PD ABM-442 DTC
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PROJECT ASSISTANCE COMPLETION REPORT

BACKGROUND DATA

A.	Project Number and Title:	936-5542.13, Cloned DNA to study Molecular Biology, Host Vector Relation, Control of Maize Rayado Fino Virus.
B.	Implementing Agency:	University of Costa Rica (UCR)
C.	PACD: Original	June 30, 1993
	First revised	June 30, 1994
	Last revised	March 31, 1995
D.	Amount obligated:	$139,906
E.	Total expenditures:	$139,906

PROJECT ASSISTANCE COMPLETION REPORT
Cloned DNA to study Molecular Biology, Host Vector Relation, Control of Maize Rayado Fino Virus

Grant Agreement 936-5542.13 with the University of Costa Rica (UCR)
May 31, 1989 - March 31, 1995

I. Summary

On May 31, 1989, the Mission signed a grant agreement with the University of Costa Rica to carry out a Program in Science and Technology Cooperation (PSTC), Research & Development (R&D), centrally funded project to investigate the complex relantionships between Maize Rayado Fino virus (MRFV), its insect vector, and tolerant and susceptible cultivars of maize. Maize Rayado Fino virus is of great economic importance as it causes significant losses in maize plantations throughout the Central and South Americas. This virus is insect transmitted, yet control of the disease by insecticides is ineffective, costly and ecollogically dangerous.

The project design includes a US-based collaborating institution, which is the Agricultural Research Service/United States Department of Agriculture (ARS/USDA) in Beltsville, Maryland.

The original PACD for this Project was June 30, 1993. However, the investigators requested a no cost extension due to several issues beyond their control, which caused delays regarding funding authorization and use of funds. Upon the request, which was previously approved by R&D/AID/W, the Mission extended the project until June 30, 1994. A second extension, through March 31, 1995, was also approved to allow the UCR to finish the construction of a greenhouse, which was delayed because of the UCR's internal regulations.

2. Project Status and Planned Outputs:

a. *Production of a more complete cDNA library of the MRFV genome and determination of the sequence of the clones in this library will allow to study the genome expression strategy of MRFV and obtain information which may lead to an understanding of its ability to replicate in both its insect and plant hosts.*

Several cDNA libraries have been sinthesized using MRFV genomic RNA as the template. In some cases, random primers have been used, in other cases, either polyadenylation followed by priming with obligo dT or alternatively, specific primers have been used to produce the first strand of cDNA. The result is a number of cDNAs representing the complete MRFV genome. Some of these

cDNAs overlap and the UCR investigators are in the continuing process of aligning the cDNAs and completing sequence analysis.

One of the most important discoveries was the identification of the gene encoding the capsid protein of MRFV. This will allow the investigators to consider the posibility of introducing resistance to MRFV in maize by the mechanism of coat protein expressions.

b. *Use of already available nucleic acid probes and antisera and synthesized cDNA probes to study the replication, accumulation, and movement of MRFV in populations of tolerant and susceptible cultivars of maize.*

An additional use envisaged for the cDNA clones generated from the MRFV template was the sensitive, quantitative detection of the virus in its plant and insect hosts. To this end, the UCR investigators have developed radioactive, and more importantly non-radioactive, strand-specific probes from selected cDNA clones. Using these cDNA probes as well as highly specific monoclonal and polyclonal antibodies to MRFV, the investigators have been able to follow the progression of MRFV infection in the host plant and to assess the tolerance and susceptibility to MRFV of selected maize cultivars and accessions obtained from CIMMYT (Mexico).

Strand specific RNA probes as well as antisera were used to analyze the accumulation of virus in selected maize accesions obtained from CIMMYT. Two experimental groups of plants were analyzed and one of them, consisting of two genotypes, proved to be tolerant, in other words, the virus was able to replicate to some level yet the appareance of symptoms was delayed and the level of symptom expression of reduced.

c. *Use of the molecular probes to study viral replication and accumulation in the insect host and relate these processes with the abilty of the insect to transmit the virus.*

One area which has essentially remained untouched has been the study of the replication and accumulation of the virus in the insect vector. This part of the project has been technically challenging, necessitating additional training not available at either institution, UCR or ARS. A graduate student received training in immunogold labeling and electron microscopy at Mississippi State University, but the experiments were not completed as the student left to pursue his doctoral thesis in Germany.

3. Planned vs. actual inputs:

Inputs, except for output c) above, were provided as planned.

4. End of Project Status:

By the PACD, for some targets the project had accomplished more than was expected at the time the proposal was written. The investigators were able to identify the coat protein gene as explained above. Maize germplasm lines exhibiting tolerance/resistance to the virus were identified. The geographic distribution of MRFV ands strain diversity have been examined - information valuable to determining factors which influence the epidemiology of the disease. The nucleotide sequence of the geographically diverse isolates is useful in order to design better constructions for engineering MRFV-resistant maize.

On the other hand, the infrastructure of the UCR was improved by the building of a modern greenhouse, and the purchase of a freezer, cold chamber, water bath, and orbital shaker. In addition, several graduate students received their M.Sc. degrees while being trained and supported from this grant.

5. Progress Towards Planned Purposes:

The *EOPS* and the *Project Status and Planned Outputs* sections above indicate that the Project accomplished its main targets and in some cases, the expected goals were surpassed.

6. Post-Project Monitoring Requirements:

None.

7. Lessons learned:

This research program has allowed the introduction of new technology into the UCR biology research department (CIBCM, Spanish acronym) by training the UCR scientists in a U.S. laboratory. Planning and performing experiments by both Costa Rican and U.S. laboratories working as a team, has yielded many interesting and useful results. Many molecular techniques that are essential to a plant pathologist, such as polymerase chain reaction (PCR), nuclear hybridization, cDNA cloning, and nucleic acid sequencing, have been acquired by the UCR scientists through this collaboration, which will greatly

enhance their ability not only to diagnose plant diseases rapidly and reliably, but also to creatively develop means for disease control.