SUMMARY RESEARCH PROGRESS REPORT FOR 2001 AND RESEARCH PROPOSAL FOR 2002

Submitted to:
San Luis Valley Research Center Committee
and the
Colorado Potato Administrative Committee (Area II)

Title: Characterization of antifungal proteins from *Phytophthora infestans* (late blight)-resistant potato progenitors

Project Leader: Dr. Jorge M. Vivanco

Project Status: Ongoing project.

Through this ongoing project we are conducting research on potential antifungal proteins from *Phytophthora infestans* (late blight)-resistant potato progenitors. The total proteins from these resistant potato progenitors will be screened and tested *in vitro* against *P. infestans*. Subsequently, these antifungal proteins will be developed as markers to screen for late blight-resistant progenitors. We believe this approach may accelerate the development of resistant potato varieties. I'd also like to point out that no late-blight resistant gene endogenous to potato has been cloned to date.

Significant Accomplishments for 2001:

Work performed in this project has been conducted by a master student (Hope Gruszewski) co-advised by Drs. Vivanco and Holm. Briefly, we have characterized five late-blight resistant accessions that are currently used in crosses at the SLVRC by Dr. Holm: J138, J138A12, J101K9, J101K6, and J103K7. All of these accessions have been shown to be resistant to *Phytophthora infestans* in field trials conducted in Toluca, Mexico. Proteins extracted from these tubers were assessed for antimicrobial activity against late blight. Accession J138 showed the highest activity, and subsequently a protein extraction and chromatography analysis was conducted in order to isolate the antimicrobial protein(s). We have been able to identify that the acidic protein fraction of J138 accounted for the antimicrobial activity. Recently, we have isolated a slightly acidic 35 kD protein that accounts for the antimicrobial activity of Accession J138 against *P. infestans*.

How can this project be of financial benefit to potato producers in the San Luis Valley?

The objective of this project is to biochemically isolate late blight-specific antifungal protein(s) from *P. infestans*-resistant potato progenitors. Subsequently, this antifungal protein will be developed into markers (antibodies) to screen for late blight-resistant progenitors. We believe this approach may accelerate the development of resistant potato varieties in the SLV. The availability of this protein would allow us to clone its gene, and to genetically engineer commercial potato varieties in order to over-express this antifungal protein. No late-blight resistant gene endogenous to potato has been cloned to date, so this research would add significantly to basic and applied knowledge regarding disease resistance. I would like to highlight that we will transform potato with an endogenous gene rather than with a gene from other taxonomically unrelated species. This will avoid much of the controversy regarding GMOs (Genetically Modified Crops). The promptness in developing new varieties and the ability to engineer resistant potato varieties will provide financial benefit to the SLV producers.

Objectives for 2002

Characterization of the antifungal protein(s). After the antifungal protein has been purified from potato tubers, N-terminal amino acid sequencing will be performed. If we encounter a blocked N-terminal region, we will obtain several internal tryptic peptides by MALDI mass spectrometry. We will also get biochemical information of the antifungal protein such as isoelectric point (pI), amino acid composition and analysis, and homology information. Homology information will let us relate the activity of the potential antifungal protein to a known gene family. We will also inoculate rabbits with the purified antifungal protein to obtain specific antibodies that can be used to develop a screen for presence of the antifungal protein. Such a kit would expedite CSU's SLVRC breeding and development program for resistant Colorado potato cultivars. Crosses will be made between resistant parent material and potato cultivars grown in Colorado will be evaluated for field resistance through collaborations with Cornell University. Simultaneously, we will construct a cDNA library using mRNA from the late blight-resistant potato progenitor(s) from which the antifungal protein was isolated.

Funding Request: $10,000

Materials and chemicals: $ 5,000
Protein analysis and sequencing: $ 3000
Travel to the SLV related to the project (PI and Hope Gruszewski-MS student): $1000
Work/study student to help with the protein separations: $ 1000

2001 – Use of Funds Report

Report funds used rounded to the nearest dollar.

1) Project Labor:
2) Project Travel: **$500** (MS student travel to SLV)
3) Project Chemical: **$1500** (Chemicals and Chromatography columns used in protein isolation)
4) Project Ag. Supplies:
5) Project Equipment: **$3,000** (Micro plate reader)
6) Project Misc:

Total Expense: **$5,000**

2002

San Luis Valley Research Center Committee

Project Outline

Project Title: Characterization of antifungal proteins from *Phytophthora infestans* (late blight)-resistant potato progenitors

Principal Investigator: Dr. Jorge M. Vivanco

I would like to respectfully request support from the San Luis Valley Research Center Committee (SLVRCC) to continue research on the antifungal proteins from *Phytophthora infestans* (late blight)-resistant potato progenitors. We believe this approach may accelerate the development of resistant potato varieties. I'd also like to point out that no late-blight resistant gene endogenous to potato has been cloned to date. My research projects center on the metabolism and biochemistry of biologically active secondary metabolites and proteins produced in plant roots and tubers, and the uses of plants for nutritional, pharmaceutical and agrochemical applications. Thus this project will fit into my research duties as a faculty member in my department.

