SUMMARY RESEARCH PROGRESS REPORT FOR 1994
AND RESEARCH PROPOSAL FOR 1995
Submitted to:
SLV Research Center Committee
and the
Colorado Potato Administrative Committee (Area II)

TITLE: Engineering Resistance to Fungal Pathogens in Potato

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PROJECT JUSTIFICATION: The objective of this proposal is to genetically engineer potato plants to obtain transgenic plants that are resistant to fungal pathogens.

Potato (*Solanum tuberosum* L.) is the most important non-cereal food crop in the world (Vayda and Park, 1990). It is susceptible to a great number of diseases, some of which are worldwide importance whereas others are of more localized significance (Rich, 1983). Approximately 160 diseases and disorders of *Solanum tuberosum* have been described (Rich, 1983) and majority of these diseases (about 50) caused are by fungal pathogens. In potato, crop loss resulting from fungal diseases is enormous (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), *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). A new group of proteins with strong antifungal activity (Roberts and Selitrennikoff, 1990; Vigers *et al.*, 1991, 1992; Huynh *et al.*, 1992, Malehorn *et al.*, 1994) have been reported recently. 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 (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). 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 (Fraley 1992; Moffat 1992; 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.*, 1992). 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 be economical to farmers and eliminate the use of fungicides that adversely effect

our environment. Several companies such as Monsanto, CIBA-GIGY and Mogen are actively engaged in using a similar approach to generate disease resistant plants.

PROJECT STATUS: Ongoing

In response to pathogen attack, plants produce several proteins. A class of pathogen-induced proteins that share significant sequence homology with thaumatin have been shown to inhibit growth of several fungal pathogens. Our goal is 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. Funding from SLV Research Center has enabled us to initiate the research and isolate the cDNAs that code for putative antifungal proteins. Funding from SLV has also helped in obtaining a seed grant from Colorado Institute for Research in Biotechnology in 1993 and an interdisciplinary grant from CSU in 1994/95. This support has greatly helped us in making considerable progress on this project.

SIGNIFICANT ACCOMPLISHMENTS FOR 1994:

A cDNA library was screened to isolate cDNAs that encode for thaumatin-like antifungal proteins. Characterization of the several cDNAs by restriction mapping indicated that there are two distinct cDNAs. The nucleotide sequence of the longest cDNAs from each group was determined. The deduced amino acid sequence from these clones showed strong homology with known antifungal proteins. Experiments are in progress to produce large quantities protein in E.coli to determine antifungal activity. (please see 1994 Comprehensive Research Report).

OBJECTIVES FOR 1994:

I. Expression and purification of proteins encoded by cDNAs for putative antifungal proteins.

II. *In vitro* assays to determine the antifungal activity of the purified proteins

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

IV. Transformation of potato to generate transgenic plants overexpressing antifungal protein.

It will take at least two to three years to accomplish the above objectives. Our emphasis during the next year will be on objectives I and II.

FUNDING REQUEST:

1994 Allocation:	$8,000
1995- Request:	$11,000

The P.I. intends to carryout the proposed work with the help of an undergraduate student. The funding 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, E. coli expression vectors, 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.

Recent Publications:

Published 47 scientific papers and review articles and 49 abstracts since 1982. Attended eighteen national and international meetings. Following are some of the recent publications.

1. Hu, X. and Reddy, A.S.N. (1995) Nucleotide sequence of a cDNA clone encoding a thaumatin-like protein from Arabidopsis. **Plant Physiol.** (in press)

2. Fordham-Skelton, A.P., Safadi, F., Golovkin, M. and Reddy, A.S.N. (1994) A non-radioactive method for isolating complementary DNAs encoding calmodulin-binding proteins. **Plant Mol. Biol. Reporter** 12: 355-363.

3. Day, S. and Reddy, A.S.N. (1994) Cloning of a family of cyclins from Arabidopsis. **Biochim. Biophys. Acta.** 1218: 115-118.

4. 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. 199: 1089-1095.**

5. Reddy, A.S.N. Calcium as a messenger in stress signal transduction. In: **Handbook of Plant and Crop Physiology**. Marcel Dekker Inc., (in press).

6. Reddy, A.S.N., Takezawa, D., Fromm, H. and Poovaiah, B.W. (1993) Cloning and characterization of two cDNAs that encode for calmodulin-binding proteins from corn root tips. **Plant Science** 94: 109-118.

7. Poovaiah, B.W. and A.S.N. Reddy (1993) Calcium and signal transduction in plants.**Cri. Rev. Plant Sci.** 12:185-211.

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.

9. Reddy, A.S.N. (1992) Auxin-regulated gene expression. In:**McGraw Hill Year Book of Science and Tech.** pp40-42.

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11. Reddy, A.S.N. and B.W.Poovaiah. (1990) Molecular cloning and sequencing of an auxin-repressed cDNA clone: Correlation between fruit growth and repression of the auxin-regulated gene. **Plant Mol. Biol.** 14: 127-136.

12. Reddy, A.S.N., P.K.Jena., S.K. Mukherjee and B.W.Poovaiah. (1990) Molecular cloning of cDNAs for auxin-induced mRNAs and developmental expression of the auxin-inducible genes. **Plant Mol. Biol.** 14: 643-653.

13. Jena, P.K., A.S.N. Reddy. and B.W. Poovaiah. (1989) Molecular cloning and sequencing of a cDNA for plant calmodulin: Signal-induced changes in the expression of calmodulin. **Proc. Natl. Acad. Sci.** 86: 3644-3648.

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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.

16. Raghothama, K.G., A.S.N. Reddy, M. Friedmann and B.W. Poovaiah. (1987) Calcium-regulated in vivo protein phosphorylation in Zea mays L. root tips. **Plant Physiol.** 83:1008-1013.

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.