2010

Title: Developing a quick and reliable method to screen potato germplasm and advanced clones for resistance against pink rot disease caused by *Phytophthora erythroseptica* Pethyb.

Funding source

First choice: CSU cultivar royalty funds from the Colorado Certified Potato Growers Association (CCPGA); Second choice: Colorado Potato Administrative Committee, area II (CPAC).

Investigators

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Scope & Objectives

Cultivated potato (*Solanum tuberosum*) is prone to several microbial diseases, and among them, pink rot, caused by *Phytophthora erythroseptica* Pethyb. is a potentially destructive disease for the potato industry. Pink rot is one of the most important soil-borne diseases (Secor & Gudmestad, 1999) for two primary reasons. First, the commercial potato fields prone to this disease incidence may range from 10 to 75%, and often times when fields are not harvested it causes total yield loss (Carrol & Sasser, 1974). Second, this disease predisposes the tubers to secondary infections, especially storage born diseases like soft rot bacteria. Infection of tubers by *P. erythyroseptica* generally occurs via stolons, but sometimes tubers can also be infected by fungal zoospores through tuber eyes, lenticels and tuber wounds (Salas et al., 2000). Symptoms, such as wilting, also appear on aerial plant parts and stunting occurs on potato vines late in the growing season. Cultivars with early vine maturity tend to be more susceptible to pink rot disease than later maturing cultivars. In the San Luis Valley (SLV) pink rot disease continuously threatens potato production especially because growers prefer to raise Russet Norkotah, the primary early cultivar grown in the SLV.

No single control measure will provide effective control of pink rot, but the disease can be managed by using a combination of cultural (crop rotation, soil acidification etc.) and chemical control methods. The most effective chemical control for pink rot is the use of mefenoxam-based

fungicides (Wicks et al., 2000), but unfortunately mefenoxam-resistant *P. erythroseptica* isolates are widely reported in the fields (Taylor et al., 2004). *P. erythroseptica* spores are also known to be highly resilient and they can survive without host or on non-host plants for at least 3-4 years, therefore crop rotation can not always be dependable and economically feasible for the growers. The most reliable, cost effective and environmentally friendly alternative to control pink rot is development of resistant cultivars, but so far no cultivars showing complete resistance to pink rot have been identified. A few reports have previously shown that some cultivars (such as Atlantic, Snowden, Russet Burbank) are moderately resistant to pink rot (Thompson et al., 2007; Salas et al., 2003) based on cultivar susceptibility tests. Recently, Thompson et al. (2007) reported that some clones derived from crossing of *Solanum berthaultii* and *Solanum tuberosum* showed more resistance to pink rot than the moderately resistant cultivars Atlantic and Snowden.

However, gene(s) and other factors responsible for this resistance have not been identified. Current efforts are being devoted on this front by the Colorado Potato Breeding and Selection Program to develop pink rot resistant varieties specifically suited for the SLV. However, the lack of clear genetic or biochemical markers to rapidly screen the available germplasm and advanced clones for their resistance has slowed down the breeding program effort to develop resistant varieties for the Colorado potato industry. Without determining the genetic/biochemical basis of plant resistance to *P. erythroseptica,* it is impossible to develop genetic markers to rapidly screen for resistant cultivars. Therefore, we propose here to identify a biochemical marker (protein) that could be used to develop a selection method to speed up the screening process for *P. erythroseptica* resistant clones in the germplasm. The recent development of "omics" technologies like metabolomics and proteomics allow us to biochemically compare the resistant and susceptible cultivars to identify potential candidates that contribute to the resistance phenotype. Once a candidate resistance protein is identified, it could be incorporated into a simple and high throughput enzyme-linked immunosorbent assay (ELISA) method to screen for resistant clones. An ELISA-based method is already in use to screen for PVY and other virus infection in plants in the SLV by monitoring the expression levels of PVY coat protein in plant tissues. **We are confident that the proposed objectives will provide a protein candidate that could be used as a marker suitable for use in a simple, quick and high-throughput method to screen for resistant clones.** This would decrease the time required to identify resistant germplasm as conventional screening methods require 2-3 months under field or greenhouse conditions. Conventional screening methods in the field are also depending on several unpredicted variables which could alter the results and make them non-replicable. The proposed new method need only

in vitro grown 2-week old seedlings and the ELISA based screening assay will take only 2-3 days to screen 90 cultivars/clones.

We propose the following objectives to complement and improve the current activities of the SLVRC potato breeding program to identify pink rot disease-resistant plants:

Objective 1

Determination of resistance to *P. erythroseptica* infection in the greenhouse. We will analyze the selected cultivars available in Dr. Holm's breeding program (SLVRC) and the clones (Etb 6-5-2, ND5822C-7, ND6956b-13, ND7443Ab-44, ND7443AB-181, ND7818-1Y and J101K6A22) developed by Dr. Thompson, North Dakota State University, using pathogenicity assays conducted with *P. erythroseptica* isolates collected from SLVRC potato fields. This objective will be performed at the SLVRC. The experiments described in Objective 1 will provide solid data about which cultivars/selections have potential resistance to the *P. erythroseptica* isolates collected specifically from the SLV region. Previous studies reported that each field isolate show different disease patterns depending on the cultivar/selection tested. This objective will identify at least three resistant and three susceptible cultivars/clones to undergo proteomic analysis in Objective 2.

Objective 2

Comparative proteomic analyses of potato tubers (wet peels and flesh) and roots of resistant and susceptible cultivars/clones derived from objective 1. This will allow us to identify proteins which are unique to plants with a resistant phenotype and could be used as markers for rapid screening of germplasm for *P. erythroseptica* resistance.

