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SOMATIC HYBRID PLANTS OF POTATO AND TOMATO REGENERATED FROM FUSED PROTOPLASTS

by

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Mesophyll protoplasts of Lycopersicon esculentum Mill. var. cerasiforme (Dunal) Alef, mutant yellow green 6, Rick and protoplasts of a liquid callus culture of the dihaploid strain HH258 of Solanum tuberosum L. were prepared and many fusion products were visible after the protoplasts were incubated together first in the presence of polyethylene glycol and then with a high Ca^{2+} ion concentration. The protoplasts were transferred to a rich medium and the resultant calli were cultured. Some calli regenerated normal green shoots which were transferred to soil or grafted onto a tomato stock. The subunit polypeptide pattern of ribulose 1,5-bisphosphate carboxylase prepared from leaf material of four regenerated plants was analyzed by isoelectric focusing. The ribulose bisphosphate carboxylase enzyme oligomer in the four plants contained the small subunit products resulting from the expression of both tomato and potato nuclear genes proving these plants to be somatic hybrids between tomato and potato. In three of the four plants the large subunit polypeptides and hence the functional chloroplast DNA were from tomato whereas in the fourth the large subunit and therefore the chloroplast DNA was derived from potato. The plant material was insufficient to establish the chromosome numbers precisely, however counts close to 50 which is near to the expected 48 were obtained for three of the hybrids whereas in the fourth a number close to 72 was observed. In the absence of a selection system against the potato parent, the analysis of ribulose bisphosphate carboxylase provides a convenient marker to demonstrate the hybrid nature of the plants.

1. INTRODUCTION

The development of methods to fuse plant protoplasts reproducibly (19,22,43) has made it possible to produce cell hybrids and to regenerate from these in certain instances plants which are somatic hybrids. Most of the somatic hybrids produced so far can also be obtained by sexual hybridization (7,27,29,30,32,36,37,38). Recently somatic hybrids have been produced by fusion of protoplasts from species which cannot be sexually hybridized either in one direction (25), at a certain ploidy level or without the aid of embryo culture (37). In cases of pure gamete incompatibility somatic hybridization is likely to be successful, but in such cases sexual hybridization by in vitro fertilization can also be a way to achieve the hybrid. In cases of zygote incompatibility either alone or in combination with gamete incompatibility, the fusion of protoplasts is unlikely to lead to hybrid plants. As discussed by ZENKTELER and MELCHERS (45) in situ observation of embryo development after sexual crosses cannot give decisive information on the type of incompatibility involved since impaired zygote development can be caused by inhibition from maternal tissues. Such inhibition is absent in callus and embryo cultures.

To the best of our knowledge sexual hybrids between potato (Solanum tuberosum, L.) and tomato (Lycopersicon esculentum Mill.) have not been described. The possibility to fuse the protoplasts between these two species is not surprising as incompatibility of cell fusion generally is absent even between animal and plant cells (6). Division and cell cultures from fusion products have been obtained in as diverse combinations as fusions of mouse and human cells or soybean (Glycine max L. Merr.) and Nicotiana glauca Grah. cells (18, 44). Regeneration of plants from hybrid callus cultures is unpredictable. In many plant species regeneration of plants from non-hybrid somatic callus tissue is not possible at present. One might expect that the chances of regenerating plants from somatic fusion products diminishes with increasing taxonomic distance between the partners involved, but the presently known somatic hybrids are too few to permit an evaluation of this hypothesis. Cases are known in which zygote incompatibility is caused by a single allele difference (15,16,26). It is of interest to note that a sexual hybrid between Petunia parodii W.C.S. and Nicotiana tabacum L. has been described (31). Although somatic hybridization between Petunia parodii and P. hybrida readily yielded plants (32) it has so far been impossible to regenerate plants from fusions of N. tabacum and P. hybrida (45).

