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AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

DATE: 8/30/88

MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18

FROM: AID/SCI, Victoria Ose

SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. C 7-198
PR Jan 1- June 30, 1988

Attachment

C7-198

Progress Report

DPE-5544-G-SS-7045

Gene and Organelle Transfer by Electroporation A Prospective Tool for Potato Improvement

by

DVORA AVIV

Period: January 1–June 30, 1988

Rec'd in SCI AUG 2 5 1988

My proposal included two major parts. One was the transfer of isolated genes. The second was the transfer of isolated organelles. In both cases I proposed to use tobacco as well as potato protoplasts as recipients.

Gene Transfer

A prerequisite to gene transfer is that the gene will be selectable. Some of the commonly used selectable markers are antibiotic resistances. Recently we isolated a large number of chloroplast mutants resistant to the following antibiotics: streptomycin, spectinomycin and lincomycin. A few of these mutants were used as starting material for the isolation of antibiotic resistant chloroplasts. In some experiments intact chloroplasts were transferred (see below) and in other experiments DNA fragments, harboring the mutated gene, were isolated and cloned. An example for the latter case is the double mutant $92str^R7spc^R1$ from which a fragment of chloroplast DNA, encoding the doubly mutated 16S RNA, was isolated and cloned (p 1–29). This DNA fragment was later used in gene transformation experiments.

In the first tranformation experiments the recipient protoplasts were derived from antibiotic sensitive tobacco. The approach was the following: as every recipient protoplast contains a large population of sensitive chloroplasts, a successful transformation of one of those chloroplasts probably would not be sufficient to confer immediate resistance upon the whole cell. A dominant nuclear gene on the other hand, is known to confer resistance shortly after being introduced into the cell. We therefore designed co-transformation experiments where the transforming DNA was a mixture containing the above mentioned chloroplast antibiotic resistance gene (p 1–29) plus another plasmid (pCAP 212) containing a nuclear gene encoding for neomycin phosphotransferase (NPTII) which confers resistance to neomycin and kanamycin. The linearized plasmids DNAs were introduced into the protoplasts by polyethylene glycol (PEG) treatment. Two weeks later kanamycin was added to the medium.

About 50 calli, resistant to kanamycin, were isolated in three different experiments. Plants were regenerated from most of them and their resistance to kanamycin was

confirmed by performing NPT II assay on leaf extracts. Seeds were collected separately from single pods and tested for steptomycin and spectinomycin resistance. In few of the pods very few seedlings showed resistance to either streptomycin or spectinomycin or both. These seedlings were isolated and transferred to the greenhouse for further analysis. The presence of streptomycin and spectinomycin resistant seedlings suggest already that the antibiotic resistant chloroplast gene was indeed transferred. Yet only detailed molecular analysis, planned for the next few months, will ascertain whether or not the DNA fragment was transferred and recombined with the endogenous chloroplast DNA. In case of a positive answer, these results will be the first example of direct gene chloroplast transformation in higher plants. These experiments were performed in collaboration with H. Fromm and A. Avni. Recently, in collaboration with A. Perl, the same DNA fragments were used to transfer antibiotic sensitive potato protoplasts.

Organelle Transfer

a. Chloroplasts

A fruitful collaboration between a Dutch team, Prof. E. Galun and myself lead to a successful transfer of chloroplasts via microinjection. In that project we contributed the chloroplast mutants (a double resistant and an albino one) and Dr. Verhoven and Dr. Blass developed the microinjection procedure. This work was submitted to the International Symposium of Plant Molecular Biology which will be held in Jerusalem, November 1988. The abstract is attached.

We are now in the process of developing a microinjection system in our own laboratory (see below). In the coming year we will attempt to transfer intact chloroplasts into either tobacco or potato protoplasts.

b. Mitochondria

Selectable mitochondrial markers are indispensable for successful mitochondrial transfer. In recent months we were involved in the isolation and characterization of an oligomycin resistant cell suspension (oli^R38) derived from *Nicotiana sylvestris*. Our results suggested that the resistance is located on the mitochondrial genome (Aviv and

Galun, 1988. Transfer of cytoplasmic organelles from an oligomycin resistant *Nicotiana* cell suspension into tobacco protoplasts yielding oligomycin resistant cybrid plants. MGG, In press). This was in fact the first report of isolation and characterization of a mitochondrial mutant, in higher plants.

Mitochondria were isolated from the oli^R38 cell line and were introduced into tobacco protoplasts by PEG treatment. The recipient protoplasts were derived from a CMS line. Plants of this line contain the nuclear genome *N. tabacum* and *N. undulata* mitochondria. Thus the mitochondrial genomes of donor and recipient could be easily distinguished. Several oligomycin resistant calli were isolated and from each one 4–5 plants were regenerated. While the first regenerated plants are already flowering, the last plants to regenerate are still at a very young stage. So far one out of 4 calli produced plants in which the typical male sterile flower of the recipient was replaced by a partially restored flower to fertility (see attached photos). This change of flower phenotype was probably caused by the transfer of the isolated mitochondria which were derived from *N. sylvestis* oli^R38 line. Molecular analysis of the mitochondrial population of the respective regenerated plants should furnish the final proof whether indeed the change in flower morphology was caused by a successful transfer of the isolated mitochondria. If the answer will be affirmative we will be the first to report the introduction of isolated mitochondria via PEG treatment.

