2005

Title: Screening of potato breeding lines for nematode resistance

Investigators: **PI:** Dr. Balakrishnan Prithiviraj, Research Assistant Professor, and **Co-PI**: Dr. Jorge Vivanco, Associate Professor, Department of Horticulture and Landscape Architecture, and Center for Rhizosphere Biology

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, act as vectors of 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. Here we propose a large-scale screening of available potato cultivars for nematode resistance using a novel technique that harnesses the potato's own defense mechanisms. In the studies performed at our 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. We propose the following objectives to complement the potato breeding program carried out at the San Luis Valley Research Center (SLVRC):

1. Screening of advanced potato breeding lines and true seeds developed by Dr. David Holm at the SLVRC for resistance to *Meloidogyne chitwoodi*.
2. Isolation and partial characterization of the active compound(s) present in the root exudates of nematode-resistant potato lines.

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 the advanced breeding lines developed at SLVRC, courtesy of Dr. David Holm. 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 (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 it as described earlier. 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. On 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 well 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, there is currently no research initiative aimed at developing nematode resistant potato cultivars in Colorado. The research proposed here will effectively complement the SLVRC breeding program by 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.

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2. **Harrison BD, Robinson DJ** (1986) Tobraviruses. In: Van Regenmortel MHV, Fraenkel-Conrat H, eds. *The Plant Viruses*. New York: Plenum Press, 339–69.
3. **Bais HP, Park SW, Weir TL, Callaway RM, Vivanco JM** (2004) How plants communicate using the underground information superhighway. Trends in Plant Science **9**:26-32.
4. **Walker TS, Bais HP, Grotewold E, Vivanco JM** (2003) Root exudation and rhizosphere biology. Plant Physiology **132**: 44-51.
5. **Bais HP, Prithiviraj B, Jha AK, Ausubel FM, Vivanco JM** (2005) Mediation of pathogen resistance by root exudation of antimicrobials. Nature (in press).
6. **Brown DJF, Boag B** (1988) An examination of methods used to extract virus-vector nematodes (Nematoda: Longidoridae and Trichodoridae) from soil samples. *Nematologia Mediterranea* **16**, 93–9.
7. **Nitao JK, Meyer SLF, Oliver JE, Schmidt WF, Chitwood DJ** (2002) Isolation of a avipin, a fungus compound antagonistic to plant-parasitic nematodes. Nematology **4**: 55-63.

Budget

Item No.	Description	Amount ($)
1.	Salary of the Investigator (4 months @ $ 3300.00 per month)	13200.00
2.	Labwares and Chemicals and Central Instrumentation Facility (Chemistry Dept., CSU) service charges	4500.00
3.	Greenhouse rental	500.00
4.	Undergraduate Salary	1000.00
	Total	**19200.00**

Budget Justification

Salary: The PI has a non-tenure appointment and his salary is supported by research grants. Salary provided by this project will allow him to dedicate 25% of his time for this project.

Undergraduates: Salary is requested for one undergraduate student to help the PI set up the screening system.

Labwares, chemicals and instrument services: This project requires a large number of plastic disposable items and glasswares along with chemicals for plant and nematode cultures; it also requires such instruments as the confocal microscope whose use is charged by the hour.

Greenhouse: The nematode used in this study cannot be cultured *in vitro*, and must be cultured along with the host plants. Therefore, greenhouse space is required to grow the host plants in pots.

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Professional Preparation

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1992	M. Sc. (Agriculture) Mycology and Plant Pathology, Institute of Agricultural Sciences, Varanasi, India
1997	Ph.D., Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India
1998-2003	Post-Doctoral Associate Department of Plant Science McGill University, Canada
2003-2004	Post-Doctoral Associate Department of Horticulture Colorado State University

Academic Appointments

2004-to date	Research Assistant Professor, Department of Horticulture & Landscape Architecture, Colorado State University

Peer-Reviewed Publications (2000 – to date) (Total: 61)