It has been reported by MELCHERS (28) that protoplasts from cell lines of dihaploid Solanum tuberosum with 24 chromosomes and the diploid Lycopersicon esculentum with 24 chromosomes can be readily fused. This can be directly seen from the protoplasts of Figure 1 which have resulted from a fusion of a colourless potato protoplast and a green tomato protoplast. Since the dihaploid potato cell line has been kept as a submersed callus culture the protoplasts used in the experiments contain only colourless proplastids. The tomato protoplasts originated from mesophyll cells of green-house grown plants and therefore contain light green chloroplasts. The cytoplasms of the fused protoplasts in Figure 1 have not yet mixed and tomato-potato fusions can therefore be recognised as protoplasts with a colourless and a green part.

The present communication presents chromosomal counts and an analysis of ribulose-1,5-bisphosphate carboxylase (RuBPCase, E.C. 4.1.1.39) of putative somatic hybrid plants regenerated from callus tissue obtained in the above mentioned fusion experiments.

Ribulose-1,5-bisphosphate carboxylase from higher plants can be dissociated into two types of subunit. Isoelectric focusing (23,35) and peptide mapping (2,21) studies of the subunits from some Nicotiana species have shown that the large subunit (MW 55,000) is maternally inherited and that the small subunit (MW 12,000-

Abbreviations: RuBPCase = ribulose-1,5-bisphosphate carboxylase: PVP = polyvinylpyrrolidone: SDS = sodium dodecyl sulphate.

15,000) is inherited in a Mendelian fashion. The large subunit gene has been located on chloroplast DNA (4,10). The small subunit is synthesized on cytoplasmic polysomes (33) and transported into the chloroplast (5,13). Isoelectric focusing of the S-carboxymethylated RuBPCase has been used to provide phenotypic markers of chloroplast and nuclear genomes to study nuclear-cytoplasmic relationships in the evolution of plant species (3,8,9,11,39) and in the analysis of sexual interspecific hybrids (35). KUNG et al. (24) have also used this approach to investigate the result of interspecific protoplast fusion within the genus Nicotiana. The present results demonstrate differences in both the large and small subunits of RuBPCase from potato and tomato which have been used to demonstrate the hybrid nature of four plants produced by fusion of tomato and potato protoplasts.

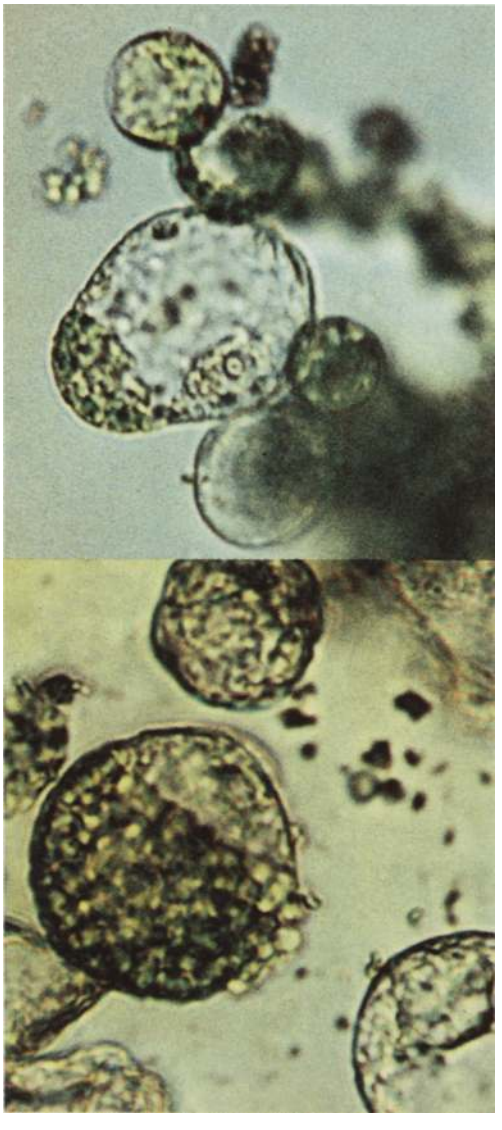

Figure 1. Protoplasts arisen by fusion of tomato and potato protoplasts. The cytoplasmic part of the tomato (Lycopersicon esculentum var. cerasiforme mut. yellow green 6) is recognizable by the green chloroplasts present in the tomato mesophyll protoplasts. The cytoplasmic part of the potato (Solanum tuberosum, dihaploid stock HH 258) is colourless as the potato protoplasts were made from submersed cultured callus cells with proplastids.