Objective 3

Development of an EIISA-based screening method using a protein marker identified in Objective 2. A unique candidate protein from plants with a resistant phenotype will be expressed in *E. coli* using standard molecular cloning methods for antibody production. Initially, we will perform the ELISA method using known resistant and susceptible cultivars/selections to pink rot disease as proof of the concept to utilize the effectiveness of the screening prior to analyze other cultivars/selections available in the breeding program. We believe that this method will be very useful for screening a large number of selections in the breeding program in a time

and cost effective manner and will prove to be a more reliable indicator of plant resistance compared to the conventional screening procedures currently used to determine by cultivar susceptibility tests.

Methodology

Cultivar resistance/susceptibility test: Several *P. erythroseptica* isolates will be isolated from diseased tubers collected from different potato fields in the SLV using standard isolation methods. Briefly, cut pieces of infected tubers will be surface sterilized and placed in Petri plates on top of wet filter paper, and incubated under continuous light to induce sporulation. Single spore transferred to water agar plates will be germinated to obtain pure cultures. Inoculation of potato tubers/stolons and disease assessment will be performed by following the method described by Thompson et al. (2007).

Proteomic analyses: Collect the infected tubers and roots at different growing stages from resistant and susceptible cutivars/clones and extract the total proteins from wet peels, wet flesh, and freeze-dried roots. Further proteomic analyses will be performed following the method described by De-la Pena et al. (2008). Comparison of total protein profiles from resistant and susceptible cultivars will allow us to identify proteins unique to the resistant cultivars that may serve as biomarkers for a high throughput screening.

Developing a screening method: After analyzing the data, we will select a candidate protein as a selection marker to develop the method to screen resistant cultivars/selections. Briefly, we express the candidate protein in *E. coli* for antibody production by employing molecular methods (which includes gene cloning, transformation, expression and protein purification) and develop a simple ELISA protocol to screen the cultivars/selections containing resistance factors that can be used in the breeding program.

Resources: We will use the resources available at CSU (on campus) and at the SLVRC to accomplish the objectives listed in this proposal. For the potato resistant and susceptible cultivars/clones we will make use of the cultivars/clones available in Dr. Holm's breeding program (SLVRC). For proteomic analyses, initial preliminary screening will be performed by

using the facilities available in Dr. Vivanco's laboratory and further comprehensive analyses will be performed by using the Metabolomic and Proteomic facility center on campus.

Relationship of proposed research to overall problem of potato growers

The development of new pink rot resistant cultivars for the SLV potato industry is the ultimate goal of this proposal. There is little information available on breeding for resistance against the pink rot pathogen because of the lack of efficient and simple screening methods to detect resistance factors. In addition, relying on quantitative resistance such as single gene resistance to develop pink rot resistant cultivars may not be stable and durable due to the co-evolution of new pathogenic races. Thus, there is a need to identify quantitative factors to implement in the breeding program for developing stable and durable resistant cultivars. We strongly believe that the outcome of the proposed research objectives will provide the basic information needed to develop markers for screening potato selections containing quantitative resistance factors to aid in the identification of potential new potato cultivars with durable resistance to pink rot disease.

Potential for results to leverage additional outside funding

The outcome of this proposal will result in the identification of resistant potato cultivar(s)/breeding line(s) to the pink rot pathogen and also to identify the factors responsible for conferring resistance to use in the potato breeding program at the SLVRC. Further, we hope to identify the quantitative resistance factors (proteins) involved in resistance to *P. erythroseptica* and develop a screening method to identify resistant selections in the potato breeding program in the second year of the project. Successful completion of this work will allow us to propose larger projects to such funding agencies such as the USDA and NSF, including a potential collaborative project with scientists at SLVRC to develop resistant potato cultivars possessing elevated levels of potential quantitative resistance factors by using conventional and molecular breeding techniques.

References

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Budget

Personnel

We request partial salary ($10,000/year) for the lead scientist (Dr. Dayakar Badri) who will conduct these studies. Dr. Badri's current salary is paid entirely from other, existing external grant budgets (no appropriated state funds), so the commitment of his time and effort to the pink rot project will require funds for this part of his salary.

Supplies

Chemicals and materials related to the cultivar susceptibility test and proteomic analyses and also the fees related to service facilities (Metabolomic and Proteomic Facility Center) for comprehensive proteomic analyses in order to analyze the resistance factors are requested. Additional funds are needed to travel to the SLV for visiting infected fields and collecting infected tubers and also maintaining the cultivars/lines in the green house. These funds will also cover the maintenance of the plant material at the SLVRC. $12,000/year.

** Due to the complexity of the project in related to objective # 3, two years of support are requested to achieve all objectives.

TOTAL: $22,000 per year; $44,000 for the length of the project (two years)

Tentative Time Table: Year 1. Conducting pathogenicity assays at SLV, sample collection, protein isolation and running 2-D SDS-PAGE gels and comparative data analyses. **Year 2.** Select the candidate protein, protein expression in *E. coli* and purification, antibody production and developing an ELISA based screening method.

Expected outcome of the proposal

The outcome of the proposed objectives will provide insights in to the quantitative factors (proteins) contributing to pink rot disease resistance. Based on this data, we could develop a method to screen for cultivars/selections with resistance to pink rot disease at the end of second year. This screening method would also be useful to identify parental material to further develop pink rot disease resistant cultivars by the Colorado Potato Breeding and Selection Program.