2001

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

Introduction:

I would like to respectfully request funding from the San Luis Valley Research Center Committee to conduct research on the 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.

Background:

Preliminary experiments indicate that a basic protein fraction obtained from potato tubers (clone J101K9) demonstrates strong antifungal activity against *P. infestans*. I will describe the activities that may be conducted over a five-year period if funding becomes available for this project.

Experimental Plan:

Year 1: Isolation of the protein (or proteins) that account for the observed inhibitory activity. The antifungal protein will be purified to homogeneity. Total protein extraction will be conducted according to Vivanco et al. (1999). Briefly, the total soluble proteins of potato tubers will be buffer-exchanged to pH 8 in an appropriate buffer such as HEPES, which facilitates the enrichment and separation of basic proteins by cation-exchange (CE) chromatography. Total proteins will be run through a CE column and basic proteins will be eluted with a linear gradient of NaCl. Protein fractions collected after CE chromatography will be screened in plate assays to determine which fraction inhibits growth of *P. infestans*. Protein fractions will then be dialyzed and re-injected into the CE column to remove any contaminant proteins and sharpen the antifungal protein. The purification process will involve both chromatographic separations and antifungal activity assays.

Year 2: 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. 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: 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

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

Year 4. 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.

Year 5. 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.

Funding:

Year 1: $10,000; Year 2: $10,000; Year 3: $10,000; Year 4: $10,000; Year 5: $10,000

Budget Justification:
Funds requested will be used for materials, chemicals, protein analysis and sequencing, travels related to the project and to hire a work/study student to help with the protein separations.