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Title: Screening of potato breeding lines for nematode resistance $25,000

Investigators: **PI:** Dr. Jorge Vivanco
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Scope and Objective: Nematodes are an important group of pests that have potential to cause significant yield losses to Colorado potato crops. Nematodes inflict losses in two ways: they can damage the potato tubers directly, causing a reduction in yield or resulting in unmarketable tubers; secondly and more importantly, nematode infection predisposes the potato plants to secondary infection by potentially devastating pathogens like *Fusarium solani* and *Verticillium dahliae* (1). Additionally, some nematodes are vectors for viral diseases like corky ringspot (2). The most efficient and environmentally safe method of disease management is the development of resistant cultivars; however, to date there has been little success in developing nematode-resistant potato cultivars. The incorporation of resistant traits by introgression is possible but its success is limited by the fact that there is no methodical screening method in place to identify nematode-resistant cultivars.

In the studies performed in the Vivanco laboratory we have found that the roots of plants secrete certain chemicals, known as root exudates, which play a major role in warding off infection (3,4,5) and have been shown to either repel or attract various nematodes. Each species has its own "signature" root exudate profile; we now know that different ecotypes and cultivars have unique profiles as well.

Recently the Holm laboratory acquired breeding lines from the USDA-ARS that are highly resistant to nematodes. These clones are PA00N44-2, PA94A10-3, PA95B1-53, PA98N5-2, PA98NM38-1, PA98NM39-1, PA99N82-4, and PA99N88-2. We propose to develop tissue culture plantlets of these materials to develop a screening technique that ultimately could be used to initiate a large-scale screening of available potato cultivars and segregating seedling populations for nematode resistance using a novel technique that harnesses the potato's own defense mechanisms. We propose the following objectives to complement the potato breeding program carried out at the San Luis Valley Research Center (SLVRC):

1. Develop a nematode resistance screening technique using susceptible and acquired resistant clones.
2. Isolation and partial characterization of the active compound(s) present in the root exudates of nematode-resistant potato lines.
3. Screening of advanced potato breeding lines and segregating populations for resistance to *Meloidogyne chitwoodi* by using tissue culture plantlets and true seeds developed by Dr. David Holm at the SLVRC for resistance to *Meloidogyne chitwoodi*.
4. Correlate results obtained in Objective 3 and Objective 2.

Methodology

Screening of potato breeding lines for nematode resistance: The proposed research will be conducted at the Department of Horticulture and Landscape Architecture (CSU), using segregating populations for nematode resistance and advanced breeding lines developed

or acquired by Dr. David Holm at the SLVRC. The nematode strain will be obtained from USDA-ARS, Prosser, WA and/or isolated from infested potato soils in Colorado (6). *Meloidogyne chitwoodi* will be maintained on greenhouse-grown potato plants. For screening, potato plants will be grown under sterile conditions in glass culture tubes or 12-well tissue culture plates containing 2-3 ml of MS medium (plant nutrient solution) using a technique developed in our laboratory (4). The tubes/plates will be placed in a rotary shaker set at 30 rpm with a day/night cycle of 16/8h at 25+2C. After the plants develop a good root system, ~100 surface-sterilized eggs (treated with 0.5% sodium hypochlorite) of *M. chitwoodi* will be transferred into each well. The 12-well plates will be returned to the shaker incubator and after one week the number of J2 juvenile nematodes will be counted; we will also record the number of J2 worms that are motile (7). These two counts will measure the effect of chemicals exuded by the roots of various potato breeding lines on the hatching of the nematode eggs. We will also study the effect of the root exudates on the nematode juveniles by transferring about 25 J2 juveniles to the culture medium in which potato plants are growing and incubating as described above. The survival of the nematodes will be monitored at one, three, and seven days after the start of the co-culture. The breeding lines that show nematode resistance will be transferred to pots in the greenhouse for multiplication and used in the breeding program. In subsequent years of this project we will propose conducting the same studies with other nematodes that affect potato production in the SLV such as *M. hapla, Pratylenchus penetrans, and Paratrichodorus* sp.

Isolation and partial characterization of the active compound(s) from nematode resistant lines: The breeding lines that show activity against the test nematodes will be used for further studies. The root exudates of these lines will be collected in large quantities and concentrated by freeze drying to avoid the breakdown of the chemical constituents of the exudates. These exudates will then be checked for their activity against the nematode. One milliliter of the exudate will be pipetted into each well of the 24-well tissue culture plate, 100 surface-sterilized eggs of *M. chitwoodi* will be transferred into each of the wells and the number of eggs hatched and the motility of the J2 juveniles will be observed after seven days of incubation. The exudates that show activity will be analyzed by High Performance Liquid Chromatography (HPLC) to isolate individual active compounds; these compounds will then be tested against the nematodes. We do not anticipate obtaining chemical identification in Year 1 of this project due to time constraints, but we will propose these studies in Year 2 of this project.

Relationship of the proposed research to overall problem: Development of disease-resistant cultivars is an important objective of the potato breeding program at SLVRC. However, to date only the introgression of nematode-resistant material into certain potato varieties has been conducted by using nematode-resistant material that has been developed by other groups through tedious and long-term screens under field conditions. There is currently no nematode resistance screening protocol in place aimed at developing resistant potato cultivars in Colorado. The research proposed here will effectively complement the SLVRC breeding program by rapidly identifying promising lines and cultivars of potato that are resistant to nematodes.

Potential for leveraging research results to obtain outside funding: The outcome of this proposal will result in the identification of several potato lines and cultivars of potato that are resistant to plant parasitic nematodes. Further, we hope to identify chemical compound(s) exuded by potato roots that are detrimental to nematodes. Successful completion of this work will allow us to propose larger projects to such granting agencies as the USDA and NSF, including a potential collaborative project with scientists at SLVRC to develop potato cultivars possessing elevated levels of potential nematidicidal compounds using conventional and molecular breeding techniques.

Bibliography

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Budget

Personnel

We need a dedicated MS student to carry out the objectives proposed in this project from start to completion. Salary and tuition for the student: $20,000/year

Supplies

Chemicals and materials related to the establishment of the screening technique and isolation of compounds are requested. Additionally, fees related to service facilities for the identification of compounds are requested (CSU Macromolecular Resource Facility). These funds will also cover the maintenance of the plant material and generating crosses in the SLVRC. $15,000/year.

** Due to the complexity of the project, two years of support are requested to achieve all objectives.

TOTAL: $35,000 per year; $70,000 for the length of the project (two years)