SUMMARY RESEARCH PROGRESS REPORT FOR 1993
AND RESEARCH PROPOSAL FOR 1994
Submitted to:
SLV Research Center Committee
and the
Colorado Potato Administrative Committee (Area II)

TITLE: Engineering Resistance to Fungal Pathogens in Potato

PROJECT LEADER(S): A.S.N. Reddy,
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PROJECT JUSTIFICATION: The long-term objective of this proposal is to genetically engineer potato plants to obtain transgenic plants that are resistant to fungal pathogens. The proposed studies are being carried out for a number of reasons that are described below:

i). The potato (*Solanum tuberosum* L.) is the most important non-cereal food crop in Colorado State as well as in the world. It is the fourth major food crop of the world and is next only to wheat, rice and maize in terms of total food production (Vayda and Park, 1990; Salunkhe and Kadam, 1991).
ii). The potato is susceptible to a great number of diseases (Rich, 1983). About 160 diseases and disorders of *Solanum tuberosum* have been described (Rich, 1983) out of which majority of the diseases (about 50) caused are by fungal pathogens. Crop loss resulting from fungal diseases is enormous in potato (Rich, 1983). The most important fungal diseases are caused by *Alternaria solani* (early blight), *Phytophthora infestans* (late blight), *Fusarium spp* (tuber rot), *Rhizoctonia solani* (black scurf) and*Verticillium dahliae* (verticillium wilt). A variety of fungicides have been developed and used to control phytopathogenic fungi of potato. The use of these fungicides is moderately successful. However, the use of fungicides is limited to minimum for various reasons. Moreover, variants of fungus that are resistant to fungicides have been found recently (Anderson, 1993).
iii). Recently a new group of proteins have been shown to have strong antifungal activity (Roberts and Selitrennikoff, 1988; Vigers *et al.*, 1991, 1992; Huynh *et al.*, 1992). These antifungal proteins have been shown to inhibit the growth of a wide range of agronomically important fungal phytopathogens including potato pathogens such as *Phytopthera infestans* (Woloshuk *et al.*, 1992), *Alternaria solani* (Huynh *et al.*, 1992) and *Fusarium oxysporum* (Huynh *et al.*, 1992). These antifungal proteins appear to be present in all plants and have been characterized from various plant species from both monocots and dicots including potato (King *et al.*, 1986; Singh *et al.*, 1987; Richardson *et al.*, 1987; Pierpoint *et al.*, 1987; Kauffmann *et al.*, 1990; Roberts and Selitrennikoff, 1990; Vigers *et al.*, 1991; 1992; Pierpoint *et al.*, 1990; Rebmann *et al.*, 1991).
iv). Isolation of genes coding for these antifungal proteins and reintroduction of the isolated genes to manipulate their expression is feasible because of the recent developments in recombinant DNA technology. Substantial progress in improving potatoes by the use of modern techniques has been realized (Cornelissen and Melchers, 1993). Transgenic potato plants, including plants derived from commercial varieties such as Russet Burbank that are resistant to potato virus X and potato virus Y, have been produced (Hemenway *et al.*, 1988; Hoekema *et al.*, 1989; Lawson *et al.*, 1990). Also, dry matter of Russet Burbank tubers was increased by about 23% by overexpressing one of the key enzymes involved in starch

biosynthesis (Stark *et al.*, 1991, 1992). Some of these genetically engineered potato plants are being tested in the field (McCammon and Medley, 1990).
v). It is easy to introduce genes into potato using *Agrobacterium* and generate a large number of transgenic plants (Sheerman and Bevan, 1988; Wenzler *et al.*, 1989a; Mitten *et al.*, 1990).
vi). The incorporation and overexpression of genes encoding potent anti-fungal proteins in plants may significantly augment the level of their resistance to fungal diseases. Generation of such transgenic potato plants will save money to farmers and eliminate the use of fungicides that adversely effect our environment. Hence, the success of the proposed project will positively contribute to sustainable agriculture.

