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

FINAL REPORT

FOR A TWO YEAR PROJECT (AUGUST 1987 TO JULY 1989);
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I. INTRODUCTION

Summary of achievements in reference to the original objectives

This Final Scientific Report summarizes research and collaboration of a two-year's project. We shall introduce this report by reiterating the main objectives of our original proposal and then compare them with the actually achieved results. We shall also indicate the collaboration between the researchers at the Weizmann Institute of Science (WIS) and the experts at the International Potato Center (CIP) and point out the achieved and the prospective contributions of this project to potato breeding for developing countries.

The overall aim of our proposed research was to bridge between existing and novel methodologies (e.g. in plant-cell manipulations and recombinant DNA techniques to be handled at the WIS) and the effort to furnish developing countries with better potato cultivars. The latter effort is an ongoing, multi-thrust, activity at CIP and its Regional Centers. While considerable success in potato improvement and management was obtained by CIP, the work at WIS could furnish the tools to vastly facilitate breeding efforts at CIP as well as at other LDCs. We proposed to focus on the organelles (i.e. chloroplasts and mitochondria) of potato and of wild relatives of potato (i.e.. *Solanum* species) containing advantageous breeding traits. We intended to characterize the chloroplast genome (plastome) of potato in respect to its DNA composition and to obtain chloroplast DNA fragments which should then serve as essential tools to characterize the plastomes of other *Solanum* species. the characterization of such genomes is important for two purposes. First, it will provide the means to positively identify *Solanum* accessions' of which there are many thousands at CIP and other potato-germplasm "banks". Many of these accessions are duplicated but were given different names on the basis of inadequate identifications. The

characterization of the accessions plastome DNA would provide a simple and quick identification procedure. A second purpose has more immediate relevance to potato improvement. Somatic hybridization and cytoplasmic hybridization by protoplast-fusion became a useful means to introduce breeding traits from wild species into target cultivars. Such traits may be found in the plastomes or in the nuclear genomes of the wild species. It is therefore essential to have a simple means to identify the plastomes in the fusion-derived plants. Furthermore, if the plastome of a wild *Solanum* species is very different from that of potato the chance of transferring this plastome into potato cultivars would be low, while plastomes of wild *Solanum* species that are very similar to those of potato should be amenable to such a transfer. A practical example will demonstrate the second purpose. Many *Solanum* species were screened in the past for resistances to pathogens and for environmental stresses. Consequently a great number of such species was identified as potential sources for such resistances in potato. In most cases sexual crosses between these species and potato are difficult or impossible. Thus protoplast-fusion could provide a breeding means. Having previous knowledge that will predict which out of several *Solanum* candidates, has the best chance as fusion-partner with potato should provide an enormous saving in cell-manipulation work.

The same considerations noted above for the chloroplast and its genome are applicable also for the mitochondrion and its genome (the chondriome). We therefore set similar objectives for the study of the chondriome in potato and other *Solanum* species. But, due to the much greater complexity and the time restriction we limited, in our proposal, the goal of mitochondria-work to the provision of means to identify the chondriomes of potato and *Solanum* species by recombinant DNA techniques. Hence, we did not intend to construct a physical map of the

potato chondriome neither to provide a phylogeny based on mitochondrial DNA in the genus *Solanum*..

We are very pleased to report that we achieved our objectives. Moreover we obtained more relevant information than planned originally. In respect to plastome characterization we analyzed in detail the chloroplast DNA of potato. This resulted in the construction of the physical map of the potato plastome (we were the first to report this map). In addition restriction fragments of potato chloroplast DNA were cloned and could then be used as probes for simple and quick identification of plastomes of other *Solanum* species. To test the usefulness of these DNA-clones we actually performed protoplast-fusion combinations between potato and 14 other *Solanum* species, regenerated the respective cybrids and used these clones to identify the plastomes in the fusion derived plants. Several hundreds of such analyses were performed and we can now state with confidence that our aim, in this respect was achieved.

A similar work was performed in respect to the characterization of the mitochondria in potato, wild *Solanum* species and protoplast-fusion derivatives. As noted above we did not analyze the potato mtDNA in detail. We found that restriction-fragments from available *Nicotiana* mitochondrial DNA furnished appropriate tools for these characterizations. We thus saved the efforts of cloning fragments from potato mitochondrial DNA.

The wild *Solanum* species serving as fusion-partners with potato were chosen in consultations with our CIP collaborators. The choice was based on two considerations. We chose species which are supposed to have different phylogenetic relationships with potato; hence the limits of compatibility could be defined. The other consideration was to use *Solanum* wild species with known breeding traits (e.g. resistances to pathogens). The

appropriate plant material was then furnished to the WIS by CIP in the form of axenic shoot cultures.

Another form of collaboration was originally proposed. It was planned that a researcher from CIP will come to the WIS and learn the methodologies of analyzing the organelle DNA. By this the transfer of advanced biotechnology should be achieved. This was actually performed as will be detailed below.

II. RESEARCH ACHIEVEMENTS

The research achievements will be presented in a concise manner. More detailed information was provided in the three semi-annual reports of this project, in our publications and in a manuscript attached to this report.

1. Propagation of axenic plant material for organelle-DNA analysis

At the onset of our project we faced a problem: plant-material samples of potato and wild *Solanum* species should be available continuously and preferably free of contaminants. The optimal material should thus be from *in vitro* cultured plants. The culture of potato shoots on culture medium, inside sterile plastic boxes, was an established procedure but such shoots grew slowly and had thin shoots with minute leaves. We suspected that this slow and suppressed growth was caused by the release of ethylene in the closed boxes. Our prediction was correct. We thus tested a number of compounds to prevent either the synthesis or the effect of ethylene. Silver thio-sulfate (STS) turned up to be the best choice. A relatively low level of STS in the culture medium caused the development of normal shoots and leaves and the yield per box was increased by a factor of 3 to 10 (Perl et al., 1988). Figure 1 demonstrates the effect of STS on potato shoot culture in plastic boxes. While this research line was not an explicit objective of our proposal our

findings not only enabled us to produce plant material efficiently - - it turned up to be of immense importance to our collaborators at CIP where the stocks of breeding lines, cultivars and *Solanum* accessions are all cultured *in vitro.* CIP experts also faced the problem of retarded growth in shoots confined in closed culture vessels. The STS addition was adopted at CIP and transferred to affiliated Regional centers in developing countries.

2. The plastome of potato

As indicated in the Introduction (and in our proposal), information on the plastome (the chloroplast genome) of potato is a required prelude to several other research lines leading ultimately to potato improvement. We therefore isolated chloroplasts from a common potato cultivar (Kathadin), extracted the chloroplast DNA (ctDNA) and subjected this ctDNA to restriction-profile analysis. Using three restriction-endonucleases (*Bam* HI, XhoI, *Pvu* II) we obtained the respective ctDNA fragments. These were cloned by the appropriate recombinant DNA procedures and then used to construct a physical map of the potato plastome. We were the first to come up with such a map (Heinhorst et al., 1988). The potato plastome was found to be a circular molecule of 155 kbp (Figure 2). This DNA molecule was basically co-linear with the previously characterized (in our Department)*Nicotiana tabacum* ctDNA (Fluhr and Edelman, 1981). Based on this co-linearity to several key plastome-genes, such genes of *Nicotiana* could be assigned to specific potato ctDNA fragments. This similarly between the plastomes of *Solanum* and *Nicotiana* could be very useful in our project as well as for future studies because it is plausible to use *Nicotiana* plastome-genes for the isolation of *Solanum* plastome-genes and vice-versa. Since the complete base sequence of *Nicotiana* ctDNA is now known (Shinozaki et al., 1986) the relation between gene expression and base sequence can now easily be studied in potato and other *Solanum* species.

Once chloroplast transformation in angiosperms will become feasible (it is possible in green algae) specific advantageous genes could then be "fished" from a given *Solanum* species and transferred to the respective transgenic potato.

3. Generation of a potato ctDNA bank

In the process of construction of the physical map of the potato palstome (as described above) we isolated almost all the respective *Pvu* II, *Bam* HI and *Xho* I restriction fragments (Table 1). These were inserted in recombinant DNA plasmids and cloned in bacteria. The potato ctDNA inserts were then isolated from the recombinant plasmids. Thus all (but one) of the *Pvu* II fragments and many of the *Bam* HI and *Xho* I fragments became available, covering the whole potato plastome.

4. Restriction analysis of ctDNA from potato cultivars and *Solanum* species.

We started meticulous analyses of the plastomes of several potato cultivars and of representative *Solanum* species. The aim was double-pronged: to provide a quick, precise and reliable procedure to determine the identity of the plastome in undefined plant materials (i.e. in *Solanum* accessions of questionable identity) and to characterize the plastome of somatic hybrids and cybrids derived from interspecific protoplast-fusions. In the latter direction we specifically asked which endonucleases will provide differential ctDNA restriction patterns between *S. tuberosum* cultivars (e.g. Desiree, Atlantic, Atzimba) and other *Solanum* species. A plethora of endonucleases were found to provide differential restriction patterns as summarized in Table 2. An example of actual ctDNA restriction profiles from potato and *S. etuberosum* (and a cybrid), obtained by digestions with *Eco* RI and *Bam* HI is given in Figure 2. While differential restriction-endonucleases were identified we found that it is rather laborious to isolate

chloroplasts and to purify ctDNA from the potato or wild *Solanum* species. Also, the ethidium bromide stained gels of digested ctDNA more often than not resulted in unclear restriction profiles. Moreover at least 10 to 30 g of plant material was required to obtain a pure sample of ctDNA, sufficient for several restriction-profiles. We thus chose to short-cut the analysis. We tested several procedures and finally found the following one to be completely satisfactory. Only one gram of plant material was required. Total DNA was extracted and cut with *Bam* HI. This digested DNA was run on an electrophoresis (agarose) gel and processed by Southern blot hybridization. The hybridization was performed with a radiolabelled potato ctDNA fragment (e.g. *Pvu* II fragment No. 3). The autoradiograms provided sufficient differential patterns between potato and all other tested *Solanum* species. The latter procedure was thus adopted as "standard".