As per last year's proposal, we have accomplished the objectives proposed for year 1 of this project, which the SLVRCC partially funded. Work performed in this project has been conducted by a master student (Hope Gruszewski) co-advised by Drs. Vivanco and Holm. Briefly, we have characterized five late-blight resistant accessions that are currently used in crosses at the SLVRC by Dr. Holm: J138, J138A12, J101K9, J101K6, and J103K7. All of these accessions have been shown to be resistant to *Phytophthora infestans* in field trials conducted in Toluca, Mexico. Proteins extracted from these tubers were assessed for antimicrobial activity against late blight. Accession J138 showed the highest activity, and subsequently a protein extraction and chromatography analysis was conducted in order to isolate the antimicrobial protein(s). We have been able to identify that the acidic protein fraction of J138 accounted for the antimicrobial activity. We foresee having the protein purified during the next two months (February-March 2002).

Experimental Plan:

Year 2 (2002): Characterization of the antifungal protein(s). After the antifungal protein has been purified from potato tubers, N-terminal amino acid sequencing will be performed. If we encounter a blocked N-terminal region, we will obtain several internal tryptic peptides by MALDI mass spectrometry. We will also get biochemical information of the antifungal protein such as isoelectric point (pI), amino acid composition and analysis, and homology information. Homology information will let us relate the activity of the potential antifungal protein to a known gene family. We will also inoculate rabbits with the purified antifungal protein to obtain specific antibodies that can be used to develop a screen for presence of the antifungal protein. Such a kit would expedite CSU's SLVRC breeding and development program for resistant Colorado potato cultivars. Crosses will be made between resistant parent material and potato cultivars grown in Colorado will be evaluated for field resistance through collaborations with Cornell University. Simultaneously, we will construct a cDNA library using mRNA from the late blight-resistant potato progenitor(s) from which the antifungal protein was isolated.

Year 3 (2003): Gene cloning and characterization. Primers will be designed based on the internal peptide sequences, which will allow us to clone the antifungal genes by RT-PCR. We will use the PCR product to screen the cDNA library in order to obtain a full-length clone. The polyclonal antibodies will also help us

screen the cDNA-expression library. Using the cDNAs as probes we will isolate genomic clones for this gene.

Year 4 (2004). Characterization of the antifungal protein's specific promoters and *Agrobaterium*-mediated transformation. The availability of a genomic clone for the antifungal protein will let us obtain insight into the regulation of its specific promoter. For example, over-expression of these antifungal proteins will be assayed in experimental systems such as cell cultures using different stress responses. Once we have obtained this preliminary information, we will transform commercial potato varieties with this late blight-specific gene. Two different promoters will be used: CaMV 35S, which is a strong and constitutive promoter, and the antifungal protein-specific promoter. After transformation the plants will be screened and different potato lines will be selected. We will transform potato varieties commercially used at the SLV, as well as from our resistant material.

Year 5 (2005). Pathogenic challenge and screening. The different potato transformants will be challenged with *P. infestans* in the greenhouse to confirm their disease resistance. Subsequently, these plants will be taken to the San Luis Valley for disease resistance trials. Alternatively, these plants will also be taken to the Toluca Valley in Mexico, which is the area with the highest diversity and concentration of *P. infestans* inoculum. We will also use the antibodies raised against the antifungal protein to screen for late blight resistant potato progenitors developed by CSU's Potato Breeding program.

PROPOSED
RESEARCH PROPOSAL FOR 2002

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

PROJECT LEADERS: Richard Zink and Robert Davidson

OBJECTIVES FOR 2002

1) Continue a full range of efficacy trials for control of pink rot, early blight, seed piece decay and Rhizoctonia to facilitate product registration, labeling and use recommendations for the San Luis Valley.

2) Continue evaluation of advanced selections from the potato cultivar development program for reactions to major pathogens such as early blight, leafroll, blackleg, ring rot and dry rot.

3) Continue and expand comprehensive research effort on powdery scab. Expand studies on pathogen biology to better understand time of disease onset so as to develop control strategies. Initiate large scale on farm trials with Omega.

4) Move into second year of three year USDA funded compost utilization study.

5) Continue collaboration with Dr. Russ Ingham, Oregon State University, in studies on the biology and management of nematodes. Expand studies to include additional chemical and biological control agents with a goal of developing a systems approach to grower specific control strategies.

6) Continue and expand comprehensive research effort on PVY to better understand how this virus perpetuates in the San Luis Valley, potential for new strains, and better strategies for control in the certified seed program.