2006

PROPOSAL TO USDA-ARS COOPERATIVE POTATO RESEARCH PROGRAM

Research Plan Title:	Potato cultivar differences in induced resistance and control of diseases during storage
ARS Principal Investigator:	Dr. Daniel Manter
ARS Management Unit: Unit	USDA-ARS-NPA, Soil-Plant-Nutrient Research
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Total Funds Requested: (ARS & cooperators budget)	$37,500

Title of Proposal: Potato cultivar differences in induced resistance and control of diseases during storage

ARS Principal Investigator: Dr. Daniel Manter, USDA-ARS, SPNRU, Fort Collins, CO
Co-principal Investigators: Dr. Sastry Jayanty, Colorado State University, Center, CO
Dr. Rob Davidson, Colorado State University, Center, CO

Aim: The objectives of this project are to characterize potato cultivar differences in host defense genes and resistance to storage pathogens, with particular emphasis on *Phytophthora erythroseptica* and *Erwinia carotovara.*

Summary of problem: Plants possess a variety of mechanisms that can allow them to successfully co-exist with the presence of pathogenic organisms. On such interaction is based on the recognition of specific cues from avirulent pathogen races (avr gene products), which are described in the gene for gene resistance theory (Boch et al., 1998; Yu et al., 1998). Alternatively, horizontal resistance is dependent upon multiple gene products and pathways that may be triggered by the invading organism or its release of an appropriate chemical cue. This general phenomenon is known as systemic acquired resistance (SAR). The general responses of SAR may vary with plant species but includes callose deposition for cell wall reinforcement (Vleeshowers et al., 2000), synthesis of phytoalexins (Kombrink et al., 1991), defence genes including the pathogenesis-related proteins (Van Loon and Van Strien, 1999) and the generation of reactive oxygen species such as hydrogen peroxide (Bolwell et al., 1999). Recent work has shown that pre-treatment of plants with various chemical compounds can induces some or all of these processes rendering the plant less susceptible to future pathogenic attack from a variety of organisms. For example, pre-treatment with a fungal derived elicitor can increase resistance to future bacterial infection, and vice versa.

There has been increasing consumer concern about chemical residues on potato tubers as well as the risk of exposure of farm staff to fungicides. Current fungicide treatments do not fully control the range of economically significant diseases such as silver scurf, black dot, soft rot, gangrene and dry rot. Treatments can be a significant input cost.

Potato is the number one vegetable crop in the U.S. with a farm gate value (value directly from the field, prior to value-added processing) of $2.7 billion in 2003. The San Luis Valley is comprised of 5 counties in Colorado and is the second leading production area of fresh-market potatoes in the U.S. Exact commercial loses to potato diseases during storage in this area have not been determined; however, even with the availability of fungicides losses to late blight are in excess of $210 million annually. The development of treatments that induce plant defense responses and broad-spectrum resistance are critical to reducing disease losses during potato storage.

Comment [DKM1]: Any data that might help this section is appreciated.

Research objectives:

1) Evaluate the efficacy of various treatments to induce SAR in different potato cultivars currently being considered for release by the CSU breeding program.
2) Evaluate the longevity of the SAR response.
3) Evaluate the efficacy of various SAR-inducing treatments to control pathogen losses during potato storage.

Research Plan:

Objective 1: The induction of SAR genes will be evaluated in tubers of at least 12 different potato varieties following treatment with elicitors (control, *P. erythroseptica* elicitin, methyl jasmonate, and β-aminobutyric acid). Measured SAR responses will include H_2O_2 production and PR gene (PR1, PR5, PR10, and NtPRp27) expression. Briefly, tuber pieces will be placed in a 5 mL vial and incubated with 2 mL dH_2O for 4 hr for pre-treatment (Conrath et al. 2003). Following pre-treatment, elicitors will be added to a final concentration of 2 mM. At the appropriate time interval, an aliquot of the bathing solution will be removed for H_2O_2 analysis and tubers will be frozen with liquid nitrogen for future RNA extraction and RT-PCR to determine defense gene expression.

Objective 2: A subset of samples from objective 1 (e.g., best elicitor treatment and four most responsive potato varieties) will be selected to determine the longevity of the SAR response. For example, after elicitor treatments, H_2O_2 and defense gene expression will be monitored 3, 7, 14, and 21 days after treatment.

Objective 3: Elicitins will be sprayed on freshly harvested potatoes with or without fungicide treatments along with controls during curing process at different concentrations. Potatoes also will be inoculated with common bacterial and fungal pathogens to evaluate resistance and susceptibility.

Comment [DKM2]: Sastry, would you mind working on this objective?

