2006

PROPOSAL TO USDA-ARS COOPERATIVE POTATO RESEARCH PROGRAM
APPLICATION COVER PAGE

Research Plan Title:
Analysis of potato skin mutant for powdery scab disease using potato gene expression array

Principal Investigator: Dr. Sastry S. Jayanty

P.I. Address: 249 East County Road, 9N
San Luis Valley Research Center
Colorado State University
Center CO-81125

P.I. E-mail: sjayanty@lamar.colostate.edu

P.I. Phone: 719-754-3594 ext 11

P.I. Fax: 719-754-2619

Total Funds Requested: $54, 000

Title of the proposal: Analysis of potato smooth skin mutant susceptible to powdery scab disease to understand biological basis of russetting.

Principal investigator: Dr. Sastry S. Jayanty, Colorado State University, Center, CO
Co- Investigators: Dr. Rob Davidson, Colorado State University, Center, CO
Dr. Dave Holm, Colorado State University, Center, CO

Aim:
Identifying the biological basis of resistance to powdery scab disease associated with russet phenotype. Our long term goals include transferring the resistance to susceptible varieties through classical breeding program.

Summary of problem:
Powdery scab disease caused by the pathogen *Spongospora subterranea f. sp. subterranea* is a major concern for potato producers in main potato production centers in North America. This is a soil borne pathogen that infects root hairs, stolon epidermal cells, lenticels, eyes and wounds of developing tubers. Infected tubers and roots may have white gall-like growths, which in turn develop into brown powdery scab lesions on tubers as they mature (Harrison et al. 1997).
Powdery scab symptoms cause significant economic losses in both fresh and seed markets. Depending on severity of symptoms, tubers are non-marketable or grade may be lowered in fresh, processing and seed markets. Even in processing, industry costs incurred for peeling is higher. Seed lots infected with powdery scab may or may not pass inspection depending on the regulations of the certifying agency and the degree of infection. Infected tubers are more susceptible to fusarium dry rot, bacterial soft rot and other pathogens during storage. Christ (1993) observed that potato varieties with smooth or light skin and red skin are more susceptible where as russet-skinned varieties are less prone to powdery scab, although root galls are common. Recently, there are reports on partial russeting in some cultivars such as Rio Grande in some potato production areas. Partial russeting and skin setting can lead to disease, loss of water and prone to skin bruise, effecting the quality of the tuber (Lulai EC, 2002).
Recently, we identified a smooth skin mutant of Russet Nugget (R. Nugget) which is devoid of the russetting phenotype and shows more susceptibility to powdery scab infection. This skin mutation offers opportunity to study the genetics of a specific phenotype and genes responsible for this important russetting phenotype. Our long term goals include transferring resistance to the susceptible varieties through a classical breeding program. A better understanding of russetting mechanisms will help in developing new cultural tools for better skin set in new varieties to enhance the native capacity of tubers for skin set.
Skin setting is an important biological process in tubers that protects against biotic stress, and has a great impact on the quality of the potatoes. Information on genes that are differentially expressed during russetting and the skin development process between mutant and wild-type gives us insight into how these processes are initiated and regulated. Microarray analysis will lead to the identification of specific candidate genes that are differentially regulated. The role of these candidate genes will be confirmed by northern analysis and gene expression will be investigated in specific potato cultivars that are more susceptible to this disease. This knowledge will help to develop markers for breeding durable resistant cultivars.
For the first time we are proposing to apply microarray analysis to economically important disease such as powdery scab in potato. We envision these studies would

increase our knowledge on how russeting plays a role in disease resistance. This will significantly impact both basic science and applied research.

Research Objectives:

1. Identifying the genes that are differentially expressed in wild-type compared to mutant.
2. Further confirming differentially expressed genes using RT-PCR or Northern analysis.
3. Testing the role of differentially expressed genes in russeting phenotype and disease resistance by checking expression pattern in smooth skin variety VS russeted variety.

Research Plan:

1 *Plant material, growth and storage conditions, RNA extractions:*
Russet Nugget and their corresponding smooth skin mutant tubers will be harvested from disease and healthy plots. Tubers will be washed with 10% Clorox. Cortex tissue and peel will be collected from healthy and infected regions of the mutant and wild-type potato tuber and will be snap-frozen in liquid nitrogen and stored at -80 °C. RNA will be extracted from frozen tissues using a hot phenol method. Each RNA extraction will be conducted in triplicate from the different sets of tuber tissue collected and purified by Qiagen kits. In all of these experiments Russet Nugget and smooth skin mutant will be used as described in detailed experimental design (Table 1).

2. *Experimental design for identifying genes that are differentially expressed in the smooth skin mutant:*
Tissue samples will be collected from infected and healthy tubers of wild type and mutant immediately after harvest. RNA will be extracted from 6 different sets of tissues in triplicates as described in table 1 to use it as query and reference samples in microarray hybridization. 18 sets of RNA samples will be hybridized to 18 different microarray slides obtained from TIGR. Hybridization and analysis will be carried out at Michigan State University Genomic Array Facility.

References:

1. Harrison JG, Searle RJ, William NA 1997. Powdery scab disease of potato – review Plant Pathology 46:1-25.
2. Christ, B.J. 1993. Powdery Scab of Potatoes--What We Know. Proceedings 12th Annual National Potato Council Seed Seminar. Pages 24-5.
3. Lulai EC 2002. The roles of phellem (skin) tensile – related fractures and phellogen shear-related fractures in susceptibility to tuber-skinning injury and skin – set development. Amer J. of Potato Res 79:241-248.

Total Budget:

Jayanty: (RNA isolation, Microarrays, Hybridization, Data analysis)

Research Associate (50% time)		15,000
Fringe Benefits	(20.3%)	3,045
Lab Supplies		35,000
Davidson and Holm		
Field activity and Materials & supplies		5,000
Total		$58,045

Table 1: Description of experimental samples for potato microarray hybridizations for gene discovery related to russeting and susceptibility of smooth skin varieties to powdery scab disease.

Experiment	Sample	Tissue	Reference	Query
1	1	Peel from mutant	Peel from R. Nugget	R. Nugget
	2	Peel from mutant	Peel from R. Nugget	R. Nugget
	3	Peel from mutant	Peel from R. Nugget	R. Nugget
2	4	Cortex Tissue from mutant	Cortex tissue from R. Nugget	R. Nugget
	5	Cortex tissue from mutant	Cortex tissue from R. Nugget	R. Nugget
	6	Cortex tissue from mutant	Cortex tissue from R. Nugget	R. Nugget
3	7	Infected peel from mutant	Healthy peel from mutant	R. Nugget
	8	Infected peel from mutant	Healthy peel from mutant	R. Nugget
	9	Infected peel from mutant	Healthy peel from mutant	R. Nugget
4	10	Infected peel from mutant	Healthy R. Nugget peel	R. Nugget
	11	Infected peel from mutant	Healthy R. Nugget peel	R. Nugget
	12	Infected peel from mutant	Healthy R. Nugget peel	R. Nugget
5	13	Infected cortex from mutant	Healthy cortex from mutant	R. Nugget
	14	Infected cortex from mutant	Healthy cortex from mutant	R. Nugget
	15	Infected cortex from mutant	Healthy cortex from mutant	R. Nugget
6	16	Infected cortex from mutant	R. Nugget cortext	R. Nugget
	17	Infected cortex from mutant	R. Nugget cortext	R. Nugget
	18	Infected cortex from mutant	R. Nugget cortext	R. Nugget