2. MATERIALS AND METHODS

2.1. Preparation of protoplasts

Protoplasts of Solanum tuberosum were prepared from the dihaploid stock HH 258 from the Max-Planck-Institut für Züchtungsforschung, Köln-Vogelsang. Plants can be regenerated from callus cultures of this line (1). This potato stock as grown in Tübingen was largely self-fertile, but non-homogeneous with regard to leaf form and flower colour. Submersed callus of this stock is kept in continuous culture at Tübingen (28).

From a culture started on Sept. 19, 1976 in LS1 medium (1) 50 ml cell suspension was combined with 50 ml E_4 solution (19) containing 0.7 M-glucose, 6 mM-$CaCl_2$, 0.7 mM-NaH_2PO_4, 3 mM-2[N-morpholino]ethane sulfonate and adjusted to pH 5.7 with NaOH. The E_4 solution also contained 2% cellulase »Onozuka« R-10 (Kinki Yakult Manufact. Co. Ltd. 8-12 Nishinomiya, Japan), 2% Rohament P (Röhm and Haas, Darmstadt, FRG) and 0.5% pectinase No. P-4625 (Sigma, St. Louis, USA). The incubation mixture had a pH of 5.8 and the treatment lasted 4-5 hours.

Mesophyll protoplasts of tomato were prepared from light green leaves of the mutant yellow green 6, in Lycopersicon esculentum var. cerasiforme. Seed of the mutant was kindly supplied by Dr. C. M. RICK, University of California, Davis. The mutant has light green leaves if grown in a shaded green-house but

Table I

Protocols of the callus tranfers and shoot regenerations of the 4 relevant groups of plants regenerated from protoplast fusions of potato and tomato.

Group	1b/2h/3 (Figures 2a and b)	
Date:		Code:
1.03.77	fusion and protoplasts plated on 8p medium	1
24.03.	dilution on liquid 8p medium	1b
4.04.	dilution on agar with 8p medium	1b/1-4
28.04.	transfer of small light calli to D2s medium (34)	1b/2h-p
12.05.	transfer of grown calli to MS13K medium (1) + coconut extract	1b/2h
26.05.	transfer of healthy tissue to MS13K medium	1b/2h
15.06.	transfer to fresh MS13K medium	1b/2h
18.07.	transfer to fresh MS13K medium	1b/2h
11.08.	transfer of healthy portions to fresh MS13K medium	1b/2h/1-3
16.09.	transfer of callus to fresh MS13K medium	1b/2h/3
16.09.	isolation of regenerated shoots (S1,S2) to NO1 medium (40) with 4g·ml^{-1} sucrose	1b/2h/3/S1, 2
27.09.	isolation of regenerated shoots (S3-S5) to NO 1 medium	1b/2h/3/S3-5
12.10.	transfer of callus to fresh MS13K medium	1b/2h/3
12.10.	transfer of shoots S1 to S5 on KW medium (29)	1b/2h/3/S1-5
26.10.	transfer of shoots to soil	
24.04.78	grafting of shoots on tomato stock var. Supravite	

Group	6a/4z/6g (Figure 3)	
Date:		Code:
1.03.77	fusion and protoplasts plated on 8p medium	6
24.03.	dilution on liquid 8p medium	6a
6.04.	dilution on agar with 8p medium	6a/1-4
6.05.	dilution in agar with 8p medium as toplayer on bottom layer with MS13K medium	6a/4z/1-8
13.07.	transfer of healthy green calli on MS13 medium	6a/4z/6a-h
16.09.	transfer of healthy tissue on MS13 medium	6a/4z/6e-h
2.11.	transfer to fresh MS13 medium	6a/4z/6g
2.11.	isolation of regenerated shoots (S1, S2) to NO 1 medium	6a/4z/6g/S1, 2
18.11.	transfer of shoots to soil	
24.04.78	grafting of shoots on tomato stock var. Supravite	