PROJECT STATUS: Ongoing

This project was started based on recent observations that plants, in response to fungal pathogen attack, produce a protein that has potent antifungal activity. This protein was purified and aminoterminal sequence of the protein was reported. Based on the existing information we proposed to first isolate cDNAs that code for these proteins and use these cDNAs to generate transgenic plants that constitutively overproduce this protein to confer resistance to fungal pathogens. SLV research center committee awarded $7,000 to initiate this research project. I used the funding from SLV research center and from our college as matching funds for a proposal to Colorado Institute for Research in Biotechnology (CIRB) to generate seed money. My proposal to CIRB received the highest rank of all the submitted grants and received a score of 100% (see attached letter). This seed money has enabled us to initiate the research and make considerable progress on the project.

SIGNIFICANT ACCOMPLISHMENTS FOR 1993:

A prerequisite for isolating a cDNA for antifungal protein is the availability of a cDNA library. We have obtained a cDNA library that is prepared from stolon tips. Using this library we have taken two different approaches to obtain the cDNAs. We have recently isolated PCR amplified fragments from the cDNA library that are being sequenced to identify the amplified products. These clones, upon the completion of characterization, will form the basis of work for this year. In an alternate approach, a heterologous cDNA probe is being used to screen the potato cDNA library. (please see 1993 Comprehensive Research Report).

OBJECTIVES FOR 1994:

I. Characterization of the isolated complementary DNAs (cDNAs) that code for an antifungal protein from potato.

II. Construction of a *Agrobacterium* based binary vector containing a modified 35S CaMV promoter and antifungal cDNA in sense orientation.

In 1995, we will be transforming the potato plants and evaluating the plants for fungal resistance.

FUNDING REQUEST:

1993 Allocation:	$7,000
1994 Request:	$8,000

The money requested in this project will be used for consumable supplies such as restriction enzymes, radioisotopes, x-ray films, nitrocellulose filter, tissue culture media, many routines chemicals and disposables and to partially support a student helper. All the necessary equipment, binary vectors and the expertise to accomplish the proposed objectives are available in the P.I's laboratory.

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Yang, M.S., Espinoza, N.O., Nagpala, P.G., Dodds, J.H., White, F.F., Schnorr, K.L. and Jaynes, J.M. Expression of a synthetic gene for improved protein quality in transformed potato plants. Plant Sci. 64, 99-111 (1989).

CURRICULUM VITAE

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RESEARCH/PROFESSIONAL EXPERIENCE:

1977 - 1979 M.S. (Botany with Genetics and Cell Biology specialization), Kakatiya Univ., India. Awarded Gold Medal
1979 - 1984 Research Associate, Molecular Biology Unit, Jawaharlal Nehru Univ., New Delhi, India (Ph.D in Plant Molecular Biology)
1985 - 1989 Postdoctoral Research Associate, Washington State Univ., Pullman, WA
1989 - 1992 Staff Scientist, Washington State Univ., Pullman, WA
1992- present Assistant Professor, Dept. of Biology, Colorado State Univ., Fort Collins, CO.

HONORS, SCHOLARSHIPS, FELLOWSHIPS:

1. Awarded Gold Medal for securing highest marks in M.S
2. Junior Research Fellowship, Council of Scientific and Industrial research, India 1980-82.
3. Senior Research Fellowship, Council of Scientific and Industrial research, India 1982-85.
4. CSIR and DST travel grant to attend a NATO Advanced Study Institute, Greece, 1984.
5. Travel grants to attend **i)** a course on "Essential techniques in gene manipulation" (1986) at Univ., Manchester Inst. Tech., London; **ii)** 13th Intnl. Conf. on plant growth substances, Calgary, Canada (1988) and **iii)** the 2nd Intnl. Cong. Plant Mol. Biol., Israel (1988).
6. Member of Bio-technology Faculty Position Search Committee, W. S. U. (1988-89).
7. Awarded genetic sequence computer analysis training fellowship by Molecular Biology Computer Research Resource Center, Dana-Farber Cancer Institute, Harvard School of Public Health, Harvard Medical School (Nov 25 - Dec 8, 1990).
8. Participated in a three week practical course on Molecular and Developmental Biology of Plants, June 30th to July 21st, 1991 at Cold Spring Harbor Laboratories, New York.
9. Member of Cell and Molecular Biology Program, CSU.
10. Member of American Assoc. of Advancement of Sciences, American Association of Plant Physiologists, Sigma XI.
11. Member of Plant Molecular Biology/Genetics Search committee, Dept. of Biology, 1993-94
12. Member of Plant Molecular Genetics Search Committee, Dept. Agronomy, 1994

Recent Publications: Published 44 scientific papers and review articles and 44 abstracts since 1982. Attended sixteen national and international meetings. Following are some of the recent publications.

