PN-AEM-444
79475

U.S.- Israel Cooperative Development Research (CDR) Program

Proposal No. C7-191

Grant No. DPE 5544-G-SS-7043-00

CHARACTERIZATION OF ORGANELLE GENOMES IN THE POTATO GERMPLASM AS AID IN BREEDING POTATOES FOR DEVELOPING COUNTRIES

PROGRESS REPORT

for the period July 31, 1988 to December 31, 1988

(Third semi-annual report)

Principal Investigator: Prof. Esra Galun
Dept. of Plant Genetics
The Weizmann Institute of Science
Rehovot 76100, Israel
Telephone: +972-8-483585
Telex: 381300
Facsimile: +972-8-466966
Bitnet: LPGRUNE1 at WEIZMANN

Collaborators: Dr. P. Gregory, Dr. J. Dodds, Dr. A.M. Golmirzaie
International Potato Center (CIP)
P.O. Box 5969
Lima, Peru
Telephone: 266920; 354354
Telex: 25672 PE

FEB 27 1989

INTRODUCTION

Following the establishment of the physical restriction map of the chloroplast genome (plastome) of potato and considerable improvements in the provision of leaves from axenically cultured shoots of potato and other *Solanum* species, for the analyses of the chloroplast DNA (ctDNA) and the mitochondrial DNA (mtDNA), as reported previously -- our main task during the recent months was devoted to actually analyse the ctDNA and mtDNA of *Solanum* species and cybrids. As indicated previously, we intended to obtain this information from a number of *Solanum* species which are presumably at different phylogenetic distances from the potato *(S. tuberosum)*. The respective information should thus provide the establishment of a rough phylogenetic dendrogram. Such a dendrogram is of prime relevance for strategies of transferring organelles (mitochondria and/or chloroplasts), coding for potentially advantageous traits, from wild *Solanum* species into potato. Thus as reported in the last semi-annual report, we asked for specific additional *Solanum* species (from the USDA). These were obtained and propagated axenically as shoot-cultures and are being analyzed. Potato leaf-cells contain a relatively high amount of starch grains in their plastids. This interferes with the purification of ctDNA. We recently found an original mean to eliminate this difficulty.

Because the overall-goal of our endeavor is to bridge between organelles from alien *Solanum* species and potato, we started to actually test organelle-transfer from a closely related species, from a relatively far-removed species and from a very far-removed species. The tests were based on donor-recipient protoplast-fusion and generation of the respective cybrids.

During three months of this reported period, we devoted intensive efforts to train a young Peruvian scientists from the International Potato Center (CIP) in the main methodologies in which we are engaged. This training is part of our collaboration as

osal of our project. This training was very successful. The trainee not only learned and practiced all the scheduled procedures but went beyond that to get acquainted with the most progressed potato-protoplast manipulation methodologies. He can now implement his acquired knowledge in Peru.

ORGANELLE ANALYSIS

Intensive efforts were put into the analysis of ctDNA and mtDNA of potato cultivars and other *Solanum* species. We supplemented the arsenal of *Solanum* species by requesting several species from the USDA (as noted in the last Semi-Annual Report). These species arrived, were duly propogated and are maintained as axenic shoot-cultures. They are now serving a dual purpose: source of protoplasts for fusion experiments (see next chapter), and source of organelle DNA. Table 1 updates our axenic-shoot cultures.

Potato leaves contain chloroplasts which are exceptionally rich in starch. This high starch content interferes adversely with the purification of ctDNA. Traditional approaches to reduce the starch, such as dark/cold storage was not efficient because the chloroplasts deteriorated before the starch was metabolized. Based on our previous experience with silver thiosulfate (STS, see Publications) and on the known effect of kinetin to maintain the integrity of chloroplasts we devised the following treatment. Leaves were harvested, water-washed and weighed. The leaves (40g) were mixed with an STS solution (20 ml, 1000 ppm) and then with a kinetin solution (20 ml, 1000 ppm). They were then wrapped in aluminium foil and maintained for 2 or 3 days at 4°C in the dark. Thereafter we started the ctDNA extraction. This "STS plus kinitin" treatment solved the starch-problem.

As noted above we are amidst the many characterizations of *Solanum* spp ctDNA and mtDNA analyses. The DNA is usually digested with either of several endonucleases in order to find which endonucleases will provide the clearest difference, in restriction profiles, between compared *Solanum* species. Tables 2 and 3 update the analyses of ctDNA and mtDNA, respectively, performed till the end of 1988.

