RESEARCH PROPOSAL FOR 1994

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

Project leaders: Nora L. Lapitan, Department of Agronomy
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Project justification:

Bacterial ring rot is an economically important disease affecting seed and table stock production of potatoes in Colorado. The disease is managed primarily by the use of disease-free seed. However, while this approach has reduced economic losses from bacterial ring rot, it cannot eradicate the pathogen, Clavibacter sepedonicum (Cms), which is often present in the absence of disease symptoms. Currently, there is no effective means to control bacterial ring rot. There are no chemical control measures for eliminating the pathogen in seed used for table stock production or for preventing certified seed from becoming contaminated by any of several possible sources.

We and others (Kriel et al. 1993) recently identified accessions of Solanum acaule, a relative of potato, that are immune to bacterial ring rot. In these accessions, cells of the pathogen are not detected in inoculated plants grown under controlled environmental conditions. Immunity is a highly desirable form of resistance, because immune plants would not be expected to carry latent infections of Cms. Resistance to bacterial ring rot has also been identified in a number of potato cultivars. These cultivars are generally omitted from potato breeding programs, because these often delay rather than preclude symptom expression and serve as latent carriers of Cms. The objective of this proposal is to develop molecular markers that can be used for screening plants carrying genes for immunity or resistance to bacterial ring rot. Molecular markers for immunity genes will enable the efficient transfer of immunity genes to potato cultivars through breeding, while markers for resistance genes can be used to enable detection of plants that may be carriers of the pathogen.

Project status:

This is an ongoing project. In 1991, 1992, and 1993, we obtained a USDA/ARS Special Agreement to locate the genes conferring resistance and immunity to bacterial ring rot by means of restriction fragment length polymorphism (RFLP) and randomly amplified polymorphic DNA (RAPD) mapping. RFLP mapping has proven to be a valuable technique for identifying genes for important traits in humans, plants, and other sexually reproducing organisms. In plants, it has been used to locate and identify genes conferring resistance to pathogens and insects, and genes for complex traits such as yield. RFLP mapping has already been used successfully in potato to identify genes conferring resistance to potato cyst nematode, potato virus X, and a quantitatively inherited insect resistance trait. A RAPD is another type of DNA marker that can be used for locating genes. Both types of markers will provide an efficient, visual tagging method to determine the presence of immunity or resistance genes. Since the DNA markers are close to the genes in the chromosomes of the plant, the presence of the genes can be inferred from the presence of the markers.

In 1991 and 1992, we obtained several accessions of potato and different Solanum species from various sources. Each accession was established in tissue culture using standard procedures. All tissue culture materials arose from a single seed, tuber, or plant to insure that tissue culture stocks of accessions represent a single, homogeneous genetic background. Twenty-three accessions were screened for bacterial ring rot immunity or resistance. Screening for resistance was done by dipping

the roots of tissue-cultured plantlets in a suspension of Cms cells, growing in the greenhouse, and observing symptom expression for 72 days. Screening for immunity was accomplished using immunofluorescent antibody staining (IFAS). In this assay, the number of bacterial cells present in stem sections harvested from inoculated plants are counted under a microscope.

In order to locate the genes for bacterial ring rot immunity and resistance, we also examined plants for levels of DNA polymorphisms using RFLP and RAPD markers. Accessions that differed in response to bacterial ring rot (i.e., immune vs. non-immune, and resistant vs. susceptible) and showed high levels of DNA polymorphism relative to each other were identified. These were used as parents in crosses to produce mapping populations. The following crosses have been made: S. sanctae-rosae (non-immune) x S. acaule (immune), S. acaule (immune) x S. infundibuliforme (non-immune), and Ute Russet (resistant) x Sangre (susceptible).

To map the genes, 100 F1 progeny from each cross will be placed in tissue culture to provide a homogeneous supply of each genotype for the bacterial ring rot assays and genetic analysis. Each individual progeny will be screened for resistance and immunity to bacterial ring rot as described above. DNA will be extracted from each plant, and screened for polymorphisms using RFLP and RAPD markers. More than 400 primers for RAPD screening are available in Nora Lapitan's laboratory. Several hundred RFLP markers have been obtained for this study. Additionally, we have generated several DNA clones that can be used for RFLP mapping. A software program called MAPMAKER will be used to establish linkage of polymorphic DNA markers to the immunity and resistance genes. Once DNA markers are confirmed to be close to the genes, the markers will be converted to a form that can be readily used in a breeding program. This will involve providing a pair of primers for use in a polymerase chain reaction (PCR) that can be used to accurately and rapidly detect the presence of the genes.

The mapping aspect of this project involves labor-intensive steps and expensive materials. The funds requested for this proposal will be used to supplement the funds received from the USDA-ARS Special Agreement (which was funded for 1994) to allow us to analyze more lines and convert RFLP or RAPD markers to PCR-based markers. Therefore, supplies and technical support provided by the SLV Research Center Committee would be used for DNA analysis in Dr. Lapitan's laboratory. Part of the funds being requested by C. Ishimaru in a separate proposal would be used for the other major aspect of this study involving bacterial ring rot assays, and tissue culture and greenhouse maintenance of mapping populations.

Objectives for 1994:

1.) Develop DNA markers for genes conferring immunity and resistance to bacterial ring rot.

2.) Convert RFLP or RAPD markers to PCR-based markers.

Funding request:
1994 request: $7,000

Itemized budget:

Technical help	$5,000
Supplies	$2,000
	$7,000