1. Bais, HP Prithiviraj B, Jha AK, Ausubel FM and Vivanco JM. 2005. Mediation of pathogen resistance by exudation of antimicrobials from roots. *Nature* (In Press).
2. Prithiviraj B, Bais HP, Weir T, Schweizer HP and Vivanco JM. 2005. Plant models for animal pathogenesis. *Cellular Microbiology* 7: 315-324.
3. Prithiviraj, B Bais, HP and Vivanco JM. 2005. *Staphylococcus aureus* pathogenicity on *Arabidopsis thaliana* unravels a novel role for salicylic acid as an anti-infective agent. *Plant Journal* (In Press)
4. Prithiviraj B, Vikram A and Kushalappa AC. 2004. Volatile metabolite profiling of onion inoculated with *Erwinia carotovora* ssp. *carotovora*, *Fusarium oxysporum* and *Botrytis allii*. *European Journal of Plant Pathology* 110:371-377.
5. Vikram A, B.Prithviraj, H.Hamzezharghani and A.C.Kushalappa, 2004. Volatile metabolite profiling to discriminate diseases of McIntosh apple inoculated with fungal pathogens. *Journal of the Science of Food and Agriculture* 84: 1333-1340.
6. Zhang, H, Prithiviraj B, Charles TC, Driscoll BT, Smith DL 2003. Low temperature tolerant *Bradyrhizobium japonicum* strains allowing improved nodulation and nitrogen fixation of soybean in a short season (cool spring) area. *European Journal of Agronomy*. 19: 205-213
7. Lui LH, B.Prithviraj, A.Vikram, A.C.Kushalappa and G.S.V.Raghavan, 2004. Volatile metabolite profiling of potato tubers inoculated with dry and soft rot pathogens. *The American Potato Research* (In Press).
8. Vikram A,.Prithviraj, B and Kushalappa, A.C. 2004. Use of volatile metabolite profiles to discriminate diseases of Cortland and Empire apples. *Journal of Plant Pathology* 86: 215-225.