Total Budget:

ARS		
Manter	**$15,000**	SAR gene expression and H_2O_2 production
CSU (cooperative agreement)		
Jayanty	**$10,000**	Storage chamber set-up and sampling
Davidson	**$5,000**	Inoculation and disease assessments
	$3,750	Indirect costs (25%)
Sub-total	**$33,750**	
Indirect costs (11.111%)	**$3,750**	
Total	**$37,500**	

References

Boch J, Verbsky ML, Roberston, TL, Larkin JC, Kunkel BN (1998) Analysis of resistance gene-mediated defense responses in Arabidopsis thaliana plants carrying a mutation in CPR5. Plant Microbe Interact 12:1196–1206

Bolwell GP, Blee KA, Butt VS, Davies DR, Gardner SL, Gerrish C, MinobayevaF, Rowntree FG, Wojtaszek P (1999) Recent advances in understanding the origin of the apoplastic oxidative burst in plant cells. Free Radic Res [Suppl] 31:137–145

Conrath U, Linke C, Jeblick W, Geigenberger P, Quick PW, Neuhaus HE (2003) Enhanced resistance to *Phytophthora infestans* and *Alternaria solani* in leaves and tubers, respectively, of potato plants with decreased activity of the plastidic ATP/ADP transporter. Planta 19:75-83

Kombrink E, Hahlbrock K, Hinze K, Schro¨ der M (1991) Molecular responses of potato to infection with *Phytophtora infestans*. In: Smith CJ (ed) Biochemistry and molecular biology of plant–pathogen interaction. Clarendon Press, Oxford

Van Loon LC, Van Strien EA (1999) The families of pathogenesis related proteins, their activities, and comparative analysis of PR-1 type proteins. Physiol Mol Plant Pathol 55:85–97

Vleeshowers VGAA, van Dooijeweert W, Govers F, Kamoun S, Colon LT (2000) The hypersensitive response is associated with host and nonhost resistance to Phytophthora infestans. Planta 210:853-864.

Yu I-C, Parker J, Bent AF (1998) Gene-for-gene disease resistance without the hypersensitive response in Arabidopsis dnd1 mutant. Proc Natl Acad Sci USA 95:7819–7824

Outlines are not part of the proposal…

Experiment 1 (ARS – Fort Collins)

Objectives: Compare the SAR response of various potato varieties. Compare various elicitors.

1. Harvest potato tubers (12 varieties)
2. Test HR response to *P. erythroseptica* elicitin, methyl jasmonate, β-aminobutyric acid and control. (all compounds tested at 2 mM except elicitin at 10 mM)
    a. HR response assays
        i. H_2O_2 production
            1. Time (3 days after treatment)
            2. Total samples: 12 varieties x 4 trt x 1 time x 6 reps = 288 samples
        ii. PR genes (PR1, PR5, PR10, NtPRp27, and COX [housekeeping gene]) <note: I'm currently optimizing these assays, all four defense genes may not prove successful…>
            1. Time (3 days after treatment)
            2. Total samples: 12 varieties x 4 trt x 1 time x 3 reps = 144 samples

Experiment 2 (ARS – Fort Collins)

Objectives: How long does the response last?

1. Select best 'elicitor treatment' and 4 varieties (*P. erythroseptica*: resistant and susceptible; *Erwinia*: resistant and susceptible) to test the longevity of response to the one of the above treatments (plus a control).
    a. HR response assay
        i. H_2O_2 production
        ii. PR genes (PR1, PR5, PR10, NtPRp27, and COX [housekeeping gene])
            1. Time (3, 7, 14, and 21 days after treatment)
            2. Total samples: 4 varieties x 2 trt x 4 time x 3 reps = 96 samples

Experiment 3 (CSU – SLV)

1. Harvest potato tubers (4 varieties). If possible, 1 *P. erythroseptica* resistant and 1 *P. erythroseptica* susceptible and 1 *Erwinia* resistant and 1 *Erwinia* susceptible.
   <Rob, do you have any varieties in mind?>
2. Place tubers in plastic chambers (15 tubers / chamber)
3. Apply treatments by dipping tubers in solutions (more uniform than spraying?) and placing in plastic chamber
    a. Elicitors
        i. *P. erythroseptica* elicitin (10 mM)
        ii. Methyl jasmonate (2 mM)
        iii. B-aminoburyric acid (2 mM)
    b. Fungicides

i. Hinokitiol (50 ppm)
ii. Metalaxyl (? ppm)
iii. Control (H_2O)

4. After 3 days, inoculate by dipping tubers in spore solutions (20,000 spores / mL) and wrapping with wet paper towels. Store in plastic chambers.
    a. *P. erythroseptica* (I will receive a culture by the end of Sept, spores will be ready late Oct)
    b. *Erwinia carotonova* (Rob has a culture, correct?)
    c. Control
5. Temperature (15 and 25 C)
6. Humidity (80 %)???
7. Score tubers (n = 15) from each variety for visible symptoms at 8 weeks
    a. Cut tubers in half longitudinally and assess area with visual symptoms. Incidence is the number of tubers infected and severity is the percent area with visible symptoms.