Group	6b/1x/2a and 2b (Figures 4 and 5)	
Date:		Code:
1.03.77	fusion and protoplasts plated on 8p medium	6
24.03.	dilution on liquid 8p medium	6b
6.04.	dilution on agar with 8p medium	6b/1-5
6.05.	dilution in agar with 8p medium as toplayer on bottom layer with MS13K medium	6b/1x/1-8
13.07.	transfer of healthy green calli on MS13 medium	6b/1x/2a-d
6.09.	transfer of healthy tissue on MS13	6b/1x/2a
2.11.	transfer to fresh MS13 medium	6b/1x/2a
2.11.	isolation of regenerated shoots (S1-S4) to NO 1 medium	6b/1x/2a/S1-4
18.11.	transfer of callus to fresh MS13 medium	6b/1x/2a
22.11.	isolation of regenerated shoots (S5-S13) to NO 1 medium, the shoots had on the callus strong roots	6b/1x/2a/S5-13
28.11.	isolation of regenerated shoots (S14-S16) to NO 1 medium medium + 50 mg/l aureomycin	6b/1x/2a/S7, 8, 12, 13
28.11.	isolation of regenerated shoots (S14-S16) to NO 1 medium	6b/1x/2a/S14-16
1-12.	transfer of shoots S7, S8, S12, S13 to NO 1 medium, as no growth occured in the presence of aureomycin	6b/1x/2a/S7, 8, 12, 13
6.12.	transfer of callus to fresh MS13 medium	6b/1x/2a
6.12.	transfer of shoots S14-S16 to soil	
6.12.	transfer of shoots (S17, S18) to NO 1 medium	6b/1x/2a/S17, 18
6.12.	transfer of shoots (S19, S20) to NO 1 medium + 25 mg.l^{-1} aureomycin	6b/1x/2a/S19, 20

6.12.	transfer of shoots (S21, S22) to NO 1 medium + 10 mg.l^{-1} aureomycin	6b/1x/2a/S21, 22
11.12.	transfer of shoots S5-S12 to soil	
23.03.78	grafting of shoots on tomato stock var. Supravite	

Group	7a/20e (Figure 5)	
Date:		Code:
1.03.77	fusion and protoplasts plated on 8p medium	7
6.04.	dilution on agar with 8p medium	7a-c
6.05.	dilution in agar with 8p medium as toplayer on bottom layer with MS13K medium	7a/14-21
11.07.	transfer of healthy green calli on MS13 medium	7a/20a-h
16.08.	transfer to fresh MS13 medium	7a/20e-g
12.10.	transfer to fresh MS13 medium	7a/20e
12.10.	isolation of regenerated shoot (S1) to NO 1 medium	7a/20e/S1
3.11.	transfer of callus to fresh MS13 medium	7a/20e
3.11.	isolation of regenerated shoots (S2, S3) to NO 1 medium	7a/30e/S2, 3
28.11.	transfer of ½ of callus to MS13 medium and ½ to MS13 medium + 50 mg.l^{-1} aureomycin	7a/20e
29.11.	transfer of shoot S6 to soil	
30.11.	transfer of shoots S5, S7 to soil	
3.12.	transfer of shoot S4 to soil	
7.12.	transfer of shoot S2 to soil	
13.03.78	grafting of shoots on tomato stock var. Supravite	

yellow green to yellow leaves when grown unshaded.

Seeds were sown on Dec. 29, 1976; leaves harvested on March 1, 1977 from large non-flowering plants were sterilized according to KELLER and MELCHERS (22). The leaf pieces were pre-incubated for 25 min in 0.5 M-mannitol at 25°C and then treated for 4 hours in enzyme solution (pH 5.8) containing 0.25% Driselase (Kyowa Hakko Kogyo Co. Ltd., Tokyo, Japan), 0.3% Cellulase »Onozuka« R-10 and 0.5 M-mannitol.

2.2. Fusion of protoplasts

The hybrid plants analysed in this paper originated in a fusion experiment of March 1, 1977. The fusion was performed by placing the protoplasts for 10 min at 23°C in a solution containing 50% (w/v) polyethyleneglycol 1540, Cat. No. 0679 Lot No. 279-8 (Polysciences Inc., Warrington Pa., USA), 0.1 M-glucose, 3.5 mM-$CaCl_2$ and 0.7 mM-KH_2PO_4. The incubation mixture was diluted with 0.08 M-$CaCl_2$ (pH 10). After 20 min the protoplasts were washed with a solution containing 0.6 M-mannitol and 0.05 M-$CaCl_2$ over a period of 25 min at 23°C.

