

- 1

PN 7-797

56067

C 7-198

PROGRESS REPORT

DPE-5544-G-3S-7045

**Gene and organelle transfer by electroporation
a prospective tool for potato improvement**

by

Dvora Aviv

Period: August 14 - December 31, 1987

Rec'd in SDI: FEB 21 1988

The proposal deals with the transfer of isolated genes and organelles into potato protoplasts. In the first stage of the proposal we will concentrate on methodology development while the second stage will deal with the application of these methodologies in potato crop improvement.

It should be noted that the actual start of this grant was mid August 1987, thus the first report period consists of 4 months only.

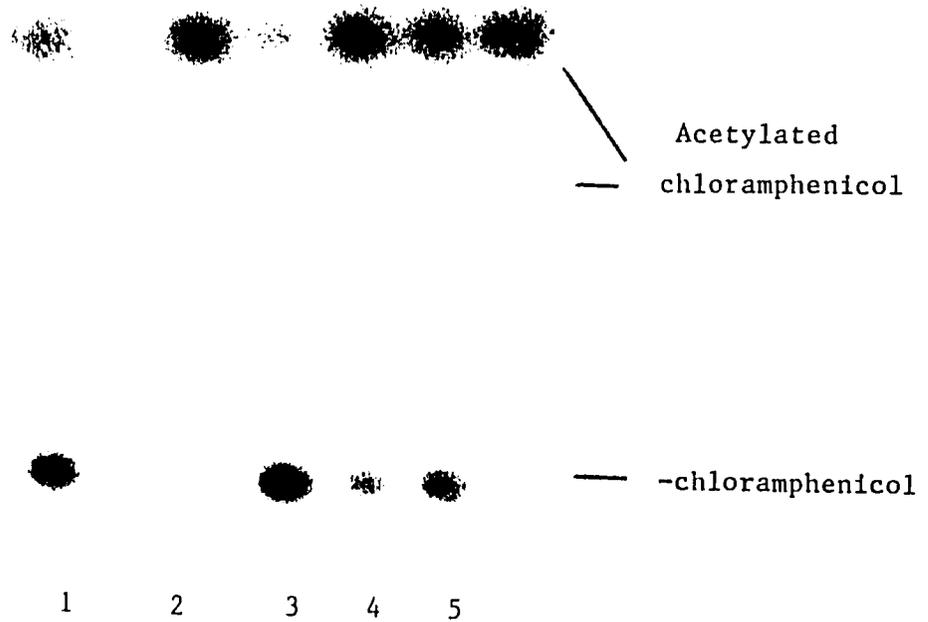
At the beginning of the period I had the opportunity to visit the International Potato Center (CIP) in Peru. This trip was financed by a previous AID grant (DFE-5542-G-55-4030-00) and was intended to discuss previous results. In the course of that visit I had met with several of CIP scientists and discussed with them also the availability of data concerning the possible role that cytoplasmic organelle could play in potato breeding programs. Unfortunately not too much hard data is available at CIP although preliminary results do suggest that cytoplasmic components may be used as source for crop improvement. There is a great need for further analysis of reciprocal crosses between potato cultivars and wild species. Following our discussions it is anticipated that CIP scientists will provide us with this crucial information.

In the first period of the proposed research, several lines of research were taken. For the gene transfer methodology we started by calibrating the uptake of plasmid DNA containing the gene for chloramphenicol acetyltransferase (CAT). This plasmid (pCAP212) was kindly provided by Dr. J. Schell (MPI, Koln, FRG). Optimization of

DNA uptake was done by measuring transient expression of CAT activity. As illustrated in the attached figure, potato protoplasts do not show any endogenous CAT activity but 24 hrs after the uptake of pCAP212 plasmid, a high CAT activity is expressed. In the coming months, we will study different parameters that may affect either uptake or expression of plasmid DNA. We will also compare uptake of DNA via electroporation versus polyethylene glycol treatment as preliminary results using *Nicotiana* protoplasts suggested that the second procedure might be as affective. We intend to use also other plasmids and to look for stable transformation as well as transient expression.

The first phase of organelle transfer will be mitochondrial isolation. We started to develop a methodology to isolate high quality mitochondria from *Nicotiana* cell suspensions. Based on the results with these mitochondria, we shall attempt the isolation of potato mitochondria. In the next few months we will develop the conditions for a successful transfer of isolated mitochondria into the proper cell recipient.

Transient Expression of Chlorophenicol Acetyltransferase (CAT).



Expression of pCAP212 plasmid DNA in potato protoplasts (CV. Bintje) as measured by CAT activity. Lane 1: Acetylation of chloramphenicol by bacterial extract (positive control). Lanes 2-5: CAT activity in 4 potato protoplasts samples 24 hrs. after plasmid uptake.