We intend to continue these analyses in order to collect sufficient information for the construction of an organelle-phylogenetic dendrogram, at least for the plastomes of key *Solanum* species. As indicated previously our choice of species is based on two considerations. We are analysing species which are presumably at various phylogenetic distances from potato and we focuss on species which are potential donors of organelles (to improve potato stress resistance and to induce cytoplasmic male sterility which has advantage for the production of hybrid true-potato-seed).

CYBRIDIZATION AND PLASMONE PHYLOGENY

A major goal of our endeavor, in this research project, is to furnish effective tools to breed better potatoes for developing countries. One of the overall objectives of the research was therefore to obtain more phylogenetic information on *Solanum* in order to provide leads on wild species which could on one hand be sources for economically important genes for potato breeding, but should also be sufficiently close, phylogenetically, to enable the transfer of the genes from alien species into potato cultivars. Focussing on organelle-coded genes, we went one step further. We therefore asked how far away , from potato, can we go and still be able to transfer chloroplasts and mitochondria (or mitochondrial genome components) from a given *Solanum* species into a potato cultivar. We based our choice of *Solanum* species on a rough ctDNA phylogeny suggested by Hosaka et al. (Jap. J. Genet. 59: 349, 1984) and on additional information. Consequently,

three species were chosen, each of them a potential source of advantageous traits. One, *S. chacoense* is considered very close to potato, the ctDNA of the latter differs only by having a T type restriction pattern rather than the W type of *S. chacoense* resulting from an apparent single deletion in potato. The second species chosen was *S. etuberosum*. This is a non-tuberous species. It is quite removed, phylogenetically, from *S. tuberosum*. The plastome of *S. etuberosum* differs from the plastome of *S. tuberosum* by at least 21 restriction sites. The third species, *S. berthaultii*, is very far removed from *S. tuberosum* but the data on the formers phylogenetic distance from *S. tuberosum* are incomplete. The phylogenetic distances between potato and the three species *S. chacoense*, *S. etuberosum* and *S. berthaultii* may be considered as relatively close, quite distant and very distant, respectively.

In three fusion experiments protoplasts of the potato cv Desiree served as recipients and were fused with gamma-irradiated protoplasts of *S. chacoense*, *S. etuberosum* or *S. bethaultii*. The respective cybrids (all having potato morphological features) were obtained and their organelle compositions were analysed. The following is a summary of the results:

- Cybrids from the fusion of potato protoplasts with irradiated *S. chacoense* protoplasts contained chloroplasts as well as mitochondria from *S. chacoense*. The plants were mostly male fertile. The cybrid plants did no show morphological abnormality. Hence organelle transfer can readily be obtained from *S. chacoense* to potato.

- From the fusion between potato protoplasts and irradiated *S. etuberosum* protoplasts we did obtain cybrid plants, which indicated that the distance between these species does not preclude cybrid regeneration. Some cybrids contained *S. etuberosum* chloroplasts. The transfer of *S. etuberosum* mitochondria into the cybrids was also observed but it was less frequent than the transfer of chloroplasts and cybrids with *S. etuberosum* mitochondria

were male-sterile. Most cybrids were abnormal in respect to having a strong green-purple pigmentation in their leaves and tubers. Fig. 1 provides an example of restriction profiles of ctDNAs from potato and a wild *Solanum* species as well as a cybrid between the two species.

- No cybrid plants could be regenerated from the fusion of potato protoplasts with irradiated *S. berthaultii* protoplasts, in spite of repeated attempts. We assume that nuclear-cytoplasmatic incompatibility, due to the great phylogenetic distance precluded the regeneration of cybrid plants. This is thus an example which shows that there is a limit even for organelle transfer - - unless the organelle-donor harbors in its plasmone a selectable marker. The last reservation stems from our recent observation that mitochondrial-genome components could be transferred, by the donor-recipient protoplast-fusion method, from a *Nicotiana* cell line which contains oligomycin-resistant mitochondria, to potato provided the cybrid colonies were cultured on a selective medium containing oligomycin .