2.3. Regeneration of the plants investigated for chromosome number and RuBPCase

The protoplasts were cultured on the very rich medium 8 p of KAO and MICHAYLUK (20) omitting riboflavin. The callus cultures obtained were kept at 24°C in rooms with weak continuous light from Osram-Fluora-fluorescence lights supplemented with weak Osram-Nitra lamps (ca. 500-1000 lux). Since the plants originated from different regimes of callus culture transfers the protocols of the 4 relevant groups of plants are detailed in Table I starting with the date and ending with the code number in each line. Representative pictures of group 1b/2h/3 are given in Figures 2a and b, of group 6a/4z/6g in Figure 3, of group 6b/1x/2a and 2b in Figures 4 and 5 and of group 7a/20e in Figure 5. The plants of 6b/1x/2a and 6b/1x/2b are morphologically similar and may be genetically identical. Both had as callus developed extensive roots, as is otherwise characteristic for regenerating tomato protoplasts. Aureomycin treatment was used to eliminate bacterial infections presumably originating from the tomato protoplast preparation.

2.4. Determination of chromosome numbers

As far as possible root tips were fixed in ethanol-acetic acid (3:1). From plants which only grew as scions shoot tips, leaf primordia and flower buds were fixed. The chromosomes were stained with orcein-acetic acid and squashed (34).

2.5. Isolation of RuBPCase

The procedure for purifying RuBPCase was based on that described previously for the enzyme from Oenothera (14) with some modifications. The leaf material, up to 6 g fresh weight, was homogenized using a Polytron (Kinematica GmbH) in 10 ml 0.2 M-sodium borate buffer pH 7.5 containing 10 mM-sodium metabisulphite, 5 mM-EDTA, 40 mM-mercaptoethanol and 1% (w/v) soluble polyvinylpyrrolidone (PVP) (K25 pharmaceutical grade, Fluka AG, Switzerland). A few drops of octanol were added to prevent foaming during homogenization and to remove the non-precipitable green material during the subsequent centrifugation. The homogenate was centrifuged at 48,000 g for 20 min. The supernatant was filtered through glass wool and was then applied to a column of Sephadex G25 (3.2. x 20 cm) equilibrated with 16.7 mM-sodium phosphate, pH 7.2 containing 10 mM-sodium metabisulphite and 20 mM-mercaptoethanol. The material in the void volume was applied to a DE52 DEAE-cellulose column (1.6 x 12 cm) equilibrated with the same phosphate buffer. After extensive washing of the column, RuBPCase was eluted with sodium phosphate buffer containing 0.4 M-NaCl. The fractions containing the enzyme were applied to a Sepharose 6B column (5.0 x 50 cm) equilibrated with 50 mM-Tris-Cl, pH 7.5 containing 100 mM-NaCl, 1 mM-EDTA and 10 mM-mercaptoethanol. Solid ammonium sulphate (351 g. l^{-1}) was added to the pooled fractions containing RuBPCase and the enzyme was stored as a precipitate at 4°C.

On some occasions the enzyme was prepared by a shorter procedure in which PVP was omitted from the homogenization buffer and the DEAE-cellulose step was excluded. The effect of this modification is discussed in section 3.1.

2.6. Preparation of RuBPCase for isoelectric focusing

Approximately 1 mg RuBPCase was dissolved in 0.25 ml 1 M-Tris-Cl, pH 8.6 containing 6 M-guanidinium chloride (Aristar grade, BDH Chemicals, England) and 2 $mg \cdot ml^{-1}$ EDTA. After flushing with nitrogen for 15 min 1 µl of mercaptoethanol was added to each sample. After a further 1 hour 2.6 mg iodoacetic acid dissolved in 1 M-NaOH (186 $mg \cdot ml^{-1}$) was added and alkylation was allowed to proceed in the dark for 20 min. The reaction was stopped by addition of a further 1 µl mercaptoethanol followed by dialysis against deionized water and freeze drying.

The freeze dried samples were re-dissolved in a small volume of water containing 8 M-urea, 2% ampholyte (LKB ampholine, pH range 5-7) and 1% Nonidet P-40 prior to isoelectric focusing.