5. Phylogeny based on plastome analysis

The ctDNA restriction patterns from the tested *Solanum* species could lead (after additional analyses of other species) to the construction of a phylogenetic dendrogram (see our previous study on *Cucumis:* Perl-Treves and Galun, 1985). Our intention was, as stated in the proposal of this project, to utilize the then available information from Hosaka and collaborators (Hosaka, 1984; Hosaka et al., 1986) and to integrate it with results of this project. But, recently Hosaka continued and expanded his studies (Hosaka and Hanneman, 1988; Hosaka et al., 1988) on the ctDNA of wild *Solanum* species and performed most of what we planned to do, in respect to phylogenetic relationships of the *Solanum* plastome. We therefore did not continue this endeavor.

6. The chondriomes of potato and other *Solanum* species

Our intention, within the present project, was to use mitochondrial DNA (mt DNA) analysis for the identification of chondriomes in potato, in wild *Solanum* species related to potato and in interspecific protoplast-fusion derivatives. Unlike in our work with plastomes, it was not intended to construct a physical map of the potato chondriome. We thus developed the appropriate procedures to purify mt DNA from potato and other *Solanum* species. The mtDNAs were digested with a number of endonuclease and the digests were separated by electrophoresis on agarose gels to obtain the respective restriction patterns. The analyzed *Solanum* species were chosen in consultation with our collaborators at CIP and most of them were actually furnished by CIP in the form of axenic shoot cultures. The restriction patterns of mtDNAs from *Solanum* species turned out to contain a great number of fragments. This number varied, obviously, according to the restriction endonuclease used but was higher than the number of fragments from endonuclease-digestion of ctDNA in this genus. There was another notable difference between the restriction patterns of ctDNA and mtDNA. Using numerous endonucleases to derive ctDNA restriction profiles, all the potato cultivars tested had virtually the same pattern, at most the potato cultivars could be divided into two groups. Not so in respect to mtDNA, where by the use of appropriate endonucleases the chondriomes of several potato cultivars. could be differentiated. Table 3 summarizes the mtDNA restriction analyses that were performed.

Although mtDNA analysis, by restriction profiles could provide a means to identify *Solanum* species, it became obvious that this procedure, based on ethidium bromide staining of agarose gels, is rather laborious and thus not very useful for routine analyses of many mtDNA samples. We therefore turned to Southern blot analysis as indicated below.

7.Southern blot analysis

We searched for a quick procedure that will differentiate between two chondriomes. This procedure should be useful for two purposes. The first purpose was to provide decisive information whether or not the chondriomes of plant samples representing two ill-defined accession numbers are different and thus should be considered separate taxa. The second purpose was to characterize the chondriomes of protoplast-fusion derived plants, where the fusion partners were from different *Solanum* species. We thus developed Southern-blot hybridization for the analysis of *Solanum* mt DNA. We found that, as with ctDNA analysis by Southern blotting, only 1 g or less plant material is required. Also, we did not have to isolate and clone mtDNA fragments from potato; mtDNA fragments from tobacco were useful as radiolabelled probes. Such fragments were available from our previous studies with *Nicotiana* cybrids (e.g. Galun et al., 1982) or could be obtained from other laboratories (e.g. the mt gene for *atp* 9). Moreover by screening about a dozen *Solanum* species we found that digestions with only one or two endonucleases will result with sufficiently different Southern-blot autoradiographs. Thus Southern blot analysis turned up to be a convenient procedure for our purposes and was ready for practical application.

8. Application of Southern-blot analysis to identify plastomes and chondriomes.

The applicability of Southern blot analyses is demonstrated by the following example. Cybrids were produced by fusing gamma-irradiated protoplasts of a given *Solanum* species (used as organelle donor) and unirradiated protoplasts from *S. tuberosum* cv Desiree (as recipient). The cybridization procedure was described in detail (Galun and Aviv, 1986) and

shall not be repeated here. In each cybridization-combination we analyzed the *Solanum* donor-species, the cybrid derivatives as well as *S. tuberosum* cv. Desiree. Both the plastomes and the chondriomes of these cybridization - combinations were analyzed by Southern-blot hybridization of the respective ct and mtDNAs. Figure 4 provides examples of three such fusion-combinations in which the plastomes were analyzed. Actually many cybrids (up to about 90) were analyzed in each of the combinations; thus the cybrids shown in Figure 4 represent only part of the results. For example 88 cybrids were analyzed from the combination between *S. etuberosum* and Desiree. Of these cybrids 57 had *S. etuberosum* plastomes (four of them are shown in Figure 4) and 31 had *S. tuberosum* cv Desiree plastomes. Table 4 summarizes the organelle analysis, by Southern-blot hybridization, of 12 fusion-combinations.

In parallel the chondriomes were analyzed by Southern-blot hybridization of the respective mtDNAs in the cybrids and fusion-partners of 12 fusion combination (Table 4). Examples of these analyses are presented in Figure 5. It should be noted that, as expected, the cybrids contained either apparently 'pure' chondriomes of one of the fusion-partners or the restriction patterns were identical to neither of the fusion partners - indicating 'novel' chondriomes that could result from recombination between the two chondriomes after protoplast-fusion.

Obviously the objective of our work was to develop methodologies which would be useful in developing countries. While we were confident that our methods were applicable in these countries we had to test it. Moreover we had to convince our collaborators at CIP that the methods are simple, easy to learn and can be performed also by a person who had little or no previous training in molecular biology. As shall be detailed in the Collaboration chapter of this report, CIP sent to the WIS a young investigator (with a M.Sc. in plant breeding from the University of Lima) who had *no*

previous training in biochemical or molecular methods. He learned all the procedures within a few weeks and independently performed all the required procedures to analyze the ctDNA and mtDNA of *Solanum* species and cybrids. We thus established that a transfer of this biotechnology is not only potentially possible but was executed in practice. There is one point worth considering. The presently developed procedure requires handling of radiolabelled material. The conditions for this may not be generally available in laboratories of LDC. But this could be circumvented: instead of radiolabelling - immunolabelling of probes and gels can be applied. The latter methods are already in use in potato breeding centers (e.g Regional centers of CIP located in different LDCs) for the detection of pathogens (e.g. viruses) in potato plants. Thus, although our procedure is apparently sophisticated- it actually is readily applicable and with minor changes can be performed in very modestly equipped laboratories.

9. **References**

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III. COLLABORATION AND BENEFIT TO DEVELOPING COUNTRIES

A tight collaboration was maintained, during the planning and execution of this project, between personelle at the WIS and CIP. The planning and setting the objectives for this project was done during several discussions between Dr. E. Galun (PI) and Dr. D. Aviv of the WIS and Dr. P. Gregory and Dr. J. Dodds of CIP. Such discussions were frequently maintained in Rehovot (at the WIS) or in Lima (at CIP) during this project. In the course of the project Dr. P. Schmiediche and Dr. A. Golmirzaie of CIP and Mr. A. Perl of the WIS joined the collaborations. In practice Dr. E. Galun visited CIP three times and Dr. D. Aviv and Mr. A. Perl visited CIP once

(only part of the expenses were from this project, some trips were financed from CIP and other sources). Dr. P. Gregory, Dr. J. Dodds and Dr. P. Schmiediche came to Israel and visited the WIS.

These discussions and reviews of the work performed were very important. For example, the CIP experts advised us about the *Solanum* species which should be included in our research work because of their relevance to potato improvement. Then these species were shipped as axenic shoots to Rehovot. The tight collaboration also was instrumental to readily apply findings in Rehovot to CIPs needs. For example the discovery (at WIS) that silver thiosulfate is very useful for the *in vitro* culture of potato (and other *Solanum* species) shoots, was quickly applied at CIP where many thousands of boxes (or vials) with shoots are cultured annually (for either transfer to "banks" or for shipment to potato breeding and propagation centers).

Most important, the goal of transfer of technology from the WIS to CIP was successfully implemented. For that Mr. Felix Serquen, a Peruvian (M.Sc. from the Agricultural University of Lima) employed as potato breeder at CIP was sent to Rehovot and stayed at the WIS for three months. He quickly learned the ctDNA and mtDNA analysis and then independently performed such analyses. The details of his program and achievements were provided in the Semi-Annual Report for July '1988-Dec. 1988.

The present project provided an additional bonus. Plant material produced (during a previous AID-supported program) at the WIS was sent for evaluation at CIP. These were cybrid potato lines with CMS - for the production of TPS hybrid varieties. The material was propagated at CIP and the plants as well as progenies from the original lines were tested during several seasons in CIP fields. The present collaboration enabled a close follow up of this material - and the utilization of these cybrid progenies was discussed by Dr. A. Golmirzaie of CIP and Drs. E. Galun and D. Aviv of WIS.

