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Progress Report

Grant No. DFE 5542-6-SS-4030-00

on

UTILIZATION OF PLANT PROTOPLAST BIOTECHNOLOGIES
FOR THE TRANSFER OF ORGANELLES (3F-29)

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As the granted money was released only several weeks ago this Progress Report includes mainly our plans for the coming months rather than a regular Progress Report.

The areas in which we already started or are planning to start soon are the following:

a. Factors affecting organelle transfer

It was shown in mammalian cell systems that elimination of mitochondria, of one of the fusion partners by rhodamine G6 pretreatment, increased somatic hybrid production. As plant protoplasts were found to be sensitive to rhodamine, we tested several rhodamines and questioned whether a rhodamine pretreatment applied to either "donor" or "recipient" protoplasts before fusion, may affect the direction of cytoplasmic organelle transfer after fusion. The experiments will be carried out first on different Nicotiana spp. If indeed rhodamine will have the expected effect we shall apply it for intergeneric fusions as well.

b. Fluorescent staining for the identification and selection of fused-protoplasts

Manual picking of fused protoplasts under the microscope by a micromanipulator requires visual identification of the fused protoplasts. Recently we started a study in which we compare the efficiency of cybrid production in a total selection scheme vs. a procedure based on micromanipulation. The identification of the fused protoplasts under the fluorescent microscope is possible due to staining of one protoplast partner with DRAFL (dichlorotriazinyl amino fluorescein) and using green mesophyll protoplasts showing 'natural' red fluorescence of chlorophyll, as the other partner. Fused protoplasts fluoresce both in red and yellow. Our study includes also a search for the optimal growth conditions for the rescue of picked fused protoplasts.

c. Mitochondrial analysis and male sterility

For the last few years we are engaged in cytoplasmic transfers in Nicotiana and the creation of alien cytoplasmic male steriles. To understand if and what role the mitochondria/nuclear interaction plays in creating male sterility we developed reliable techniques for mitochondrial analysis. The technique is based on Southern hybridization of mtDNA with several radioactive mtDNA probes. We plan to continue our studies in this direction and to gain more insight into the role of nuclear/mitochondria interactions in creating male sterility.

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d. Development of "protoplast systems" for various potato varieties

Recently we established tip cultures stocks of various cultivated potato. The different cultivars include Desiree, Spunta, Mirka and Binje. The aseptic stocks provide a source material for protoplast isolation. Different conditions will be tested for optimal protoplast isolation, culture and regeneration. Once the 'protoplast to plant' system for each cultivar will be established we shall proceed to fusion and organelle transfer experiments.

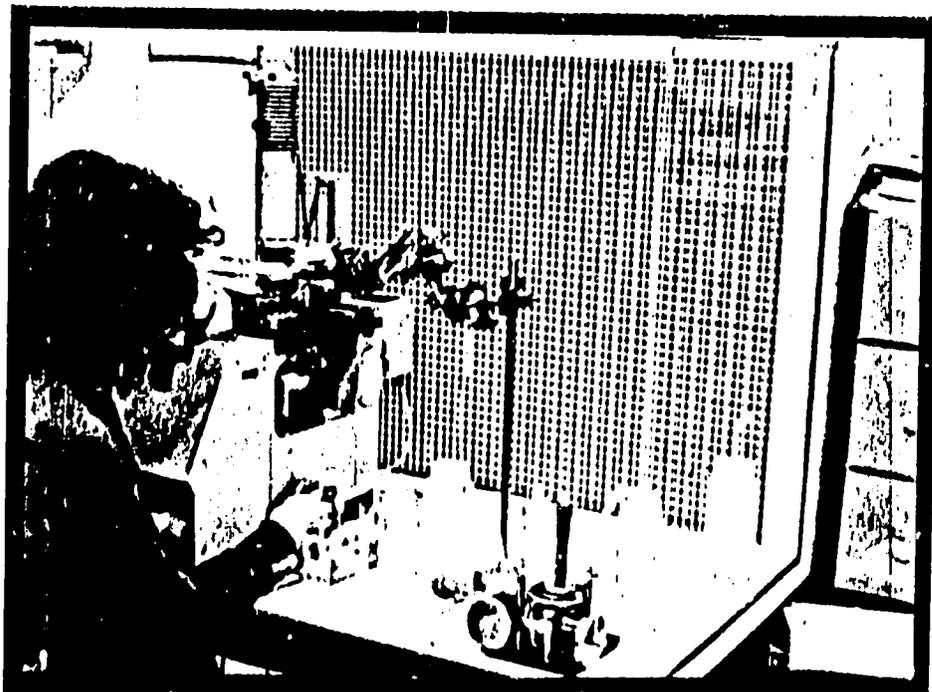
e. We contacted our collaborator, Dr. C. Brown of International Potato Center Lima, Peru requesting specific information and seeking true seeds of potato and relevant wild species in order to start towards applied aims as soon as possible. We also inquired about the first visit in the frame of this project for detailed future plans and exchange of plant material.

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Isolation by a micromanipulator of heterofused protoplast following fluorescent staining of one fusion-partner