2.7. Isoelectric focusing of RuBPCase

Isoelectric focusing was performed at 5°C on a flat bed apparatus designed and built by Mr. JAN RASMUSSEN at the Carlsberg Laboratory. A 4.8% polyacrylamide gel slab (24 x 11.5 x 0.2 cm) containing 8 M-urea, 2% ampholyte (LKB ampholine, pH 5-7), 1% Nonidet P-40 and polymerized with riboflavin was prepared essentially as described by VESTERBERG (41). 50 µl samples were placed in wells cut in the gel near the cathode. A potential of 300 V was applied for 1 hour followed by 700 V for 16 hours and 1000 V for the last 30 min. The gel was fixed, stained and destained according to procedure 'D' described by VESTERBERG et al. (42).

Figure 2. Potato-tomato hybrid 1b/2h/3

a) growing on its own roots with filled flowers (possibly because of abnormal chromosome number). Flower colour: very dilute purple (from potato?) but also weak yellow (from tomato?)

b) fibrous roots and rhizome, which is slightly thickened and has produced a shoot with filled flowers. An underground flower bud is also present.

Figure 3. Potato-tomato hybrid 6a/4z/6g
The plants have anthocyanin rich leaves. The large plant is grafted on tomato var. Supravite and the small plants grow on their own roots.

Figure 4. Potato-tomato hybrid 6b/1x/2b
The fruits have probably arisen by parthenocarpy. Results of back crosses not yet known. This hybrid has probably the same genetic constitution as 6b/1x/2a (cf. 2.3).

Figure 5. Potato-tomato hybrid shoots grafted on tomato stock.
Left: 6b/1x/2a had as callus strong root formation as is characteristic for tomato callus.
Right: 7a/20e was very difficult to regenerate and to free from infections.

Figure 6. Comparison of flowers from dihaploid potato (top), potato-tomato hybrid 6b (middle) and tomato var. Supravite (bottom). Inset: flower of hybrid 1b.

2.8. RuBPCase subunit separation

The subunits of S-carboxymethylated RuBPCase were separated by gel filtration in 50 mM-ammonium bicarbonate containing 0.5% SDS on a Sephadex G100 column as described previously (14).

3. RESULTS

3.1. RuBPCase from Solanum tuberosum, stock HH258 and Lycopersicon esculentum var. cerasiforme, mutant yellow green 6

The purification procedure as described was successfully applied to the isolation of RuBPCase and gave approximate yields of 3-5mg protein per gram fresh weight leaf material. The RuBPCase prepared from tomato and potato was essentially homogeneous by the criterion of SDS-polyacrylamide gel electrophoresis (C. POULSEN, personal communication). If PVP was absent from the grinding medium and the DEAE-cellulose chromatography step was omitted a pronounced effect on the isoelectric focusing behaviour of the RuBPCase large subunit was observed, as described later, presumably due to interaction of the protein with polyphenols.

Figure 7 shows the polypeptide pattern obtained after isoelectric focusing of S-carboxymethylated RuBPCase from tomato and potato together with the isolated subunits to allow assignment of the individual bands to the two subunits. It can be seen that the small subunit of tomato RuBPCase (tracks 1 and 2) gave rise to 3 bands of different intensity in the pH 5 region of the gel. In the same region of the gel the potato RuBPCase small subunit (tracks 5 and 6) stained as 5 polypeptides of which one was prominent. The patterns of tomato and potato small subunit bands were clearly distinguishable. The number of small subunit polypeptide bands could not be reduced by variation in the extent of reduction or alkylation, and indeed under identical conditions the small subunit of Oenothera hookeri RuBPCase produced a single band (A. A.