COLLABORATION

In accordance with our collaboration schedule a young scientist came from CIP to our laboratory in Rehovot. Felix Serquen is a Peruvian (with two M.Sc. degrees) employed at CIP. He arrived in early October for a training period of 3 months. The choice of Felix Serquen, by Dr. A.M. Golmirzaie of the Potato Breeding and Genetics Department of CIP (authorized by Dr. Peter Gregory, the Director of Research), turned out to be a very successful one. Mr. Serquen speaks fluently English, thus there were no language barriers. We were a little worried upon his arrival because he had very little experience in biochemical or plant-molecular methodologies. Our worries faded when Mr. Serquen started to work. The training included three aspects:

- Extraction and purification of mtDNA from potato cultivars and other *Solanum* species; the mtDNAs were digested with endonucleases, run on gels and the respective restriction profiles were obtained. Thus in addition to the potato cv Desiree three other species *(S. commersonii, S. berthaultii* and *S. chacoense)* as well as fusion derived cybrids were analyzed.

- Extraction of DNA, endonuclease digestion and restriction analyses of chloroplast DNAs were performed on the *S. tuberosum* cv Desiree, *S. berthaultii,* and *S. etuberosum* as well as on a cybrids resulting from fusion between *S. etuberosum* (donor) protoplasts and Desiree (recipient) protoplasts.

- Although not scheduled formally in our training program Mr. Serquen insisted to be trained also in protoplast manipulations. Hence, protoplasts were isolated from leaves of axenically cultured shoots of two potato cultivars (LT-7 and TS-1) which are important in tropical lowlands, as parental lines for the production of TPS hybrids. In addition protoplast fusion experiments were performed.

Due to diligence and intelligence, Mr. Serquen learned all these three methodologies and can transfer them to Peru. This was much beyond our expectations.

PUBLICATIONS

A. Articles

Galun, E., Aviv, D., Breiman, A., Fromm H., Perl, A., and Vardi, A. 1987. Cybrids in *Nicotiana, Solanum* and *Citrus:* isolation and characterization of plastome mutants, pre-fusion treatments, selection and analysis of cybrids. In: D. von Wettstein and N.-H. Chua (eds) Plant Molecular Biology, NATO ASI Proceedings, Copenhagen, June 1987, Plenum Press, N.Y. pp. 199-207.

Galun, E., Perl, A. and Aviv, D. 1988. Protoplast fusion-mediated transfer of male-sterility and other plasmone-controlled traits. Applications of Plant Cell and Tissue Culture-Ciba Foundation Symposium 137, pp. 97-112.

Perl, A., Aviv, D. and Galun, E. 1988. Ethylene and *in vitro* culture of potato: suppression of ethylene generation vastly improves protoplast yield, plating efficiency and transient expression of an alien gene. Plant Cell Reports 7: 403-406.

B. Papers at an International Congress

Galun, E., Perl, A., Bottcher, U. and Aviv, D. 1988. Plant organelle manipulations: means to evaluate plasmone/nuclear interaction. Proceeding 2nd Intern. Congress, Plant Molecular Biology, Jerusalem, November 1988.

Perl, A., Aviv, D. and Galun, E. 1988. Organelle DNA and CMS in *Solanum* cybrids. Proceedings 2nd International Congress, Plant Molecular Biology, Jerusalem, November 1988.

Perl, A., Aviv, D. and Galun, E. 1988. Silver thiosulfate vastly improves protoplast manipulations in potato. Proceedings 2nd International Congress, Plant Molecular Biology, Jerusalem, November 1988.

Table 1. *Solanum* species which are presently cultured as axenic shoots at the Department of Plant Genetics (Update: January 1989).

S. demissum	*S. bukasovii*
S. chomatophilum	*S. nigrum*
S. tarijense	*S. venturii*
S. raphanifolium	*S. commersonii*
S. piurae	*S. etuberosum*
S. microdontum	*S. berthaultii*
S. polydenium	*S. chacoense*
S. sparsipillum	*S. brevidense*
S. stoloniferum	*S. tuberosum (12 different cultivars)*

ivars and *Solanum* species which were used for the analysis of their plastomes by restriction patterns of the respective ctDNA (update: December 31, 1988).