We are pleased to state that all the objectives, set at the onset of this project, that concern potential benefit to potato improvement in LDC - were achieved. These objectives were detailed in our proposal and shall not be repeated here. But in general terms, we provided a very useful means to characterize and identify the chloroplasts and the mitochondria of *Solanum* species especially of those species that have breeding relevance to potato. The methods are such that they are readily applicable also in modest laboratories and shall therefore be very useful in any project where wild *Solanum* species will serve in potato breeding. Moreover the technology of the analysis of *Solanum* organelles was actually transferred from the WIS to CIP.

PUBLICATIONS

The results of this project were published in the following papers.

Heinhorst,S., G.C. Cannon, E. Galun., L. Kenschaft and A. Weissbach. 1988. Clone bank and physical and genetic map of potato chloroplast DNA. Theor. Appl. Genet. 75: 244-251.

Perl, A., D. Aviv and E. Galun. 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.

Galun, E., D. Aviv, A. Breiman, H. Fromm, A. Perl and A. Vardi. 1987. Cybrids in *Nicotiana, Solanum* and *Citrus:* Isolation and characterization of plastome mutants, pre-fusion treatment, selection and analysis of cybrids. In: Plant Molecular Biology (NATO Adv. Study Inst.) D. von Wettstein, N.H. Chua (eds) Plenum Press.

Galun, E., A. Perl and D. Aviv. 1988. Protoplast-fusion mediated transfer of male-sterility and other plasmone-controlled traits. Ciba Foundation

Symp. No. 137, Kyoto. In: Applications of Plant Cell and Tissue Culture. Wiley, Chichester, eds. pp. 97-112.

In addition the following manuscript was submitted for publication (copy attached to this Report).

Perl, A., Aviv, D. and Galun (1990). Protoplast-fusion derived *Solanum* cybrids: application and phylogenetic limitations (submitted).

Also, the results of this project were included at numerous lectures at international meetings, among them a meeting sponsored by AID in Washington D.C.:

Aviv, D., Perl, A. and Galun, E. (1988) Manipulation of cytoplasmic organelles of potato - a complement to potato breeding. PSTC Biotechnology for Health and Agriculture Conference, June 6-9, 1988, Washington DC.

Figure 1. Potato shoots cultured in boxes containing media without (right) or with (left) 2 $\mu g.ml^{-1}$ silver thiosulfate.

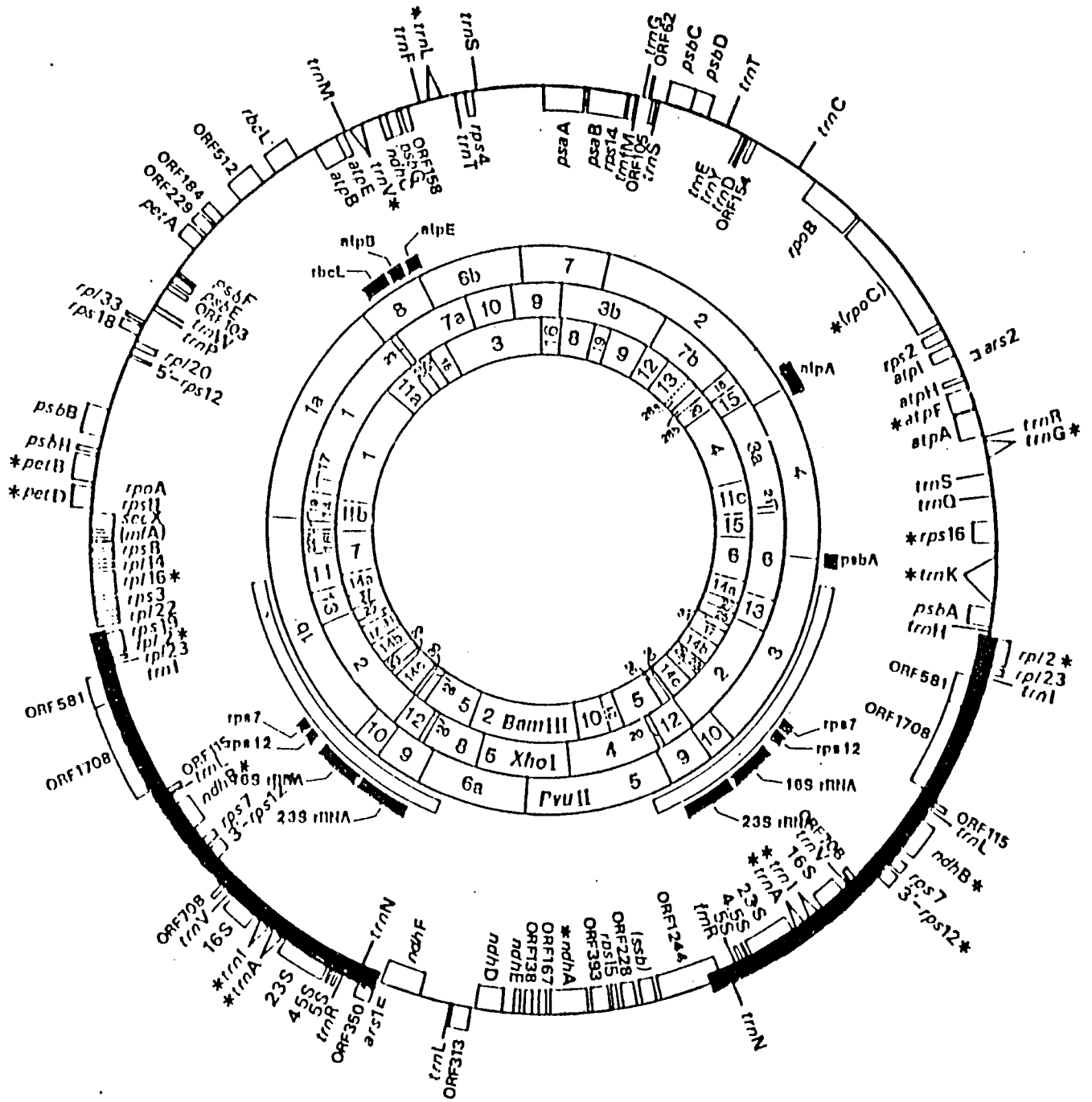

Fig. 2. Inner circle: The physical map of the *Solanum tuberosum* plastome, based on digestions with three restriction endonucleases (from our study: Heinhorst et al., 1988); outer circle the gene-map of the tobacco (*Nicotiana tabacum)* plastome (from: Shinozaki et al. 1986), aligned with the *S. tuberosum* plastome map.

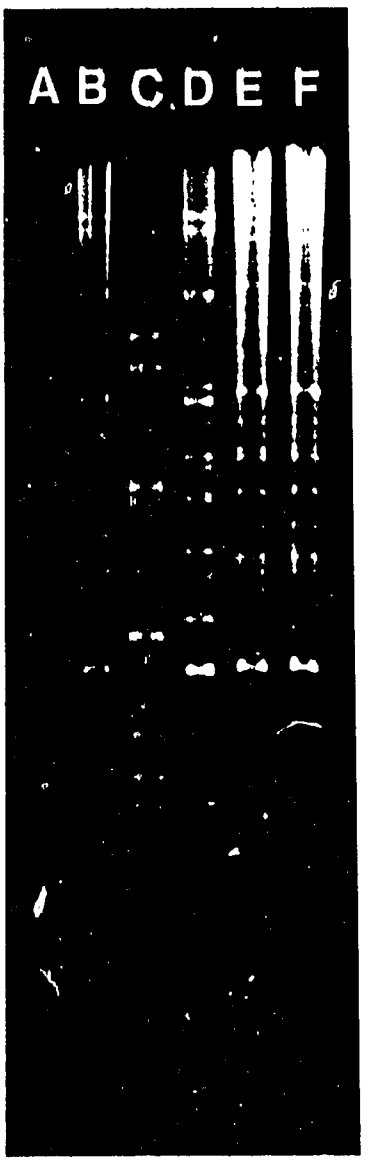

Fig. 3. Restriction profiles of ctDNAs from potato (*S. tuberosum* cv. *Desiree*), from *S. etuberosum* and from a cybrid between these two species. lane designation: A & B.- *S. etuberosum* C & D - cybrid; E & F - potato. ctDNAs were restricted with *Eco*RI (lanes A & C) or with *Bam* HI (lanes B, D, E & F).