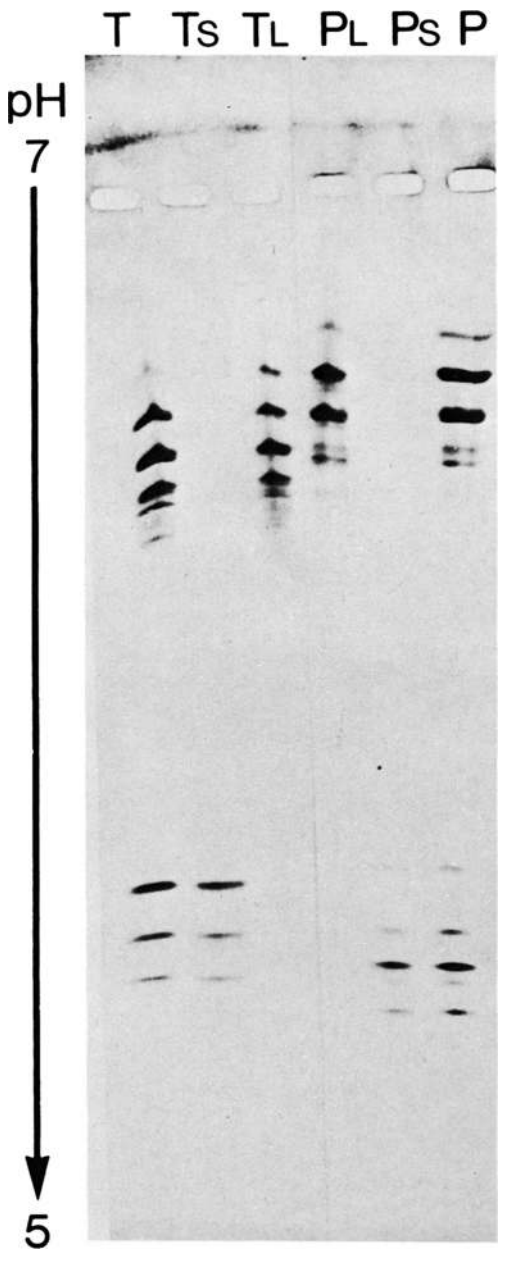

Figure 7. Isoelectric focusing of S-carboxymethylated RuBPCase and its isolated subunits from tomato and potato. (T) Tomato total enzyme, (T_S) tomato small subunit, (T_L) tomato large subunit, (P) potato total enzyme, (P_S) potato small subunit, (P_L) potato large subunit.

HOLDER, unpublished observation). For the large subunit a cluster of bands was obtained in the high pH region. It can be seen that the potato large subunit (tracks 4 and 6) focused at a higher pH than the tomato large subunit (tracks 1 and 3).

The effect of the purification procedure is demonstrated by comparing the pattern of large subunit bands from tomato prepared by the shortened procedure (Figure 7, tracks 1 and 3) with the pattern obtained when PVP was included during homogenization and the DEAE-cellulose chromatography was performed (Figure 9, track 1), the latter procedure giving a much simpler distribution of polypeptide bands. When the potato enzyme was prepared by the shortened procedure the protein was yellow in solution and the large subunit produced more stained bands, the two most intense were in positions identical to the strongest of the tomato large subunit. This artefact, possibly due to the high content of polyphenols in potato leaves (17) had no effect on the number or position of the small subunit polypeptide bands.

3.2. RuBPCase of the somatic hybrids

The plants grown from protoplast fusion products were analyzed by isoelectric focusing of the S-carboxymethylated enzyme prepared by the long purification procedure. In Figure 8

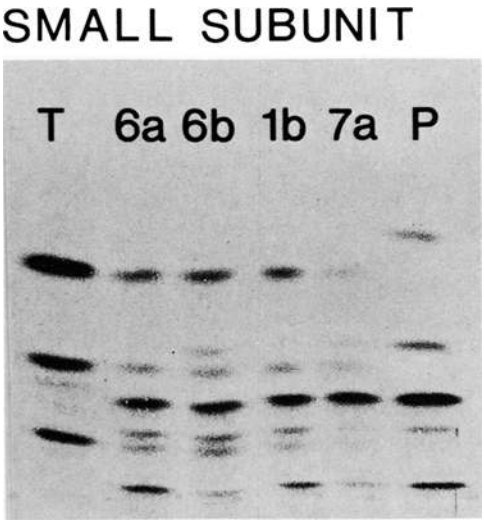

Figure 8. Isoelectric focusing of S-carboxymethylated RuBPCase small subunit from tomato, potato and the hybrids. (T) tomato, (P) potato, (6a) 6a/4z/6g, (6b) 6b/1x/2a, (1b) 1b/2h/3, (7a) 7a/20e.

