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PROGRESS REPORT

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Utilization of Plant Protoplast Biotechnologies for the Transfer of Organelles

by

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During the present report period, one of us (E.G.) visited our collaborators at the International Potato Center (CIP), in Lima, Peru. In this second visit we further discussed different aspects of our project as was fully detailed in the report of this visit. During May, Dr. John Dodds, from CIP visited our laboratory for several days. We had very fruitful discussions concerning our present and future research plans. According to our requrest Dr. Dodds also brought to us different shoot-cultures of potato lines (see Appendix 1) that should be useful in future research. Dr. Dodds received from us a considerable number of shoot cultures which were derived from different fusion experiments (see Appendix 3). These will be checked for PSTV (viroid) at CIP and then the resulting plants will be evaluated in appropriate locations (especially for male-sterility/ fertility) in addition to the evaluation of sib-clones of these shoots in Israel.

Characterization of chloroplast and mitochondrial DNAs.

To identify correctly the organelle composition of fusion derived plants, the DNA restriction patterns of chloroplast and mitochondrial DNAs from the fusion-partners should be first studied. To this end we set out to isolate, separately, chloroplast and mitochondria from each of the potato lines used as donors and recipients in our fusion studies. Their DNA was extracted, digested with each of several restriction endonucleases and electrophoresed in order to obtain the respective restriction profiles. We followed the methods developed by us previously for *Nicotiana* spp. and found that the quality of EtBr stained

mtDNA gels were as good as those obtained with Nicotiana while the quality of the potato cpDNA was much inferior. We therefore had to change different parameters in the procedure. We believe that one of the most crucial factors influencing cpDNA quality is the physiological state of the plant from which the chloroplasts are isolated. In previous experiments we noticed that the starch content of potato chloroplasts is very high, interfering with cpDNA isolation. Even maintaining the plants in complete darkness for 24-48 hr prior to chloroplast isolation was not sufficient to eliminate the starch. Recently we acquired a controlled growth chamber where the potato plants are kept at about 18°C, and light intensity is much reduced. We hope that plants growing under these conditions will serve as a better source for chloroplast isolation.

Another important modification which considerably improved the quality of the potato cpDNA was made at the sucrose gradient step. In Nicotiana, the procedure calls for a discontinuous gradient of 20%, 45% and 60% sucrose. When the gradient was changed to 15%, 30% and 52% the yield of clean chloroplasts was significantly improved.

At this stage we have found that Serrana cpDNA restriction pattern can be distinguished from S. tuberosum with any one of several restriction enzymes. e.g. BamHI, EcoRI, XhoI and the cpDNA of the CMS line Y245.7 can be distinguished from S. tuberosum cultivars using Hind III: We therefore intend to use these enzymes for the chloroplast analysis of putative cybrid plants when either Serrana or the CMS line Y245.7 were used as as donors.

Fusion experiments and cybrid plants production

The first three fusion experiments yielded already flowering plants derived from independently isolated calli. In two of these experiments donor protoplasts were derived from a CMS plant (Y245.7) and recipient protoplasts were derived from either Atzimba (Exp.I) or Atlantic (Exp.II). The aim of these fusions was to transfer cytoplasmic male sterility to parental lines used for hybrid true-potato-seed (TPS) production. The putative cybrid plants are currently used for organelle and pollen analysis (see previous section). To study pollen sterility/fertility the plants should be grown under optimal conditions for flowering. This we achieved only recently when we acquired a new growth chamber where we can control both light and temperature conditions which are optimal for potato flowering. As noted above, we also provided Dr. Dodds with 32 shoot cul ures for field evaluation in Peru. (See Appendix 3).

Rhodamine 6-G treatment

Summary of the first set of results concerning the use of rhodamine 6-G (R6G) pretreatment of *Nicotiana* protoplasts was submitted to Plant Cell Reports and is now in press (see attached preprint).