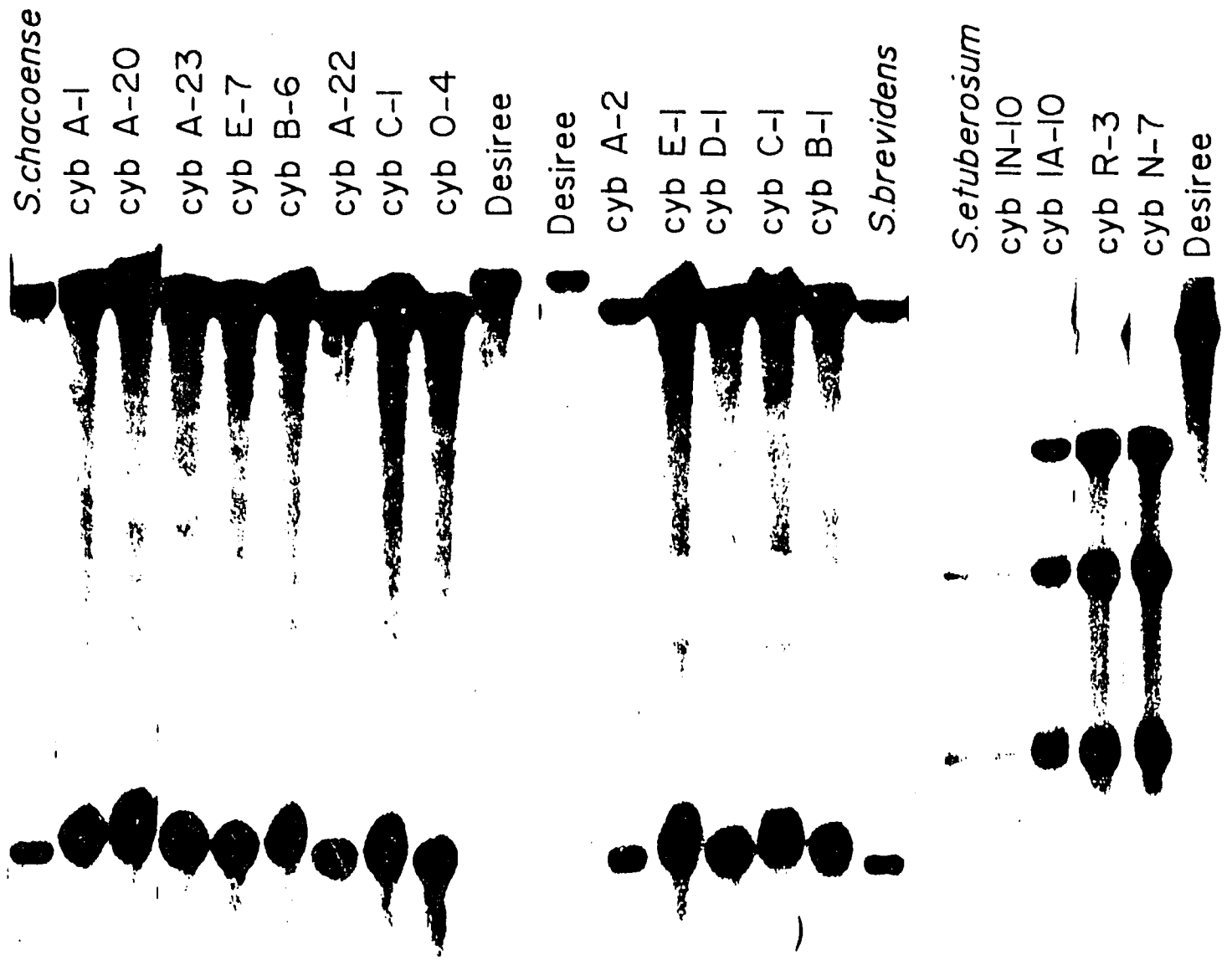

Fig. 4. Examples to demonstrate the utilization of Southern blot hybridization for the identification of *Solanum* plastomes. Derivatives of three fusion combinations were analyzed and compared to the respective fusion-partners. In these examples all cybrids harbored the wild *Solanum* plastomes; in other analyses the potato (Desiree) plastomes (not shown) were retained in the cybrids. The DNAs were digested with *Bam* HI and probed with a radioactive *Bam* No. 3 potato ctDNA fragment.

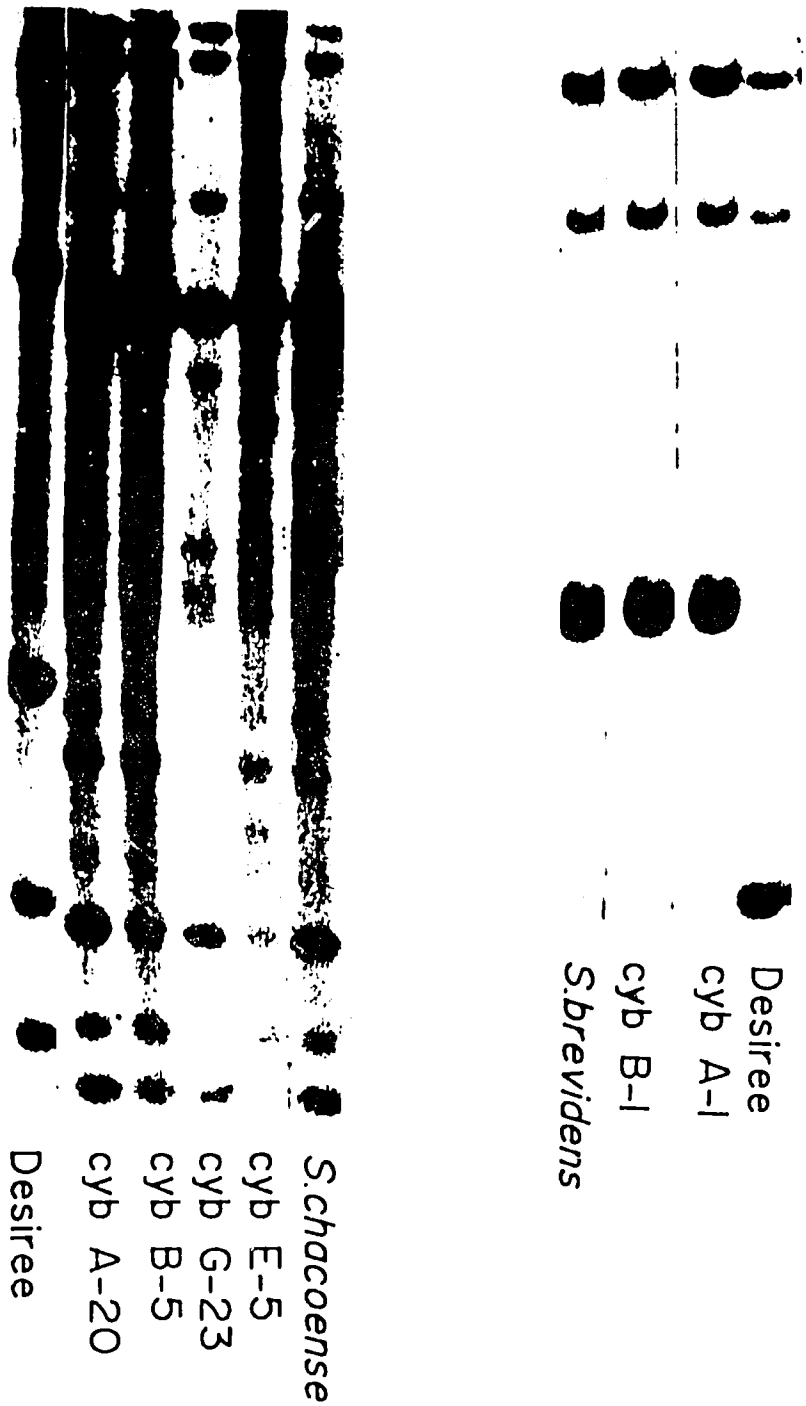

Fig. 5. Example to demonstrate the utilization of Southern blot hybridization for the characterization of *Solanum* chondriomes. Derivatives of two fusion combinations were analyzed and compared to the respective fusion-partners. In the *S. brevidens* (donor) and *S. tuberosum* (recipient) cv Desiree (recipient) recombination the cybrids contained apparently the donor's chondriomes while in the other combinations (*S. chacoense* with Desiree and others not shown) some cybrids contained 'novel' chondriomes. The DNAs were digested with *Hind* III.(*S. chacoense* / Desiree) or with *Eco* RI (*S,.brevidens* / Desiree) and hybridized with radiolabelled *Nicotiana* mtDNA fragments (pmtSylSa2 or pmtSylSa8).

Table 1: Numbers and sizes in Kbp of *S. tuberosum* cv. Katahdin ctDNA restriction fragments generated by *Pvu*II, *Xho*I and *Bam*HI. The numbers in brackets on the right of each column indicate the stoichiometry of fragments located in the inverted repeats.

Fragment Numbers	*Pvu*II	Fragment Numbers	*Xho*I	Fragment Numbers	*Bam*HI
1	a 19.9*	1	16.9	1	14.4*
	b 20.9*	2	12.2 (2X)	2	13.9
2	19.7*	3	a 11.2	3	12.3*
3	17.8*		b 11.2	4	9.2*
4	15.2*	4	10.8	5	6.1 (2X)
5	14.2*	5	9.7	6	5.1
6	a 10.0	6	8.8	7	5.0*
	b 10.0*	7	a 8.3*	8	4.4*
7	8.4*		b 8.3	9	4.0
8	6.1*	8	5.8*	10	3.9*
9	4.2*(2X)	9	5.7	11	a 3.8
10	2.5*(2X)	10	5.3		b 3.8*
		11	3.8		c 3.8
		12	3.2*(2X)	12	3.7*
		13	3.0*(2X)	13	3.2*
		14	2.9	14	a 3.0 (2X)
		15	2.2		b 3.0* (2X)
		16	2.1		c 3.0* (2X)
		17	1.5	15	2.5
		18	1.4	16	2.4
		19	0.82*	17	2.15
		20	0.75(2X)	18	2.1*
		21	0.7	19	2.05
		22	0.5	20	1.92
		23	0.3	21	1.35
				22	1.33
				23	1.25 (2X)
				24	1.22
				25	1.20*(2X)
				26	1.17 (2X)
				27	1.10*(2X)
				28	a 1.07
					b 1.07
				29	0.4 (2X)
				30	0.36 (2X)
				31	0.3 (2X)
Sum	155.6		156.5		151.7

*Clones of these fragments are available.