9. Prithiviraj B, Zhou X, Souleimanov A, Kahn, WM and Smith DL 2003. A host-specific bacteria-to-plant signal molecule (Nod factor) enhances germination and early growth of diverse crop plants. *Planta* 216: 437-445
10. Smith DL, Costa C, Ma B. Madakadze C, Prithiviraj B, Zhang F, and Zhou X. 2003. From Chemical to agronomy: Cropping systems in the humid northeast. Journal of Crop Production 9: 455-499.
11. Wajahatullah MK, Prithiviraj B and Smith DL. 2003 Photosynthetic responses of corn and soybean to foliar application of salicylates *Journal of Plant Physiology* 160: 485-492.
12. Wajahatullah MK, Prithiviraj B and Smith DL. 2003. Chitin and Chitosan Oligomers Increase Phenylalanine Ammonia-Lyase and Tyrosine Ammonia-lyase Activities in Soybean. *Journal of Plant Physiology* 160: 859-870.
13. Wajahatullah MK, Prithiviraj B and Smith DL. 2003. Effect of chitooligosaccharides on photosynthesis of corn and soybean. *Photosynthetica* 40: 621-624.
14. *Soulemanov A, Prithiviraj B and Smith DL. 2002. Lipo-chitooligosaccharide Nod Bj V(C18:1, MeFuc)* of Bradyrizhobium japonicum *promotes early growth of soybean and corn* . Journal of Experimental Botany *53: 1929-1934.*
15. Smith, D. L. Prithiviraj, B. Zhang, F. 2002. Rhizobial signals and control of plant growth. [Book chapter] Nitrogen fixation: global perspectives. Proceedings of the 13th International Congress on Nitrogen Fixation, Hamilton, Ontario, Canada, 2-7 July 2001. CABI Publishing,Wallingford, UK: 327-330.
16. Zhang H, Prithiviraj B, D'Aoust F, Charles TC, Driscoll BT and Smith DL.2002.The effect of temperature and genistein concentration on Lipo-chitin oligosaccharides (LCOs) production by *Bradyrhizobium japonicum* mutants and strains. *Soil Biology and Biochemistry* 34: 1175-1179.
17. Zhang H, D'Aoust F, Charles TC, Driscoll BT, Prithiviraj B and Smith DL . 2002.*Bradyrhizobium japonicum* Mutants Allowing Improved Soybean Yield In Short Season Areas With Cool Spring Soil Temperatures. *Crop Science* 42: 1186-1190.
18. Zhang H, D'Aoust F, Charles TC, Driscoll BT, Prithiviraj B and Smith DL . 2002. *Bradyrhizobium japonicum* mutants allowing improved nodulation and nitrogen fixation of field grown soybean in a short season area. *Journal of Agricultural Science* 138: 293-300.
19. Zhang H, D'Aoust F, Charles TC, Driscoll BT Prithiviraj B and Smith DL . 2002. Low temperature tolerant *Bradyrhizobium japonicum* strains allowing improved soybean yield in short season areas. *Agronomy Journal* 94: 870-875.
20. Soulemanov A, Prithiviraj B and Smith DL. 2001. Isolation and characterization of the major nod factor of *Bradyrhizobium japonicum* strain 532C. *Microbiological Research* 157: 25-28.
21. Lian, B., Prithiviraj, B., Souleimanov, A. and Smith, DL.2001. Evidence for the production of chemical compounds analogous to nod factor by the silicate bacterium Bacillus circulans GY92. *Microbiological Research* 156: 289-292
22. Singh UP, Prithiviraj B, Singh M, Sarma BK and Ray AB. 2001. Role of garlic (*Allium sativum* L.) in human and plant diseases. *Indian Journal of Experimental Biology* 39: 310-322 .
23. Madakadze IC, Prithiviraj B, Madakadze IC, Stewart KA, Peterson PR, Coulman BE and Smith DL. 2001. Variation in base temperature for germination in warm season grasses. *Seed Science and Technology* 29: 31-38.
24. Singh UP, Prithiviraj B and Sarma BK. 2001. Development of *Erysiphe pisi* on pea leaves as affected by calcium and calmodulin modulators. *Microbiological Research* 156: 65-70.
25. Singh U P, Singh S K, Sugawara Koya, Srivastava J S, Sarma B K, and Prithiviraj B. 2001. Studies on sclerotium formation in Curvularia species. *Mycobiology*. 29: 154-159.
26. Singh, S.K., Tuli, L., Singh, U.P. and Prithiviraj, B. 2001.Effect of temperature, light/darkness and relative humidity on germination and sporulation of *Alternaria tenuissima*. *Indian Phytopathology* 54:128-130.

27. Prithiviraj B, Souleimanov A, Zhou X and Smith DL. 2000. Differential response of soybean (*Glycine max* (L.) Merr.) genotypes to lipo-chitooligosaccharide Nod Bj V-(C18:1, MeFuc). *Journal of Experimental Botany* 51:2045-2051.
28. Prithiviraj B, Souleimanov A and Smith DL. 2000. Differential response of soybean cultivars to lipochitin oligosaccharide Nod Bj (V18:1 MeFeu) of *Bradyrhizobium japonicum*. *Canadian Journal of Plant Science*. 80: 223.
29. Dashti N, Prithiviraj B, Zhou X, Hynes RK and Smith DL. 2000. The combined effects of plant growth-promoting rhizobactria and genistein on nitrogen fixation in soybean (*Glycine max* (L.) Merrll.) at suboptimal root zone temperatures. *Journal of Plant Nutrition* 23: 593-604.
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Advisors

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Professional Preparation

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1999	Ph.D., Plant Pathology-Biotechnology, The Pennsylvania State University
1999-2000	Post-Doctoral Associate Biotechnology Center for Agriculture and the Environment Rutgers University

Academic Appointments

2004-to date	Director Center for Rhizosphere Biology Colorado State University
2004-to date	Associate Professor Department of Horticulture Colorado State University
2002-to date	Affiliate Faculty Member Cell and Molecular Biology Graduate Program Graduate Degree Program in Ecology Colorado State University
2000-2004	Assistant Professor Department of Horticulture Colorado State University