Recently, together with A. Perl, we attempted to transfer oli^R38 mitochondria into potato protoplasts. This experiment is at an early phase; we hope to present the result in the next semi-annual report. To test whether tobacco mitochondria could be compatibly transferred into potato we first employed the 'donor-recipient' method in which γ-irradiated oli^R38 donor protoplasts were fused with potato recipient protoplasts. Over 50 calli were recovered after oligomycin selection and over 100 plants were regenerated and transferred into the greenhouse. Their molecular analysis is still underway. We do not have yet any positive indication that tobacco mitochondria, or part of them were transferred into potato.

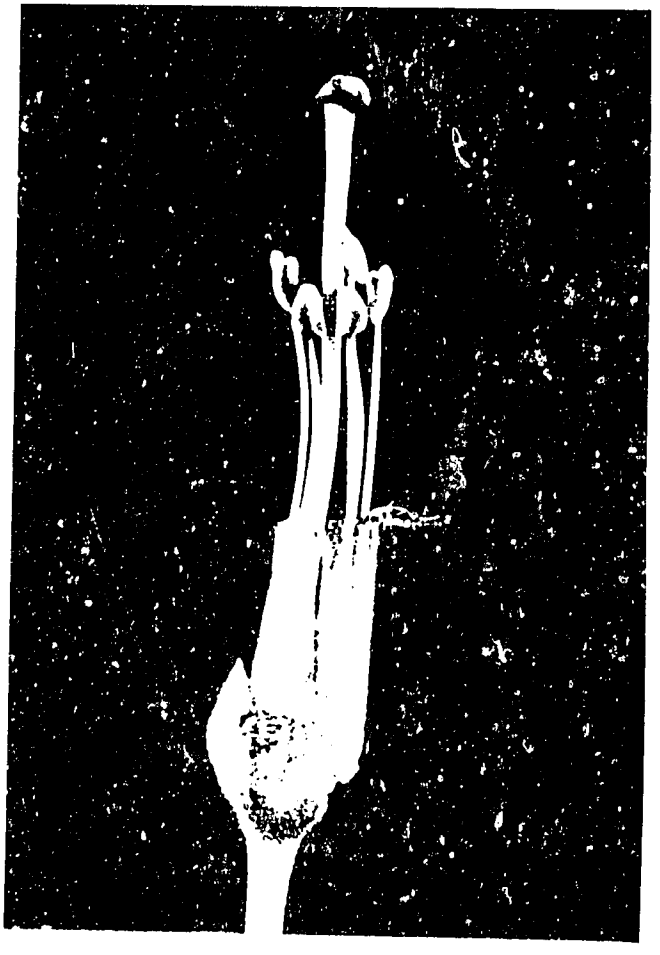
Microinjection System

Previously we already detailed in length why a microinjection system is superior to electroporation system for the introduction of isolated organelles. My request for the acquisition of a microinjection system, replacing the electroporation system, was approved last April and we are now in the process of ordering the different components of that system. It may take some time before all parts will be installed. Meanwhile we were fortunate that our Ph.D. student Avi Perl, was accepted to a microinjection course organized by Prof. Graessmann. The course will be held in Berlin in the coming Fall. His experience will certainly contribute to a fast establishment of our microinjection system.

The research described in this report was done in collaboration with Prof. Esra Galun.

Morphological changes of recipient CMS plants following the introduction of isolated *oli*^{R}38 mitochrondria.

A typical male sterile flower.



A partially restored flower.

JERUSALEM 1988

ABSTRACT FORM

I am interested in Oral presentation (Yes) / No

Most closely related Symposium or Workshop number: Sym. 7.2 or W.S. 7.5

DIRECT CELL TO CELL TRANSFER OF CHLOROPLASTS BY MICROINJECTION

H.A. Verhoeven[1], J. Blass,[1], D. Aviv[2] and E. Galun[2]; [1]Res. Inst. ITAL, Wageningen, The Netherlands and [2]Dept. of Plant Genetics, The Weizmann Institute of Science, Rehovot, Israel.

The feasibility of transferring chloroplasts from one protoplast to another protoplast by microinjection, was studied. A micropipette ('needle') with a microdrop of mercury was attached to a controlled air-pressure pump. This formed the basic injection system. Donor tobacco protoplasts containing *N. undulata* cytoplasm and chloroplasts with streptomycin and spectinomycin resistances were used as chloroplast source. Recipient protoplasts were from an albino *N. tabacum*. The needle was inserted into immobilized and evacuolated recipient protoplasts; chloroplasts were taken up by the needle and reinjected immediately into adjacently located recipient protoplasts. The overall survival rate of injected recipient protoplast was 30%. Out of 50 successful injections, as verified by red fluorescence of the recipient protoplasts, 3 calli were obtained. These calli contained cells with fluorescing chloroplasts which are being regenerated into plants. Molecular characterization of the chloroplasts of the future cybrid plants should provide evidence for a direct cell-to-cell transfer of chloroplasts by microinjection

BEST AVAILABLE DOCUMENT