2013 14

**Title of Proposal: Developing an effective screen for powdery scab resistance in potato germplasm smooth-skin and russet-skin type cultivars**

**Most relevant funding source: *CCPGA***

**Investigator names and department:**
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**Summary of Problem**

Powdery scab disease caused by *Spongospora subterranea f. sp. subterranea* is one of the major concerns for potato producers in production regions throughout the world. 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 later develop into brown powdery scab tuber lesions as they mature (Harrison et al. 1997). Christ (1993) observed that potato cultivars with smooth or light skin (i.e whites and reds) are more susceptible; whereas russet-skinned cultivars are less prone to powdery scab, although root galls are common. There are reports on partial russeting in some cultivars such as Rio Grande Russet. Partial russeting and irregular skin set can lead to disease, loss of water and susceptibility to skin bruise, effecting tuber quality (Lulai EC, 2002).

**Nature, scope, objectives of proposed research and preliminary results**

Powdery scab symptoms cause significant economic losses in both fresh and seed markets. Depending on the severity of symptoms, tubers could become non-marketable or grade quality may be reduced in fresh and seed markets. 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 also more susceptible to secondary infections, such as fusarium dry rot, bacterial soft rot and other pathogens during storage. We are proposing to identify markers for powdery scab susceptibility to help breeders in the cultivar selection and breeding process.

Potato genotypes that are being identified with tuber-powdery scab tolerance are usually turned out to be russet skin types (Houser and Davidson 2010; Nitzan et al. 2010). In spite of this finding act, there has not been substantial amount of research effort to understand genetics and biochemistry of powdery scab disease tolerance in russet skinned potatoes. It is important to understand the functional genes and related biochemical pathways to confirm disease tolerance in these potatoes. As mentioned above, certain storage proteins such as LOXs and patatins have a role in certain disease tolerance mechanism to certain diseases. Our recent report suggests that the storage protein levels of various potato genotypes are significantly different (Perla et al. 2012). In this context, the objective of this study was to understand the role of LOX and patatin and other protease inhibitor proteins, in the powdery scab tolerance in of russet skinned tubers.

**Table 1**. Kendall's tau correlation between tuber DSI, unmarketable tubers, total protein, LOX protein and patatin-lipase levels of tubers

| Variables | Unmarketable Tubers | Total Protein | LOX (PB) | LOX (DB) | Patatin-L (PB) | Patatin-L (DB) | Tuber Skin[a] |
|---|---|---|---|---|---|---|---|
| DSI | **0.688**** | -0.045 | -0.225 | **-0.494*** | 0.180 | 0.000 | **-0.691*** |
| Unmarketable tubers | | -0.076 | -0.076 | -0.328 | 0.227 | -0.025 | -0.553 |
| Total protein | | | 0.156 | 0.022 | 0.200 | **0.644***** | 0.163 |
| LOX (PB) | | | | **0.600**** | 0.244 | 0.333 | **0.683*** |
| LOX (DB) | | | | | -0.067 | -0.067 | **0.618*** |
| Patatin-L (PB) | | | | | | **0.556**** | 0.163 |
| Patatin-L (DB) | | | | | | | 0.358 |

[a]Genotypes were ranked on the basis of the roughness of tuber skin, where 1 = smooth and 2 = rough (russet skin).

DSI = Disease severity index of tubers. PB = Protein basis. DB = Dry weight basis. Patatin-L = Patatin-lipase. Values in bold are significantly different from zero.

*, ** and *** suggest that the correlation is significant at *P*-value 0.05, 0.01 and 0.001, respectively (two tailed tests).

Our initial results reveal significant variation was found between the genotypes with respect to the total tuber protein content of the tubers. Kendall's tau correlation between different parameters suggests that the tuber DSI was positively correlated with unmarketable tubers (Table 1). LOX protein levels on dry weight basis, otherwise known as the physiological levels of LOX protein, were negatively correlated with the tuber DSI. On the other hand, LOX protein levels on protein basis, otherwise known as the relative abundance of LOX protein within the proteome, did not reveal any significance for LOX protein in the powdery scab disease tolerance of potato tubers. Furthermore, tuber russet skin is positively correlated with the physiological levels of LOX protein as well as the relative abundance of LOX protein within the proteome of the tissue around the skin.

In another experiment (Fig 1) when we compared chitinase A2 & A3 gene expression in Mutant Healthy Peel (MHP) and Mutant Infected Peel (MIP), we observed elevated gene expression in MHP suggesting that chitinase may be having a role in resistance mechanism for powdery scab.

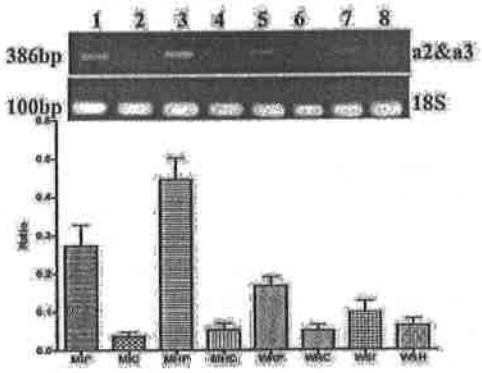


Figure 1

**Research Objectives:**

1). Conduct biochemical (enzymatic) analysis of potato tubers with different skin-types.
2). Screening different pathogen related proteins, chitinases, LOX, patatin and other protease inhibitors and phytoalexins and other related genes.
3). Understanding the importance of the physiological levels of these proteins in the context of the disease tolerance mechanism.

**Research Plan:**

Ten potato genotypes with different tuber skin types will be selected from the potato breeding program at the San Luis Valley Research Center (SLVRC) to test evaluate for powder scab susceptibility under in a greenhouse environment. Included will be different cultivars with different flesh colors and skin-types. Five grams of fresh tissue will be collected from smooth and russet skin type tubers ground under liquid nitrogen using a freezer mill. The frozen powder will be stored at -80°C. The frozen samples will be analyzed for activity in smooth and russet skin type cultivars.

Smooth and russet skin type tubers will be analyzed for pathogen related protein expression. We will use a variety of techniques to find differences in the protein expression and their level of expression and also their activity. The total protein concentration in of the sample extractions was determined according to the procedure of by Perla et al (2012). One dimensional SDS-PAGE will be performed with a Mini-PROTEAN® Tetra electrophoresis system. These proteins after separation will be detected using immunodetection with specific antibodies and also depending on enzymes that are probed activity staining procedure will also be employed.

Once target proteins are identified molecular markers will be designed and tested initially on resistant and susceptible cultivars to confirm our results followed by evaluation of advanced selections and greenhouse testing.

**Relationship of proposed research to overall problem for potato growers:**

The development of potato cultivars with powdery scab tolerance will help growers tremendously especially in case specialty cultivars.

**Timeline and expected short term (1 yr) and longer term (3-5 yrs) outcomes.**
1st years: Screening number of target enzymes
2nd year: Conforming laboratory results with green house and field trails.

**Detailed annual budget (personnel, materials and supplies, travel, equipment, services) and a budget justification.**

| | |
|---|---|
| **Total budget** | **45, 000 (for two years)** |
| **Requested funding for 2013-14:** | **22,500.00** |
| Research Associate (50%) | 10,000.00 |
| Equipment | 5,000.00 |
| Chemicals | 3,000.00 |
| Travel | 1,500.00 |