Table 2. Potato cultivars and *Solanum* species which were used for the analysis of their plastomes by restriction patterns of the respective ctDNA

Cultivar or species	Restriction endonucleases used
S. tuberosum cv. Desiree	*Bgl* I, *Bam* HI, *Eco* RI, *Xho* I, *Ava* II, *Pvu*II, *Hind*I
S. tuberosum cv. Atlantic	*Pst*, *Bam* HI, *Eco* RI, *Kpn*, *Hind* III, *Sma*, *Ava* II
S. tuberosum cv. Atzimba	*Hind* III, *Bam* HI, *Eco* RI, *Bgl*I, *Pvu*II, *Sma*
S. etuberosum	*Bam* HI, *Xba* I, *Hind*III, *Pvu* II, *Eco* RI
S. nigrum	*Bgl* I
S. bukasovii	*Bam* HI
S. stoloniferum	*Bam* HI
S. acuale	*Bam* HI
S. chocoense	*Pvu* II, *Sal* I, *Bam* HI
S. brevidens	*Bam* HI
S. berthaulti	*Bam* HI
S. commersonii	*Bam* HI, *Hind* III, *Eco* RI, *Sal* I
S. venturii	*Bam* I, *Bgl* I, *Hind* III, *Eco* RI, *Sal* I
S. demissum	*Bam* HI, *Hind* III, *Eco* RI, *Pst* I, *Pvu* II, *Sal* I
S. microdontum	*Bam* HI
S. raphanifolium	*Bam* HI, *Bgl* I, *Hind* III, *Eco* RI, *Sal* I, *Xba* I
S. tarijense	*Bam* HI

Table 3. Potato cultivars and *Solanum* species which were used for the analysis of their chondriomes by restriction patterns of the respective mtDNAs.

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
S. acuale	*Eco* RI
S. demissum	*Eco* RI
S. etuberosum	*Eco* RI
S. microdontum	*Eco* RI
S. raphanifolium	*Eco* RI
S. tarijense	*Eco* RI
S. stoloniferum	*Eco* RI

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

Table 4. Transfer of plastomes and chondriomes (or chondriome components) from *Solanum* species donors to *S. tuberosum* (cv. Desiree). Plastomes and chondriomes were identified in protoplast-fusion derivatives by restriction analysis of the respective organelle DNAs. (updated on December 31, 1989).

Organelle donor species	Organelle DNA identified in cybrids	
	Plastome	Chondriome
S. acuale	+[1]	+
S. berthaultii	-	+
S. brevidens	+	+
S. bukasovii	*[2]	*
S. chacoense	+	+
S. commersonii	+	+
S. demissum	+	?
S. etuberosum	+	+
S. microdontum	+	?
S. polyadenium	?[3]	?
S. raphanifolium	+	-
S. stoloniferum	+	+
S. tarijense	+	?
S. venturii	*	*

[1] Plus and minus signs indicate transfer or no transfe. respectively

[2] Asterisk indicate that this fusion combination did not yield cybrids, in spite of repeate attempts.

[3]. A question mark indicates that the final answer will be available within 4 to 6 weeks.

Protoplast-fusion derived *Solanum* cybrids: application and phylogenetic limitations

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Summary. We established interspecific *Solanum* cybrids in order to study the intra-gene nuclear-organelle compatibility and the introgression of advantageous plasmone-coded breeding traits into potato. Cybridization was performed by the donor-recipient protoplas fusion procedure. We found that the plastomes of *S. chacoense, S. brevidens* and *S. etuberosum* could be transferred into the cybrids having *S. tuberosum* nuclear genomes; like-wise were chondriome components transferred from the former species into these cybrids. The combination with *S. chacoense* as organelle donor and potato as recipient resulted in green fertile plants with potato morphology. By using *S. etuberosum* as an organelle donor and potato as recipient, male sterile cybrid plants, most of them having pigmentation abnormalities, were obtained. The combination of *S. brevidens* with potato resulted in pale green (almost albino) regenerants. The latter albino plantlets had b h th chloroplast DNA and the mitochondrial DNA of the donor *(S. brevidens)* and did not survive the transfer into the greenhouse. An immediately applicative result of this study the *de novo* establishment of male-sterile plants in a potato cultivar. Such plants should l useful as seed-parents in the production of hybrid, true-potato seeds.

Key words: Cybridization - *Solanum tuberosum* - Male sterility - Phylogenetic relationships

Introduction

The ability to derive functional plants from isolated protoplasts paved the way for innovative cell-genetics studies in angiosperms. One approach, started in our laboratory (Zelcer et al. 1978) was to establish plants having the nuclear-genome of one species but

plastomes and chondriome components of another species (see reviews: Galun and Aviv 1986; Galun et al. 1987,1988). This approach, mediated by the donor-recipient protoplast-fusion, was based on the utilization of x- or γ-irradiated protoplasts serving as organelle donors and recipient protoplasts which contributed the nucleus of the derived cybrids. The procedure was improved by several means as pre-fusion exposure of protoplasts to antimetabolites (e.g. iodoacetate - Sidorov et al. 1981; Rhodamine 6-G - Aviv et al. 1986) and the utilization of donors or recipients with plastome (e.g. Belliard et al. 1978; Medgyesy et al. 1980; Menczel et al. 1983) or chondriome markers (Aviv and Galun 1988). These protoplast-manipulation methods were supplemented with efficient protocols to identify plastome and chondriome compositions in the derived cybrids (Galun and Aviv 1986). Hence a powerful tool was established to obtain cybrids containing novel plasmones. Consequently, cybrids were obtained in several angiosperm genera, in addition to *Nicotiana*, where the procedures were initially developed. Representatives of the former genera are *Citrus* (Vardi et al. 1987,1989); *Brassica* (Barsby et al. 1987; Morgan and Maliga 1987; Chatterjee et al. 1988; Thomzik and Hain 1988) and *Daucus* (Ichikawa et al. 1987; Tanno-Suenaga et al. 1988). It is noteworthy to recall that in some studies aimed to produce only somatic hybrid plants, cybrids, in addition to somatic hybrids, were derived from fusions where neither of the partners was x- or γ-irradiated (e.g. Gleba et al. 1984; Kushnir and Gleba 1987; Bottcher et al. 1989).

The evidence from the many reported interspecific cybrids of *Nicotiana (loc cit)* indicated that these cybrids were similar or identical, in their morphological features, to the recipient fusion partner. In none of these cybrids could pigmentation deficiency or morphological defects be causally correlated to alien plasmones. The only reproducible manifestation of alien chondriome components in interspecific cybrids was *de novo* cytoplasmic male-sterility (e.g. Aviv et al. 1984, Kumashiro et al. 1988 - in *Nicotiana* Tanno-Shenaga et al. 1988 - in *Daucus)*. *Solanum* is a vast genus containing about 1600

species (D'arcy 1978). This genus therefore contributes attractive material to investigate alien plasmone/nuclear-genome compatability by the donor-recipient protoplast-fusion.

Procedures to regenerate functional plants from isolated protoplasts of potato *(S. tuberosum)*, a major food source, were reported by several authors (Gunn and Shepard 1981, Thomas et al. 1982, Bockelmann and Roest 1983, Haberlach et al. 1985, Tavazza and Ancora 1986, Masson et al. 1987). In the past, somatic hybridization but not cybridization was used, mainly in order to introduce advantageous breeding traits from wild *Solanum* species into potato (Butenko and Kuchko 1980; Binding et al. 1982; Barsby et al. 1984; Ehlenfeldt and Helgeson 1987; Austin et al. 1985a,b, 1986; Kemble et al. 1986; Sidorov et al. 1987; Fish et al. 1987, 1988). With the exception of one study (Sidorov et al. 1987) - where an asymmetric hybridization was attempted to introduce advantageous genetic traits from *S. pinnatisectum* into *Solanum tuberosum x phureja* hybrids - no cybridization, between potato and other *Solanum* species, was recorded.

In the present work we intended to study the compatibility between the nuclear-genome of potato and organelles from alien *Solanum* species. Consequently we employed the donor-recipient protoplast-fusion procedure and chose organelle-donors that varied in their phylogenetic distance from potato. We employed Southern blot hybridization of chloroplast and mitochondrial DNAs to determine the organelle-compositions of the fusion-derived cybrid plants and related these compositions to morphological abnormalities and floral-member malformations (i.e. male sterility) in the respective cybrids.

Materials and methods

Plant material

Shoot sections of *Solanum tuberosum* cv. Desiree bearing one node were cultured axenically in Magenta GA7 boxes containing 50 ml of solidified (1% agar) Nitsch medium (Nitsch 1969) with only 1% sucrose. Silver thiosulfate (STS) was added (2 mg l^{-1}) to this medium in order to produce more leaf-tissue and to increase the yield of protoplasts per unit tissue mass (Perl et al. 1988). The sections rooted, produced new shoots and were harvested after about three weeks' culture at 25° C under 16 h cool-white inflorescent illumination providing 60-70 $\mu Em^{-2}s^{-1}$. The harvested shoots served for either reculture or for protoplast isolation.

Seeds of *Solanum chacoense* (PI 472816), *Solanum etuberosum* (PI 498311), *Solanum brevidens* (PI 245764) and *Solanum berthaultii* (PI 498107), were kindly supplied by Dr. John Bamberg (Potato Introduction Station, Sturgeon Bay, Wisconsin USA). The seeds were surface sterilized with diluted commercial sodium hypochlorite (1% available chlorine), washed three times with sterile distilled water and germinated. The resulting seedlings were further cultured as axenic shoots, as detailed for *S. tuberosum*.