1. Reddy, A.S.N., Farida, S., Bayette, J. and Mykles, D. (1994) A calcium-dependent proteinase activity in Arabidopsis root cultures. **Biochem. Biophys. Res. Commun.** In press.
2. Day, S. and Reddy, A.S.N. (1994) Cloning of a family of cyclins from Arabidopsis. **Biochim. Biophys. Acta. (Gene Structure & Expression)**. In press.

3. Reddy, A.S.N. Calcium as a messenger in stress signal transduction. In: **Handbook of Plant and Crop Physiology**. Marcel Dekker Inc., In press.
4. Reddy, A.S.N. (1994) Cell cycle regulation in plants. In: **Handbook of Plant and Crop Physiology**, Marcel Dekker Inc., In press.
5. Reddy, A.S.N., Takezawa, D. Fromm, H. and Poovaiah, B.W. (1993) Isolation and characterization of two cDNAs that encode for calmodulin-binding proteins from corn root tips. **Plant Science** 94: 109-118.
6. Poovaiah B.W. and Reddy, A.S.N. (1993) Calcium and signal transduction in plants. **CRC Cri. Rev. Plant Sci.** 12: 185-211.
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8. Reddy, A.S.N., A.J. Czernik., G. An and B.W. Poovaiah (1992) Cloning of the U1 small nuclear ribonucleoprotein particle 70K protein from *Arabidopsis thaliana*. **Biochim. Biophys. Acta**. 1171: 88-92.
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15. Reddy, A.S.N. and B.W. Poovaiah. (1987) Inositol 1,4,5-trisphosphate induced calcium release from corn coleoptile microsomes. **J. Biochem.** 101:569-573.
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17. Reddy, A.S.N., A. Raina, S. Gunnery and A. Datta. (1987) Regulation of protein synthesis in plant embryo by protein phosphorylation, I. Purification and characterization of a cAMP-independent protein kinase and its endogenous substrate. **Plant Physiol.** 83:988-993.
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19. Reddy, A.S.N., S. Chengappa and B.W. Poovaiah. (1987) Auxin-regulated changes in protein phosphorylation in pea epicotyl segments. **Biochem. Biophys. Res. Commun.** 144:944-950.
20. Reddy. A.S.N., M. Friedmann and B.W. Poovaiah. (1988) Auxin-induced changes in protein synthesis in the abscission zone of bean explants. **Plant and Cell Physiol.** 29:179-183.

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June 25, 1993

Dr. A.S.N. Reddy
Assistant Professor
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Colorado State University
Fort Collins, Colorado 80523

Dear Dr. Reddy:

I am pleased to inform you that the Colorado Institute for Research in Biotechnology (CIRB) has chosen to fund your proposal, *"Engineering Resistance to Fungal Pathogens in Potato,"* at a level of $15,000 for the period July 1, 1993, through June 30, 1994.

By accepting this award, you agree to prepare a final report on the project and to send two copies of it to me by July 31, 1994. The report should be two to three pages and should include: (1) a brief description of the research progress and accomplishments; (2) a status summary of any related external proposals that are planned or submitted (give funding agency, proposal title, P.I. name(s), duration, dollar amount, and status); (3) a letter from any industrial partner stating their level of support (only if their participation was different than that indicated in your proposal). This letter is extremely important as it will be required in CIRB's year-end financial report to CATI. You are also asked to make a presentation at the CIRB Biotechnology Symposium in September 1994.

Please sign the enclosed Memorandum of Understanding and return it to me at your earliest convenience. Once I have received the memorandum, CIRB will contact your institution's research contract administrator to set up an account for your use. If you have any questions, please call Dr. Robert Davis at the University of Colorado (492-7314) or Dr. Vincent Murphy at Colorado State University (491-1791).

Congratulations on your award!

Sincerely,

Virginia P. Orndorff
Director, Biotechnology Programs

VPO:kt

cc: James R. Myers, San Luis Valley Research Center
John C. Raich, CSU, College of Natural Sciences
Bruce A. Wunder, CSU, Dept. of Biology

PS -
Dr. Reddy - your proposal was the highest ranked of them all, with one score of 100%. - the highest ever for a CIRB Seed Grant!
G. O.

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