Cultivar or species	Restriction endonucleases used
S. tuberosum cv. Desiree	*BglI*, *BamHI*, *EcoRI*, *XhoI*, *AvaII*, *PvuII*, *HindIII*
S. tuberosum cv. Mirka	*BglI*
S. tuberosum cv. Atlantic	*Pst*, *BamHI*, *EcoRI*, *Kpn*, *HindIII* *Sma*, *AvaII*
S. tuberosum cv. Atzimba	*HindIII*, *BamHI*, *EcoRI*, *Bgli*, *PvuII*, *Sma*
S. tuberosum cv. Kufri-jyoti	*BamI*, *EcoRI*
S. tuberosum cv. 245.7*	*BamHI*, *HindIII*, *Sma*, *EcoRI*, *XhoI*, *AvaII*
S. tuberosum cv. Serrana	*BamHI*, *EcoRI*, *Kpn*, *XbaI*, *PvuI*, *HindIII*, *Pst*, *Sma*, *AvaII*.
S. etuberosum	*BamHI*, *XbaI*, *HindIII*, *PvuII*, *EcoRI*
S. nigrum	*BglI*.

*cv 245.7 contains, most probably, the cytoplasm of *S. stoloniferum*

;ivars and *Solanum* species which were used for the analysis of their chondriomes by restriction patterns of the respective mtDNAs (update: December 31, 1988).

Cultivar or species	Restriction endonucleases used.
S. tuberosum cv. Desiree	*Cla*I, *Pvu*II, *Pvu*I, *Bgl*II, *Sal*I
S. tuberosum cv. Atlantic	*Sal*I, *Bam*HI, *Bgl*II, *Eco*RV
S. tuberosum cv. Atzimba	*Cla*I, *Bgl*II, *Pvu*I, *Pvu*II, *Sal*I
S. tuberosum cv. Kufri jyoti	*Bgl*II *Pvu*I, *Pvu*II, *Cla*I, *Eco*RV, *Sal*I
S. tuberosum cv. Serrana	*Pvu*II, *Bgl*II, *Bam*HI, *Sal*I
S. tuberosum cv. 245.7*	*Sal*I, *Bam*HI, *Bgl*II, *Pvu*I, *Pvu*II
S. brevidense	Eco*RV*, Cla*I*, Pvu*I*, Pvu*II*, Bgl*I*
S. chacoense	Cla*I*, Eco*RV*, Bgl*I*, *Pvu*I, Pvu*II*, *Sac*I, Sac*II*
S. berthaultii	*Sal*I, *Sac*II, *Sac*I, Eco*RV*, Cla*I*, Pvu*II*
S. commersonii	*Sac*II, *Sac*I, *Eco*RV, *Cla*I
S. venturii	*Eco*RV.

*cv 245.7 contains, most probably, the cytoplasm of *S. stoloniferum*

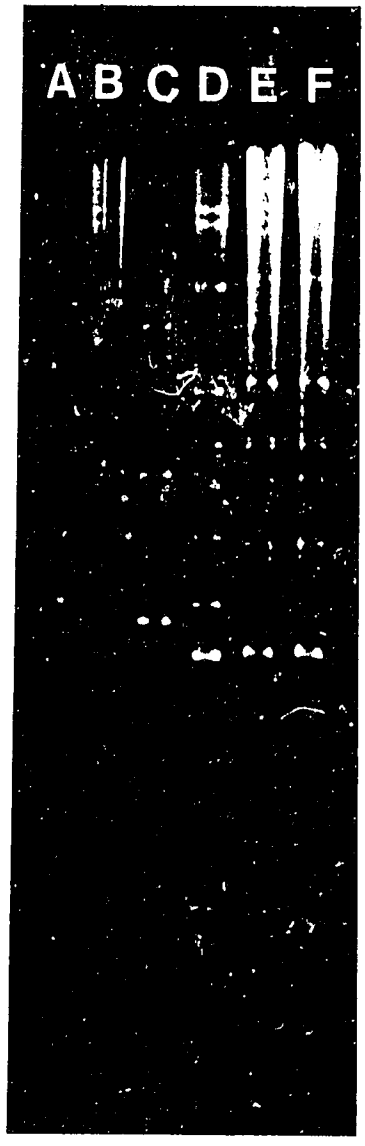

Fig. 1. Restriction profiles of ctDNAs from potato (*S. tuberosum* cv. Desiree), from *S. etuberosum* and from a cybrid between these two species. Lane designation: A & B - *S. etuberosum* C & D - cybrid; E & F - potato. ctDNAs were restricted with *Eco*RI (lanes A & C) or with *Bam*HI (lanes B, D, E & F).