Protoplast isolation and pre-fusion treatments

One gram of leafy shoots harvested from axenic cultures was pricked, using a multineedle pricker and incubated in 10 ml of maceration fluid as described previously (Perl et al. 1988). For protoplasts isolation from the four wild *Solanum* species the maceration fluid contained also 0.1% Driselase (Fluka). Donor protoplasts were γ-irradiated (10KR). Recipient protoplasts were treated with 0.25 mM iodoacetate (recrystallized) for 30 min before fusion (Medgyesy et al. 1980).

Protoplast fusion and culture

Protoplast fusion was performed with the addition of 100 mg l^{-1} STS during the exposure of protoplasts to $Ca(NO_3)_2$ (following the polyethylene glycol treatment) and the fused

protoplasts were cultured in liquid medium as described (Perl et al. 1988). After 3-4 weeks of culture in liquid medium, with periodical reduction of the osmolarity, calli were transferred to solidified (0.8% agar) regeneration medium consisting of MS (Murashige and Skoog 1962) medium supplemented with 0.25 mg l^{-1} zeatin-riboside and 0.1 mg l^{-1} gibberellic acid. Regeneration commonly commenced after further 3-4 weeks. Shoots were rooted on a hormone-free MS medium and transferred to the greenhouse for further observation. In cases of sluggish regeneration the calli were transferred to regeneration medium with 4 mg l^{-1} o-coumaric acid.

Characterization of organelle DNA

DNA extraction and analysis were performed according to Mettler (1988). Thus, total DNA was extracted from 1 g of leaf tissue, digested with endonuclease run on agarose gel (0.8%), transferred to a GeneScreen Plus membrane and hybridized with a nick-translated probe. For chloroplast DNA (ctDNA) analysis the DNA was digested with *Bam* III and hybridized with a potato ctDNA clone, P153 (Heinhorst et al. 1988). For mitochondrial DNA (mtDNA) analysis the total DNA was digested with one of several endonucleases and fragments from *Nicotiana* mtDNA (Aviv et al. 1984) or maize mitochondrial ATPase subunit 6 (Dewey et al. 1985) were used as radiolabelled probes.

Pollen germination

Pollen was collected from several flowers immediately after anthesis and germinated in a hanging drop of a pollen-medium described by Pallais et al. (1984).

Results

In all the fusion combinations we used protoplasts derived from axenic leafy shoots. Protoplasts from the potato cultivar Desiree served as recipients and were exposed to iodoacetate treatment. The latter protoplasts were fused with γ-irradiated protoplasts from one of the four *Solanum* species serving as donors of organelles. Repeated control experiments indicated that when Desiree protoplasts were exposed to iodoacetate but not fused with donor protoplasts the protoplasts succumbed in culture and no cell-division occurred. Also, none of the four wild *Solanum* protoplasts produced cell colonies after γ-irradiation; neither did colonies form when the polyethylene glycol treatment was deleted from the fusion of donor to recipient protoplasts. We therefore assumed that the fusion-derived calli and plants resulted from heterologous fusion events.

S. chacoense *protoplasts as donors*

A total of 38 plants were regenerated from this fusion combination. All the plants had the normal morphological (presumably nuclear-genome coded) features of potato (i.e. Desiree). Root tip squash from 5 randomly collected plants indicated normal tetraploid mitotic karyotypes. The plants attained anthesis and had normal flowers. The pollen of these flowers germinated normally. The plastome composition of 17 plants was evaluated by ctDNA analysis as exemplified in Fig. 1. These analyses indicated that 10 plants contained the plastome of Desiree while 7 plants contained the plastome of *S. chacoense* (Table 1). No fusion derived plant was hetroplastomic nor was plastome recombination indicated. The chondriomes were characterized in the same 17 plants by mtDNA analysis. The analysis of 4 cybrids is demonstrated in Fig. 2 and the total data are summarized in Table 1. Six out of the 7 plants, found to contain the ctDNA of *S. chacoense,* contained also the mtDNA of the latter species; all 6 plants except one (cyb G-23) had apparently pure mtDNA patterns of *S. chacoense.* Cybrid G-23 exhibited a novel mtDNA pattern that was similar

(but not identical) to the pattern *S. chacoense* mtDNA. Eleven plants apparently retained the mtDNA pattern of the recipient fusion partner (Desiree). Our analysis does not reveal those *S. chacoense* or novel mtDNA fragments which do not hybridize with the radioactive probe. Hence it can not be excluded that (some or even all) these eleven plants contained components of the *S. chacoense* chondriome.

S. etuberosum *protoplasts as donor*

A total of 117 plants were regenerated from this fusion-combination and all had the normal morphological features of potato (i.e. Desiree). Root tips squashes from 5 randomly collected plants indicated normal tetraploid mitotic karyotypes. It should be noted that *S. etuberosum* is a tuber-less wild species and is thus not closely related, phylogenetically, to *S. tuberosum* . Never the less all fusion derived plants produced normal potato tubers. Plastome composition was evaluated by Southern blot analysis of the ctDNA in 88 fusion derived plants (Table 1). Out of the latter, 31 contained the plastome of the donor (examples in Fig. 3) while 57 fusion-derived plants retained the Desiree (i.e. *S. tuberosum)* plastome. As with *S. chacoense* as a donor, there was no indication of heteroplastomes or recombination in the ctDNA. Putative cybrids containing chondriome components of *S. etuberosum* were detected by pollen germination tests. Eighteen plants were found to be male sterile. Among them 5 plants had malformed anthers with almost no pollen (Fig. 4) while the other 13 sterile plants had structurally normal anthers but their pollen did not germinate. Apparently pure mtDNA of *S. etuberosum* was detected in 13 cybrid plants (Table 1) regardless to the phenotypic expression of male-srerility in those plants (Fig. 5), Most of the cybrids with *S. etuberosum* mtDNA contained also the ctDNA of *S. etuberosum.* Only two of the former cybrids retained the plastome of *S. tuberosum.* In 75 of the fusion-derived plants we detected the mtDNA pattern of *S. tuberosum.* It can not be excluded that some of these plants contained components of the *S. etuberosum* chondriome that were not revealed by the radiolabelled probe. Moreover, 7 calli (among

them calli N, IN and S, see Fig. 5) regenerated plants with *S. etuberosum* mtDNA as well as plants which retained the recipient (Desiree) mtDNA pattern. This fact indicates that these 7 calli were derived from a fusion event and not a result of recipient escapees. In contrast to the fusion with *S. chacoense* as donor of organelles, where no abnormalities were revealed among the fusion-derived plants, in this combination with *S. etuberosum* two types of abnormalities were observed. One, male sterility (with or without floral-member malformation), was noted above. The second was an abnormal pigmentation presumably by anthocyanin. It was expressed in the leaves and the stems of part of the cybrids. This phenomenon, not expressed in the donor species, was correlated in the cybrids with the transfer of *S. etuberosum* plastomes. Cybrids which received only mtDNA components but not chloroplasts from the donor did not reveal any pigmentation abnormalities.

S. brevidens *protoplasts as donor*

Only 5 calli were derived from this fusion (Table 1) and they regenerated sluggishly. Regeneration did occur after 3 months resulting in 10 plantlets (2 from each callus). All these plantlets were pale green-albino (Fig. 6) and were propagated as axenic shoot-cultures. Attempts to grow these plantlets autotrophically in the greenhouse were unsuccessful. These putative cybrid shoot cultures were morphologically similar to shoot-cultures of *Solanum tuberosum* but for the deficient pigmentation. Root tip squashes from 5 randomly collected plantlets indicated normal tetraploid mitotic karyotypes. The organelle composition of 5 plantlets (each representing one callus) was evaluated by Southern blot hybridization of their respective ctDNAs and mtDNAs. All 5 plantlets had the ctDNA pattern of *S. brevidens* (Fig. 7); likewise all these plantlets had the mtDNA pattern of *S. brevidens* (examples in Fig. 8). These results indicate that cybrids of this combination contained both the chloroplasts and the mitochondria from *S. brevidens.*

S. berthaultii *protoplasts as donor*

The calli derived from this fusion combination did not change their structure upon transfer to regeneration medium (that is lacking auxins). The calli maintained a friable growth pattern. In this respect they differed from the calli derived from the previously described fusion-combinations. The latter calli became compact upon transfer to regeneration medium. We thus suspected that the calli derived from the fusion with *S. berthaultii* were over-producing endogenous auxins and that this over-production retarded regeneration. Several compounds known to enhance auxin degradation were thus added to the regeneration medium. One of these compounds, o-coumaric acid, strongly promoted regeneration and shortened the time from transfer to regeneration medium to differentiation of plantlets by 3-4 weeks (Fig. 9). It should be noted that o-coumaric acid is considered as a natural co-factor of IAA oxidase and is known to enhance IAA oxidation (Gortner and Sutherland 1958). This improvement in regeneration procedure yielded 78 plants. All these plants were morphologically similar to Desiree (i.e. *S. tuberosum)* suggesting they had *S. tuberosum* nuclei. Root tips squashes from 5 randomly collected plants indicated normal tetraploid mitotic karyotypes. The organelle composition of 75 plants (from 22 calli) was analyzed by Southern blot hybridization (Table 1). All the plants retained *S. tuberosum* plastomes. Only 8 plants out of 75 had apparently pure *S. berthaultii* chondriomes (data not shown). Four out of these 8 plants attained flowering and were found to be male-sterile. Our data can not exclude the possibility that the majority of the plants regenerated from this fusion-combination were escapees rather than cybrids. On the other hand in the 8 verified cybrids the chondrioms were transferred from *S. berthaultii* without the co-tarnsfer of plastomes.

