have maintained a good relationship during the tenure of the BARD grant, and have met each other both at the Volcani Center, at UCR, and at International meetings. We have been able to support each others efforts, especially as it applies to increasing our understanding of the complexity of CTV, the diseases it causes in our respective countries, and understanding the importance of using improved detection methods at a time when we are willing to consider the disease "tristeza" as a complex, rather than a single entity.

EVALUATION OF ACHIEVEMENTS

The most significant accomplishment is the recognition that the tristeza complex caused by CTV can be analyzed by physical as well as biological methods. The techniques used to date, especially the dsRNA analysis, suggest that isolates that were believed to be complex, based on biological properties, do have complexity at the physical level, and that some of this complexity can be reduced by biological sieves, such as host passage. The importance of these conclusions is that we must continue to use appropriate technology to devise laboratory tests that can distinguish between isolates that have or do not have disease inducing potential. The authors of one of the citations (Dodds et al, 1985) were presented with the Wallace Award for presenting the outstanding paper at the 9th Conference of the International Organization of Citrus Virologists (Argentina, 1983).

PUBLICATIONS

Publications which resulted from the project.

RIVERSIDE (DODDS)

Dodds J. A., Tamaki S. J. and Roistacher C. N. 1985. Indexing of citrus tristeza virus double-stranded RNA in field trees. Proc. 9th Conf. IOCV: 327-329.

Dodds J. A., Jordan R. L., Heick J. A., Tamaki S. J. 1985. Double-stranded RNA for the diagnosis of citrus and avocado viruses. Proc. 9th Conf. IOCV: 330-336.

Dodds J. A. 1986. The potential of using double-stranded RNAs as diagnostic probes for plant viruses. pp. 71-86 In "Developments and Applications in Virus Testing" (R. A. C. Jones and L. Torrence, eds.). Association of Applied Biologists, Wellesbourne, England.

Dodds J. A., Valverde R. A., and Mathews D. M. 1987. Detection and interpretation of double-stranded RNA in plants and plant pathogenic fungi. Chapter 14. pp 309 - 326. In "Viruses of Fungi and Simple Eukaryotes" (Eds Koltin, Y. and Liebowitz, M.) Marcel Dekker, New York. 434pp.

Roistacher C. N. and Dodds J. A. 1987. Means of obtaining protective strains from seedling yellows (CTV-SY) and stem-pitting tristeza viruses (CTV-SP) and testing them for cross protection in citrus. Phytophylactica 19: 119-203.

Dodds J. A., Jarupat T., Lee J. G., and Roistacher C. N. 1987a. Effects of strain, host, time of harvest and virus concentration on double-stranded RNA analysis of citrus tristeza virus. Phytopathology 77: 442-447.

Dodds J. A., Jordan R. L., Roistacher C. N., and Jarupat T. 1987b. Diversity of citrus tristeza virus indicated by dsRNA analysis. Intervirology 27: 177-188

Dodds J. A., Jarupat T., Roistacher C. N., and Lee J. G. 1987. Detection of strain specific double-stranded RNA in Citrus spp. infected with citrus tristeza virus. Phytophylactica 19: 131-137

Dodds J. A., Jarupat T., Roistacher C. N. 1988a. Effect of host passage on dsRNA profiles of selected strains of citrus tristeza virus. Proc. 10th Conf. IOCV: 39-45.

Roistacher C. N., Dodds J. A., Bash J. A. 1988b. Cross protection against citrus tristeza seedling yellows (CTV-SY) and stem-pitting (CTV-SP) viruses by protective isolates developed in greenhouse plants. Proc. 10th Conf. IOCV: 91-100.

VOLCANI (BAR-JOSEPH and ROSNER)

Bar-Joseph, M., D. J. Gumpf, J. A. Dodds, A. Rosner, and I. Ginzberg. 1985a. A simple purification method for citrus tristeza virus and estimation of its genome size. *Phytopathology* 75: 195-198.

Bar-Joseph M., Segev D., Twizer S. and Rosner A. 1985b. Detection of avocado sunblotch viroid by hybridization with synthetic oligonucleotide probes. *J. Virol. Methods* 10: 69-73.

Bar-Joseph, M. Segev D., Blickle W., Yesodi V., Franck A., and Rosner A. 1986. Application of synthetic DNA probes for the detection of viroids and viruses. pp. 13-23 In "Developments and Applications in Virus Testing" (R. A. C. Jones and L. Torrence, eds.). Association of Applied Biologists, Wellesbourne, England.

Beckmann J. S., and Bar-Joseph M. 1986. The use of synthetic DNA probes in breeders rights protection: a proposal to superimpose an alpha numerical code on the DNA. *Trends in Biotechnology* 4: 230-232.

Bar-Joseph M., Giband M. and Yesodi V. 1987. Viruses, viroids and diseases of unknown etiology observed in avocado groves in Israel. *S. Afr. Avocado Growers Assoc. Yrb.* 10: 126-128.

Evenor D., Bar-Joseph, M and Izhar S. 1988. Attempts to detect extra genomic factors in cytoplasmic male-sterile petunia lines. (in press).

Chevalier D., Rosner A., and Bar-Joseph M. (in preparation). Double stranded RNA patterns from *Nicotiana glauca* plants naturally infected with viruses in Israel.

Yesodi V., Bar Joseph M., von Wechmar B. and Segev D. (in preparation). Genomic analysis and typing of tobacco mosaic virus isolates and strains using synthetic oligonucleotides.

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