Briefly, we proposed that a short R6G treatment, of either donor protoplasts or recipient protoplasts, might determine the mitochondrial composition of the cybrid plants. Careful molecular analysis of over 30 cybrid plants derived from two independent fusion experiments in which

recipient protoplasts were treated with 15 μg/ml R6G, revealed that under these conditions no complete elimination of the mitochondria of the treated protoplast was observed. Although, in some fusion experiments only the mitochondria of the partner which was not treated with R6G were revealed in the cybrids, the dose of R6G could be a decisive factor in mitochondrial elimination. To test the possibility, a second set of experiments was performed in which donor protoplasts were pretreated with 10, 15, 20 or 25μg/ml R6G. The analysis of cybrid plants derived from this experiment is still underway. At this stage it can be already concluded that the number of developing calli decreased as the R6G dose was increased. Thus many calli were derived from fusions where 10 and 15μg/ml R6G was used, less so when the dose was increased to 20μg/ml and only few when the dose was increased to 25 μg/ml. We anticipate that after completion of the mitochondrial analysis of all cybrid plants, would be able to conclude whether complete elimination of mitochondria could be achieved by increasing R6G dosage. Obviously, the prospective elimination of mitochondria from one of the fusion partners (commonly from the recipient) should furnish a very valuable tool in controlled organelle-transfer, not only in Nicotiana but in all genera where cybridization by the donor-recipient protoplast-fusion can be achieved.

Thus, turning to the potato-system, we found that potato protoplasts are much more sensitive to R6G then Nicotiana protoplasts. We therefore decreased the dose to 1-2μg/ml and shortened the treatment to one hour and will investigate the use of R6G to eliminate mitochondria from the recipients of potato cybrids.

Appendix 1

Sterile shoot cultures of various potato lines received from Dr. John Dodds.

CIP #		NAME
702677	AJN	Yari
703241	AJN	Pinta Yari
700313	STN	SS-1356
700670	STN	EE-1762
701014	JUZ	Huataqui
701165	STN	Calhua Rosada
701266	STN	Llameina Blanca
702078	JUZ	Yurac Kalpi
702142	STN	Yana Moro Pitiquina
702305	JUZ	Chimu Luke
702443	JUZ	Pariña
702445	JUZ	Piñaza
702610	STN	Unknown
702815	STN	Mari
703258	JUZ	Ckanchali Morado
703260	JUZ	Yuracc Ckanchillo
703283	ADG	Chejchi
703243	ADG	Imilla Blanca
702937	CUR	Ugro Shiri
702455	CUR	Luke
703254	TBR	Low Bay
703252	TBR	Clavela
703240	ADG	Sani Imilla
702514	ADG	Chata Blanca Ojo Morado
700921	ADG	Ccompis
703264	ADG	Puca Quitish
703244	GON	Zapallo
701830	GON	Garhuash Huayro
703272	PHU	Alka Pinu
703269	PHU	Chaucha Roja
703286	STN	Yana Pituwaya
378017.2		LT-7
760147.7		PI-123-05.02
575020		I-1150
720084		CFK-69.1

Appendix 2

Seeds of Solanum spp. obtained either from C.I.P. or from the U.S.A.

S. acaule	PI 205508	S. infundibuliforme	PI 435075
S. berthaultii	473331	S. pinnatisectum	186554
S. brevidens	218228	S. piurae	473501
S. chacoense	230580	S. polyadenium	275238
S. chomatophilum	266387	S. raphanifolium	296126
S. commersonii	243503	S. sanctae-rosae	230464
S. demissum	160230	S. tarijense	414150
S. etuberosum	498311	S. venturii	218220
S. fernandezianum	320270	S. vernei	458370

Appendix 3:

Fusion-derived plants sent to the International Potato Center (CIP) for further evaluation.

EXP.	Donor of of cytoplasm	Recipient	Designations of calli and plants	Goal in fusion	Total plants per fusion
I	Y245.7 (CMS)	ATZIMBA	B 1-5 C 1-3 D 1-3 E 1-3 F 1-3 G 1-3 H 1-3 J 1-5	convert to CMS	29
II	Y245.7	ATLANTIC	A 1-3	convert to CMS	3
III	Desiree (tub)	LT-1	12 plantlets each one represent a different callus	restored fertility increase heat tolerance	12

In all cases the donor protoplasts were X-irradiated and the recipient protoplasts were treated with R-6G.