Discussion

The results of this study indicate that the donor-recipient protoplast-fusion technique is amenable for potato and may lead to the production of cybrid plants with the nuclear

genome of potato and alien organelle and/or organelle controlled features from different wild *Solanum* species. We have confirmed that the DNA-DNA hybridization analysis, using specific probes can be used to identify cybrid plants. The morphology of the fusion derived plants suggested that they were normal tetraploids and this was verified by karyotype analyses. In a parallel study (unpublished) we analyzed the meiotic and mitotic karyotypes of 21 *Solanum* cybrids derived from a different donor-recipient protoplast-fusion; all but one of the cybrids had apparently normal chromosomes, identical to those of the recipient fusion partner *(S. tuberosum)*. Hence regeneration of potato plants from protoplasts, even after donor-recipient fusion, does not necessarily result in observed morphological or karyotype somaclonal variation, as indicated by some previous reports on protoplast-derived potato plants (e.g. Shepard et al. 1983). In spite of the morphological similarity, among the fusion-derived plants from a given combination (e.g. *S. etuberosum* as donor) as well as between these plants and the potato cultivar Desiree, the cybrid character of some of the fusion-derived plants could be determined even before organelle analysis, due to their male-sterility. In this research we intended to study primarily organelle/nuclear-genome compatibility-barriers among *Solanum* species. We used *S. tuberosum* as our "standard" nuclear-genome "host" (recipient) and four other *Solanum* species as sources of alien organelles. To choose appropriate organelle-donor species having different phylogenetic relatedness to potato we also considered the information reported by Hosaka and collaborators (Hosaka et al. 1984; Hosaka 1986; Hosaka et al. 1988a, 1988b). These investigators used the endonuclease restriction profiles of ctDNA from *Solanum* species to evaluate plastome phylogeny in this genus. They found that the plastome of *S. chacoense* is very similar to that of *S. tuberosum* (the difference was revealed in only one restriction site). Our results showed that indeed *S. chacoense* chloroplasts (as well as chondriome components) could be transferred to potato without causing any morphological changes or male sterility in the respective cybrids. On the other hand Hosaka and

collaborators found a considerable phylogenetic distance between *S. tuberosum* and *S. etuberosum* plastomes: 29 differences in restriction sites were revealed. Our results showed that cybrids could be obtained from the fusion of *S. etuberosum* as organelle donor and *S. tuberosum* as recipient, and that the respective cybrids were morphological similar to the recipient fusion partner but two abnormalities were revealed. Some had abnormal pigmentation and about one fifth of the analyzed cybrid were male sterile (with or without floral-member deformation). All the abnormally pigmented cybrids had *S. etuberosum* chloroplasts and all the male sterile had apparently pure *S. etuberosum* chondriomes. The rate of plastome transfer in this fusion combination was higher then the rate of mitochondrial transfer, but the two organelles were independently transferred, in conformation of similar independent transfer of organelle in *Nicotiana* cybrids (e.g. Aviv et al. 1984). Also in conformation with the previously studied organelle transfer in *Nicotiana* (Aviv et al. 1984) organelle sorting out was not completed at the callus stage: cybrids having different organelle composition may be derived from the same callus.

The species *S. brevidens* is considered even more far removed from *S. tuberosum* than *S. etuberosum*. The fusion combination of the former species with *S. tuberosum* resulted in pale green-albino cybrids. Only a few plants could be regenerated and in all of them there was a co-transfer of chondriome and plastome from *S. brevidens*. It should be noted that because of pathogen resistance traits, *S. brevidens* was a favorite species in somatic hybridizations with potato (Austin et al. 1985a; Austin et al. 1986; Barsby et al. 1984; Ehlenfeldt and Helgeson 1987; Fish et al. 1987,1988; Kemble et al. 1986). In contrast to our cybrids, the somatic hybrids derived from the fusion between protoplasts of *S. tuberosum* and *S. brevidens* did not show morphological abnormalities. A possible explanation for this difference is that the somatic hybrids contain the nuclear genome of both species thus there is no manifestation of alloplasmonic incompatibility. An extreme case of intertribal nuclear/organelle combination was reported by Thanh et al. (1988)

between *Nicotiana* and *Salpiglossis*. In that case the transfer of *Salpiglossis* chloroplasts into *Nicotiana* nuclear background or vise versa resulted in normal green cybrids. On the other hand, interspecific alloplastomic incompatibilities are known from sexual crosses in *Oenothera* (Kutzelnigg and Stubbe 1974; Tilney-Bassett 1978). In the latter genus there exists a biparental transmission of chloroplasts in sexual hybrids thus novel chloroplast/nuclear relations can be established by sexual crosses.

When *S. berthaultii* was used as donor and its irradiated protoplasts were fused with iodoacetate treated potato protoplasts - none of the resulting plants had the donor chloroplasts. Furthermore, only 8 out of 75 analyzed, fusion-derived plant had the donor chondriome: thus most of the fusion-derived plants could have been escapees. On the other hand since unfused and iodoacetatated Desiree protoplasts never attained cell division it is reasonable to assume that the initial fusion products were heteroplasmonic and that the *S. tuberosum* organelle composition was a result of later sorting-out. It is noteworthy that all cybrids with *S. berthaultii* chondriomes which attained flowering were found to be male-sterile. This correlation between male sterility and chondriome/nuclear-genome composition is in line with finding in other genera where alien chondriomes were considered to be causally related to male sterility (see: Hanson and Conde 1985; Pring and Lonsdale 1985; Galun and Aviv 1986; Galun et al. 1988 for reviews).

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Figure legends

Fig. 1. Southern blot hybridization of ctDNA from *S. tuberosum* cv. Desiree (recipient), *S. chacoense* (irradiated donor) and their cybrids. The ctDNA was digested with *Bam* HI and probed with a *S. tuberosum* ctDNA *Bam* III fragment-P153.

Fig. 2. Southern blot hybridization of mtDNA from *S. tuberosum* cv. Desiree (recipient), *S. chacoense* (irradiated donor) and their cybrids. The mtDNA was digested with *Hind* III and probed with pSylSa8.

Fig. 3 Southern blot hybridization of ctDNA from *S. tuberosum* cv. Desiree (recipient), *S. etuberosum* (irradiated donor) and their cybrids. The ctDNA was digested with *Bam* HI and probed with a *S.tuberosum* ctDNA *Bam* HI fragment-P153.

Fig. 4 Anthers of potato and cybrid flowers: A. Malformed anthers of a CMS cybrid plant derived from a fusion between *S. etuberosum* (donor) and *S. tuberosum* (recipient). B. Normal stamens of a *S. tuberosum* cv. Desiree flower.

Fig. 5. Southern blot hybridization of mtDNA from *S. tuberosum* cv. Desiree (recipient), *S. etuberosum* (irradiated donor) and their cybrids. The mtDNA was digested with *Eco* RI and probed with a mtDNA fragment of maize ATPase subunit 6.

Fig. 6. Regenerated albino shoots derived of a fusion between *S. brevidens* (donor) and *S. tuberosum* cv. Desiree (recipient).

Fig 7. Southern blot hybridization of ctDNA from *S. tuberosum* cv. Desiree (recipient), *S. brevidens* (irradiated donor) and their cybrids. The ctDNA was digested with *Bam* HI and probed with a *S. tuberosum* ctDNA *Bam* HI fragment-P153.

Fig 8. Southern blot hybridization of mtDNA from *S. tuberosum* cv. Desiree (recipient), *S. brevidens* (irradiated donor) and their cybrids. The mtDNA was digested with *Eco* RI and probed with pSylSa2.

Fig 9. Promotion of shoot regeneration from calli by o-coumaric acid. The calli were derived from the fusion of *S. tuberosum* protoplasts (recipient) with *S. berthaultii* (donor) protoplasts. Shoot regeneration was scored 21 days after transfer to regeneration medium. each point represents 10 calli.

Table 1. Organelle composition of protoplast-fusion derived plants (calli) based on Southern blot hybridizations with organelle-specific DNA probes. Protoplasts of the potato cultivar Desiree serve as recipients in all fusion combinations.

Donor	Fusion derived plants(calli)		Organelle analysis				
			Plastome		Chondriome		
	Total	Analysed	Donor	Recipient	Novel	Donor	Recipient
S. chacoense	38(10)	17(7)	7	10	1	5	11
S. etuberosum	117(36)	88(33)	31	57	0	13	75
S. brevidens	10(5)	5(5)	5	0	0	5	0
S. berthaultii	78(28)	75(22)	0	75	0	8	67

Fig. 4. Pure cyb.