Recent Peer-Reviewed Publications

Five most relevant publications (Total: 50)

1. Walker, T.S., Bais, H.P., Halligen, K.M., Stermitz, F.R., and Vivanco, J.M. (2003) Metabolic profiling of non-polar compounds in root exudates of *Arabidopsis thaliana in vitro*; study of dynamic interface for the comprehensive characterization of rhizospheric interactions. Journal of Agricultural and Food Chemistry 51:2548-2554
2. Bais, H.P., Vepachedu, R., Gilroy, S., Callaway, R.M., and Vivanco, J.M. (2003) Allelopathy and exotic plant invasion: from molecules and genes to species interactions. Science 301:1377-1380
3. Walker, T.S., Bais, H.P., Grotewold, E., and Vivanco, J.M. (2003) Root exudation and rhizosphere biology. Plant Physiology 132:44-51
4. Bais, H.P., Fall, R, and Vivanco, J.M. (2004) Biocontrol efficiency of *Bacillus subtilis* (6051) against *Pseudomonas syringae* (DC3000) in *Arabidopsis thaliana* roots is facilitated by biofilm formation and surfactin production. Plant Physiology 134: 307-319
5. Bais, H.P., Prithiviraj, B., Jha, A.K., Ausubel, F.M., and Vivanco, J.M. (2005) Root exudation of antimicrobials mediates pathogen resistance. Nature (in press)

Five other significant publications:

1. Bais, H.P., Vepachedu, R., Lawrence, C.B., Stermitz, F.R., and Vivanco, J.M. (2003) Molecular and biochemical characterization of an enzyme responsible for the formation of hypericin in St. John's wort (*Hypericum perforatum* L.). Journal of Biological Chemistry 278:32413-32422

2. Bais, H.P., Park, S.W., Weir, T.L. Callaway, R.M., and Vivanco, J.M. (2004) How plants communicate using the underground information superhighway. Trends in Plant Science 9:26-32
3. Steeghs, M., Bais, H.P., de Gouw, J., Goldan, P., Kuster, W., Northway, M., Fall, R., and Vivanco, J.M. (2004) Proton-transfer-reaction mass spectrometry (PTR–MS) as a new tool for real time analysis of root-secreted volatile organic compounds (VOCs) in *Arabidopsis thaliana.* Plant Physiology 135:47-58
4. Vivanco, J.M., Bais, H.P., Stermitz, F.R, and Callaway, RM (2004) Root Allelochemistry strongly contributes to *Centaurea diffusa* invasive behavior. Ecology Letters 7:285-292
5. Weir, T., Park, S-W., and Vivanco, J.M. (2004) Biochemical and physiological mechanisms mediated by allelochemicals. Current Opinion in Plant Biology 7:472-479

Synergistic Activities

- Development of international internships on Andean agriculture for CSU students.
- Involvement in minority programs at CSU.
- Development of a Medicinal Plants/Plant Biochemistry/Ethnobotany/Bioactive Plants course at CSU
- Panel Member, Metabolic Biochemistry, National Science Foundation, 2004
- Member, USDA-Technical National Committee for W-122 Project, 2002-to date

Collaborators

Dr. Fred Ausubel (Harvard Medical School), Dr. Ray Callaway (U Montana), Dr. Joost de Gouw (NOAA), Dr. Erich Grotewold (Ohio State U), Dr. Ray Fall (University of Colorado), Dr. Simon Gilroy (Penn State U), Dr. Martha Hawes (U Arizona), Dr. David Holm (Colorado State U), Dr. Mark Paschke (Colorado State U), Dr. Marinus Pilon (CSU), Dr. Gerardo Rubio (UBA), Dr. Herbert Schweizer (Colorado State U), Dr. Frank Stermitz (Colorado State U).

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Past Postdoctoral Scholar Sponsor

Ajay Jha (CSU), Neelam Sharma (CSU), Ramarao Vepachedu (National Jewish Center)

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