PD-AAX-218
54404

PROGRESS REPORT

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

Utilization of plant protoplast biotechnologies for the transfer of organelles.

by

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Period: January 1st to June 30th, 1985.

During the present report period, Prof. Esra Galun visited CIP, established good contacts with several scientists with whom he discussed our project and brought back potato seeds of Renacimento and shoot cultures of several potato varieties. A report on this trip was submitted by Prof. Galun on March, 1985.

I. Shoot cultrues of various potato lines and protoplast isolation.

The shoot culture lines that we received from CIP include:

LT-1 (CIP 377257.1)	DTO-28 (CIP 800169)
Atzimba (CIP720045)	Kufri Jyoti (CIP800258)
Atlantic (CIP 800827)	Chata blanca (CIP702514)
Ticauasi (CIP 720019)	Sereno

As we obtained only 1-2 shoots per line it took us almost 3 months to establish a reasonable size of vigorously growing shoot culture stock from each of the above listed lines. Now we are in a position where we have enough shoot material to start protoplast isolation studies. For each of the lines we will determine enzymes composition and concentrations and media ingredients for optimization of protoplast isolation and division.

II Isolation of plastome mutants.

1. Potato plastid mutants. Unfortunately the Renacimento seeds that we recently obtained from CIP do not germinate inspite different pretreatments that we tried. We hope to get from CIP either a new batch of seeds of the same variety or seeds of another variety. In the meantime we continue the mutagenesis studies with the Y245.7 line that we mentioned in the last report (Section IV).

In the green house we have already several variegated plants derived from NMU treated seeds. So far these plants did not flower.

In order to obtain antibiotic plastid resistant mutants, dose curves of streptomycin, erythromycin, spectinomycin and lincomycin were established for Y245.7. This line was found to be very sensitive to the last two antibiotics. 60 ug/ml of spectinomycin and 400 ug/ml of lincomycin cause complete bleaching of the cotyledons while up to 1 mg/ml erythromycin or 3 mg/ml streptomycin do not bleach the cotyledons. We will therefore attempt first at isolation of spectinomycin and lincomycin resistant mutants.

2. Tobacco plastid mutants. We have already a large collection of plastid mutants affected in either pigmentation or antibiotic sensitivity. We are now in the process of constructing a "bank" of Nicotiana lines containing several genetic markers in their plastomes. We have already several double mutants (e.g. str^R pig^- or str^R $spec^R$)

and we try to construct triple mutants. It should be stressed that it takes about 2 months from the time of seed mutagenesis until a putative mutant plantlet is transferred to the greenhouse and three more months until the seeds of the putative mutant can be tested. Usually the mutant plant contains a mixed population of mutated and non-mutated plastids and another generation is needed to obtain a pure mutant line. So that process of mutant isolation is quite lengthy.

III. Cybrid plant production: attempts to control organelle composition by pre-fusion treatment of protoplasts.

As was detailed in the previous report (Sections II and III) we have set up a series of experiments to test the possibility of specific elimination of mitochondria from protoplasts by pre-treatment with Rhodamine 6-G (R6G).

Protoplasts of all donors were derived from green mesophyll cells and protoplasts of all recipients were derived from albino mesophyll cells. All donors were irradiated before fusion. In some combinations the donor was also treated with R6G, in others the recipient was treated with R6G and yet in others R6G treatment was omitted.

The successful fusion combinations yielded many calli crom which many plants were regenerated while others yielded only few calli with few plants. e.g. the following reciprocally treated pair:

(a) rus/rus/rus + tbc/str^{R} pig^{-35}/tbc (R6G)*

(b) rus/rus/rus (R6G)+ tbc/str^{R} pig^{-35}/tbc

Combination (a) yielded 18 calli and 90 plants while combination (b) yielded only 2 calli with 11 plants.

In previous fusion experiments when R6G was omitted, the mitochondrial composition of the fusion derived plants (as defined by male fertility/sterility) was about 50% of donor type and 50% of recipient type. If indeed a R6G treatment is effective in mitochondrial elimination then in combination (a) the rus mitochondrial composition (or male sterility) among the fusion derived plants is expected to be greater than 50% and in combination (b) the tbc mitochondrial composition (or male fertility) should be greater than 50%.

Indeed, in combination (a) only one callus gave rise to fully male fertile plants. The other 17 calli gave rise to plants which ranged from partial male fertility to sterility. In combination (b) none of the two calli produced fertile plants (but of course the total number is too low). Of the 11 plants of combination (a) which were tested for mitochondrial composition 7 were of rus type and 4 were of tbc type. (See attached figure). Actually this is the first evidence of the transfer of rus chondriome into tobacco background.

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* On the left is the donor and on the right is the recipient. For each plant the nuclear, plastome and chondriome composition ...../...../..../ is given from left to right.

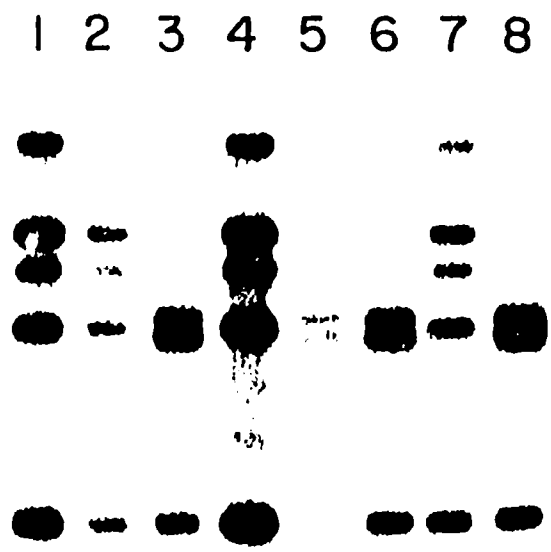
These preliminary results suggest that R6G probably does eliminate (or reduce) the mitochondria of the treated protoplast. To firmly establish this important observation we repeated the experiments with the following combinations.

(c) tbc/und lin^{R}17/und + tbc/str^{R} pig^{-35}/tbc (R6G)

(d) rus/rus/rus (R6G) + tbc/str^{R} pig^{-35}/tbc

(e) tbc/und str^{R}/ und (R6G) + tbc/str^{R} pig^{-35}/tbc

Combination (c) yielded about 50 calli which were transferred to regeneration medium. Combinations (d) and (e) are at an earlier stage.



g. 1. Southern blot hybridization of SalI digest of mitochondrial DNA rived from N. rustica (lane 1) N. tabacum (lane 8) and 6 cybrid plants anes 2-7). Note the identity of cybrids 2,4 and 7 with N. rustica.