S.chacoense
cyb E-5
cyb G-23
cyb B-5
cyb A-20
Desiree

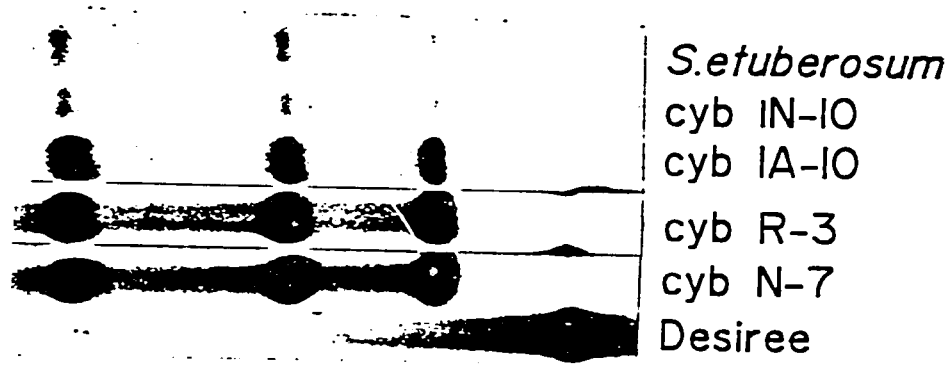

Fig 3. Peroxidase

A
B

ایزوزایم های پروتئینی

Perl et al. Fig 6

Fig. 4. Isoenzyme

Fig 2. Potato tuber

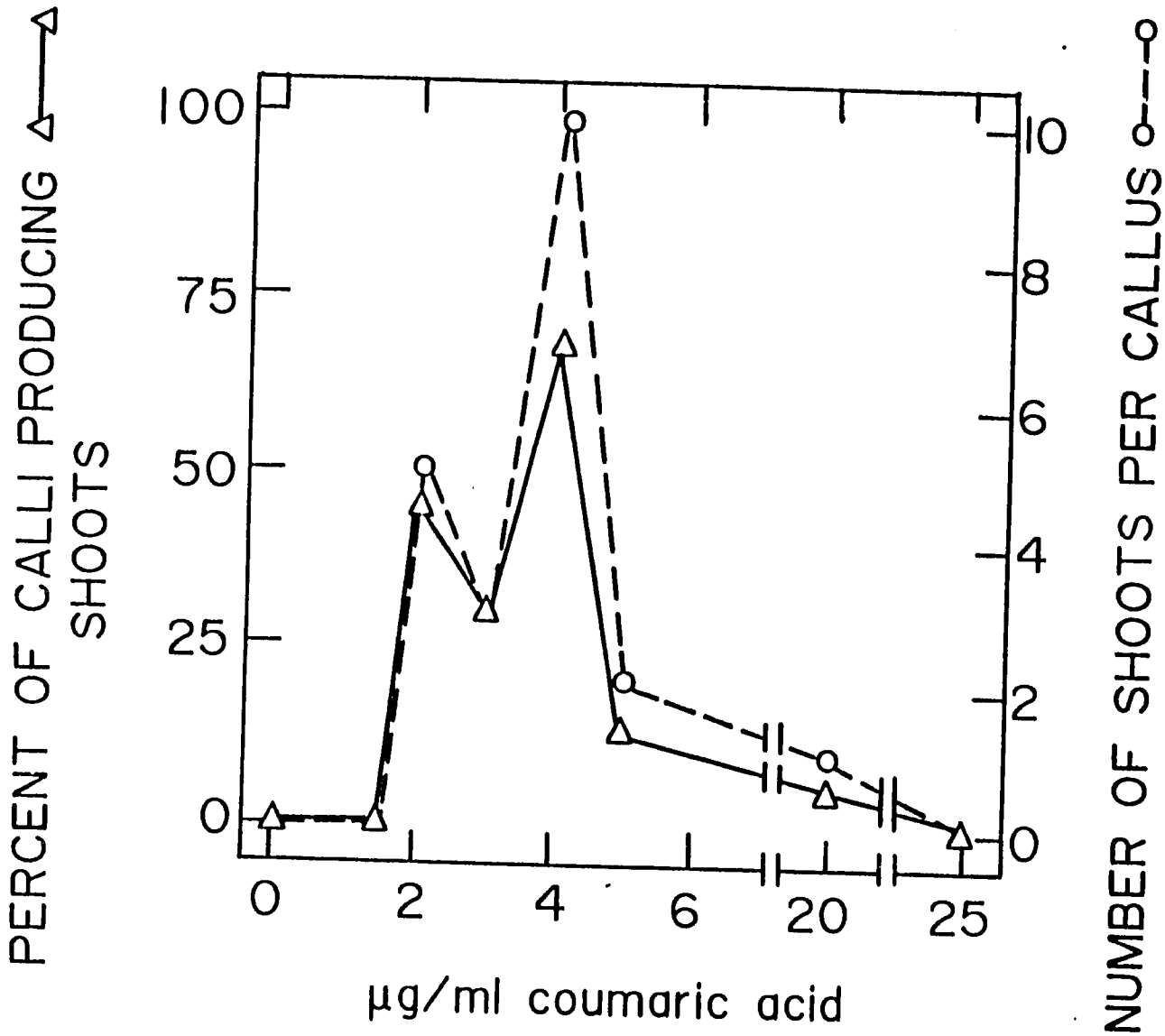

Fig 9 Pear et al.

מכון ויצמן למדע
THE WEIZMANN INSTITUTE OF SCIENCE
76100 REHOVOT · ISRAEL רחובות 76100 · ישראל

OFFICE OF RESEARCH CONTRACTS
AND PROJECTS

המחלקה לחוזי מחקר
ולפרויקטים

C 7-191

Our Ref. 3814 מספרנו
Your Ref. DPE-5544-G-SS-7043 מספרכם

February 4, 1990

Ms. Joyce Frame
Grant Officer
Agency for International Development
U.S.-Israel Cooperative Development (CDR) Program
Room 720, Building SA-18
Washington, DC. 20523
U.S.A.

Dear Ms. Frame,

Re: Final Scientific Report

Further to the final scientific report forwarded to you on January 31,90, by Prof. E. Galun, I enclose herewith the scientific report submitted by Dr. John H. Dodds, the Peruvian co-principal investigator on Project Number DPE-5544-G-SS-7043 entitled:

"Characterization of Organelle Genomes in the Potato Germplasm as an aid in Breeding Potatoes for Developing Countries".

Thank you very much for your kind collaboration,

PPC/CDIE
BOSTID

Sincerely yours,

N. Guter

Ms. Nurit Guter
Acting Director

cc: Dr. Charles Lawson, Scientific Attache (attached copy of the report)
Prof. E. Galun
Dr. J. Dodds

Cable address:Weizinst(Israel) מען למברקים Phone:08-482465,483284 טלפון Telex381300 WIX אלקס Fax. 8-466966 פקס

PROJECT

US ISRAEL CDR PROGRAM

'Characterization of Organelle Genomes in Potato Germplasm as Aid in Breeding Potatoes for Developing Countries'

DPE-5544-G-55-7043-0

Report

Dr. John H. Dodds
International Potato Center
P.O. Box 5969
Lima, Peru

Until October 1989

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INTRODUCTION

The common cultivars of potato belong to Solanum tuberosum ssp. tuberosum or to S. tuberosum ssp. andigena but the "cytoplasms" (i.e. the plastome and the chondriome) of many cultivars are undefined. Furthermore, in developing areas ranging from Bolivia, through Peru and Chile to Argentina there are numerous local potato cultivars which belong to other six or seven Solanum species. There is surprisingly very little knowledge on the cytoplasmic relationships between these cultivared species and other Solanum species which are related to potato. Whatever is known is based on cytogenetic studies rather than on direct analysis of the respective chloroplast and mitochondrial genomes.

The information to be gained by this project is of prime importance to potato-improvement and is of special relevance to breeding potatoes for the developing countries. Presently there is very little or no information on the cytoplasmic (i.e. plastomes and chondriomes) constitution of many breeding lines and of potential sources (accessions) having advantagous breeding traits, in the potato germplasm. Moreover the cytoplasmic relatedness between potato cultivars and such sources (which are used for cross-breeding) is unknown.

The potato germplasm collection at the CIP has 4000 accessions. The collection is recorded and computerized and partially characterized in respect to several features and breeding traits and is accessable to retrieval by several characteristics. But, the collection lacks descriptions of the plastomes (the chloroplast genomes) and the chondriomes (the mitochondrial genomes). characterization of the plastome and the

chondriome should not only readily reveal synonyms and group questionable accession in a coherent manner but should also provide very useful breeding information.

IDENTIFICATION OF MATERIALS TO SEND TO ISRAEL

CIP has already been able to identify material of wide cytoplasmic backgrounds and send this material directly as pathogen tested in vitro plantlets.

CIP has also facilitate the provision of seeds through the IRI genebank in Strogeon bay Wisconsin.

TECHNOLOGY TRANSFER TO CIP

This project is based on the development of molecular techniques in Israel, and the transfer of these technologies to CIP for further potential transfer to other developing countries.

CIP has already purchased the appropriate electrophoretic equipment and has been working on the refinement of the procedure.

In the near future CIP intends to send a Peruvian assistant to work in the laboratory of Prof. Galun to gain on site training on the use of these techniques. We shall then implement these kinds of studies on the germplasm collections at CIP

It is worth noting that this transfer process has been relatively shown since importation of certain equipment/chemicals into Peru has been delayed by repeated public sector strikes affecting importations.

FUTURE PLANS

The following points still need to be addressed in the coming months:

(1) New germplasm to be sent to Israel to broaden the present studies.

(2) Training of 1 CIP assistant in the finer points of the technique.

(3) Complete technology transfer to CIP so we can use this technique on a routine basis. The details of this will be further discussed when the PI's meet in 1990.