SUMMARY RESEARCH PROGRESS REPORT FOR 1994 AND RESEARCH PROPOSAL FOR 1995

Submitted to :
SLV Research Center Committee
and the
Colorado Potato Administrative Committee (Area II)

Title: Molecular Markers for Bacterial Ring Rot Immunity and Resistance Genes

Project leaders: Nora L. Lapitan, Department of Soil and Crop Sciences
Carol Ishimaru, Department of Plant Pathology and Weed Science

Project justification:

The goal of this project is to develop DNA markers for genes conferring immunity and resistance to bacterial ring rot in wild potato. DNA markers for immunity genes will serve as tags to enable efficient transfer of the genes to potato cultivars, while DNA markers for resistance genes can be used to detect and eliminate plants that may be potential carriers of the pathogen. Furthermore, the results of this project will lay the foundation for isolation of immunity genes based on their genetic map positions. Cloning of immunity genes will provide the fastest and most efficient approach of producing immune potato cultivars. Onced cloned, immunity genes can be incorporated into susceptible potato by means of transformation. With this approach, an immune potato cultivar can be produced in less than two years compared to approximately 15 years required for breeding. This project will therefore expedite the incorporation of a desirable form of resistance to bacterial ring rot into cultivated potato.

Project status:

This is an ongoing project. Since 1991, we have received funds on a yearly basis from the USDA Special Cooperative Agreements to locate the genes conferring immunity and resistance to BRR by means of molecular mapping techniques. In 1994, the Potato Administration of Colorado provided $7,000 to supplement the molecular mapping work. These funds made it possible to pay for additional hours of technical help and for laboratory supplies that were both necessary in achieving our objectives for 1994.

The search for BRR immunity genes began by evaluating twenty-one accessions representing seven *Solanum* species for BRR reactions in greenhouse studies and assessing for the presence of *Clavibacter michiganensis* subsp. *sepedonicus* (Cms) cells by an immunofluorescent antibody (IFAS) assay. An accession of *S. acaule* and *S. phureja* were found to be immune to BRR. The immune *S. acaule* accession was crossed with a non-immune accession of *S. infundibuliforme* to generate a segregating F1 population. In the IFAS assay, the F1 progeny were observed to segregate in a ratio of 3 non-immune: 1 immune, indicating the presence of two dominant genes at different loci. RFLP (restriction fragment length polymorphism) markers distributed among the 12 potato chromosomes were screened for polymorphisms in the *S. acaule* parent. One marker, CP105-3, was found to be associated with BRR immunity.

The next step in molecular mapping of BRR immunity genes is to determine the genetic distance between the marker CP105-3 and the immunity genes. Depending on the results, additional screening of RFLP markers may be necessary to identify ones that are very near the immunity genes. These objectives will be accomplished in progeny of the selfed immune *S. acaule* parent. This will involve germinating 100 F1 progeny, growing in the greenhouse, establishing each F1 plant in tissue culture, screening for BRR reactions in the greenhouse, assessing for presence of Cms cells by IFAS, extracting DNA from F1 plants, and performing molecular experiments to analyze each F1 for RFLPs. These steps would best be accomplished with a full-time postdoctorate and a part-time technician. The USDA Cooperative Agreements has funded this project for another year, but at a level lower than requested. A full-time postdoctorate will be hired on the USDA grant. The funds requested from this project would be used to pay for additional technical help and for some supplies to be used in molecular mapping work to be conducted in Dr. Lapitan's laboratory. Part of the funds being requested by C. Ishimaru in a separate proposal would be used for other aspects of this study involving bacterial ring rot assays, and tissue culture and greenhouse maintenance of mapping populations.

Significant accomplishments for 1994:

- One hundred F1 progeny from the cross between *S. acaule* and *S. infundibuliforme* were established in tissue culture, evaluated for BRR reaction in the greenhouse, and assessed for the presence of Cms cells by IFAS.

- Results of greenhouse evaluations and IFAS assays were analyzed. It was determined that immunity in *S. acaule* is controlled by two dominant genes at different loci.

- A total of 147 marker/enzyme combinations was used to screen the F1 progeny for polymorphisms. Marker CP105-3 was determined to be associated with BRR immunity.

Objectives for 1995:

1.) Determine genetic distance between marker CP105-3 and genes conferring immunity to bacterial ring rot.
2.) Identify RFLP markers that are near the immunity genes.

Funding request:
1994: $7,000

1995 request: $5,000

Itemized budget:

Technical help	$3,000
Supplies	$2,000
	$5,000