

**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: APPLICATION OF FOURIER TRANSFORM INFRARED SPECTROSCOPY
TO IDENTIFY POTATO CULTIVARS**

PROJECT LEADERS: Cecil Stushnoff, David Holm and Robert Davidson

PROJECT JUSTIFICATION:

Adaptation of tissue culture technology by the potato seed industry, the release of superior new cultivars from plant breeding programs, and eminent release of transgenic patented new cultivars from plant biotechnology programs have heightened the need for an accurate, repeatable, rapid technique to identify potato cultivars. There is a need to characterize and archive new cultivars to support visual descriptions. In particular, potatoes are often propagated in a controlled, disease-free environment by means of a mini-tuber technique, but various cultivars increased by this technique are often extremely difficult to identify visually.

Previous research targeted at these needs has centered on either electrophoretic analysis of isoenzymes or on direct analysis of DNA, using polymerized chain reactions (PCR). Although both are valuable techniques, especially PCR which can be used to identify lines that differ by only a few genes, the procedures are involved are complex and costly.

We suggest from preliminary data that potato cultivars can be identified by means of IR spectroscopy, using a fine powder prepared from freeze-dried tubers and a specially designed sampling accessory. Use of a freeze dried powder would provide a simple, inexpensive method of shelf storage of archival material, as well as a simple, quick, inexpensive means of analyzing samples based on a computer library of spectra.

PROJECT STATUS: Ongoing

Previous work suggested a need to explore improved sample preparation techniques and to test the possibility that computer libraries of spectra could be used to identify powders prepared from minitubers. As well, there was a need to determine the effects of storage on stability of protein and starch bonds used in developing fingerprinting spectra. This report describes the progress attained toward those goals and the work which yet remains to be completed.

SIGNIFICANT ACCOMPLISHMENTS FOR 1994:

Results obtained in 1994-95 strongly suggest that spectra from FTIR spectroscopy of very fine freeze-dried powders can be used to fingerprint and identify potato cultivars. The following procedures produced the best results.

1. Freeze-dried, finely screened whole tuber powders produced better spectra than either soluble or insoluble peel or tuber fractions.
2. Sifting through a 400 mesh (38 micron) screen is essential.
3. Comparisons made on small, but different segments of the infrared spectrum, especially in the amide I and amide II overtone regions, have markedly improved sensitivity and accuracy of identification.
4. Minituber and whole tuber unknowns were correctly identified from minituber libraries. Libraries of 10-month old tuber powders were not suitable to screen minituber powders for unknowns.

OBJECTIVES FOR 1995:

1. Complete preparation and analyses of stored tubers.
2. Test identification potential at the onset of tuber initiation from field samples.
3. Determine if leaf samples can be used for identification purposes.
4. Modify the software program to simplify analyses.
5. Obtain samples of similar cultivars from different regions in the US to test impact of environment on identification parameters.
6. Disclose procedure for possible patent application.
7. Attempt to improve precision, enabling identification of somatic mutant clones differing by only a few genes.
8. Test applicability to distinguish transgenic lines with genes for increased starch content, BT insect resistance, protein coat virus resistance and herbicide resistance. This would need to be explored with Monsanto Company.

FUNDING REQUEST: 1994 Allocation: \$4528. 1995 Request: \$5023

1. Summer student to complete analyses of stored tubers and freeze-dried powders. An undergraduate work-study student, proficient in analytical chemistry and computer science, has been trained to conduct infrared studies. John Carminati is available to work this summer (11 weeks X 40 hours X \$7.00/hour X 2.5% = \$3157)
2. Analysis of field tubers and greenhouse minitubers during the growing and storage season to expand the cultivar database library and to confirm results from last year; to be shipped by UPS from SLV (approximately monthly from June 1994 to March 1995 (work study assistance for 2 semesters, \$700)
3. Travel to SLV; two trips for meetings and discussion if required (\$300)
4. Partial expenses for service contract on the infrared spectrometer (.25 X \$2,192 = \$548)
5. Consumable supplies; glassware, filters, 400 mesh screen. (\$300)
6. Total = \$5023