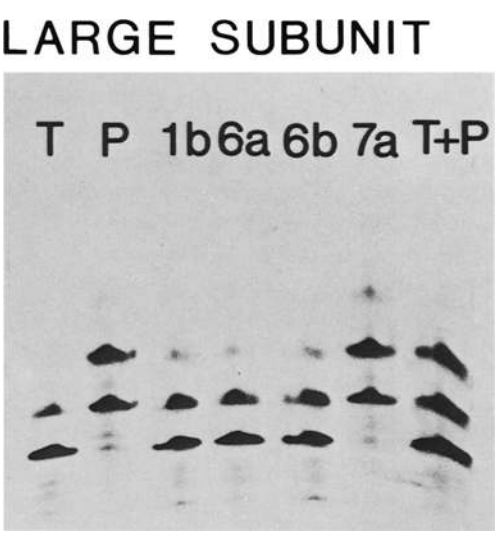

Figure 9. Isoelectric focusing of S-carboxymethylated RuBPCase large subunit from tomato, potato and the hybrids. (T) tomato, (P) potato, (T+P) mixture of tomato and potato, (1b) 1b/2h/3, (6a) 6a/4z/6g, (6b) 6b/1x/2a, (7a) 7a/20e.

the pattern of small subunit bands from these plants is compared with those of tomato and potato. It can be seen that all 4 plants contain the 3 prominent tomato small subunit bands. In addition they all contain the prominent potato small subunit band and two of the faint bands. One of the faint potato small subunit bands is absent in all the hybrids and one is present in only two of the four (6b/1x/2a and 7a/20e); the reason for this is unclear. Relative to the potato bands the staining intensity of the tomato small subunit bands in 7a/20e is considerably less than in the other plants. These results show that the native RuBPCase in the four plants contains the small subunit products resulting from the expression of both tomato and potato nuclear genes and prove these plants to be somatic hybrids between potato and tomato.

The RuBPCase large subunit polypeptides from the hybrid plants are compared with the tomato and potato large subunit polypeptides in Figure 9. For each large subunit only two prominently stained bands were observed, together with some faint bands. The position of the bands from 1b/2h/3, 6a/4z/6g and 6b/1x/2a was identical to those of tomato. The position of the bands of the large subunit from 7a/20e was identical to those of the large subunit from potato. This is interpreted to indicate that in 3 of the 4 hybrid plants examined the functional chloroplast DNA was derived from tomato whereas in the fourth the chloroplast DNA was derived from potato.

3.3. Chromosome counts

In control experiments cultured tomato protoplasts gave rise to callus which developed roots but never shoots. The four regenerated groups of plants investigated here are normal green and do not show the recessive yellow green marker of the tomato used. It can therefore be excluded that the plants are tomatoes. The analysis of RuBPCase (cf. section 3.2) revealed the plants to be somatic hybrids. Ideally their chromosome number should be 48 and the plants amphidiploid and perhaps fertile.

The material was insufficient to establish the chromosome numbers precisely. The numbers counted are presented in Table II. Hybrids 1b/2h, 6a/4z and 6b/1x gave counts around 50 chromosomes which is close to the expected number but in all cases a few extra chromosomes were counted. The poorly growing hybrid 7a/20e could be a hexaploid with 72 chromosomes. It was noted that this hybrid

Table II

Chromosome counts of the somatic hybrids of potato and tomato

Callus	Shoot regenerate	Number of chromosomes
1b/2h/3	S1	≧ 50
–	S3	54 - 56
6a/4z/6g	S1	50 - 54
–	S2	≧ 50
–	S3	no division found
–	S6	50 ± 2
–	S8	54
6b/1x/2a	S1	50 - 52
–	S5	≧ 52
–	S14	no division found
7a/20e	S2	no division found
–	S6	72
–	S7	≧ 60
–	S12	no division found

plant contains significantly less staining bands of the tomato small subunit, which may indicate this plant to have arisen by triple fusion of two potato and one tomato protoplast.

3.4. Morphology of the somatic hybrids

The plants were transferred into soil under unfavourable conditions and grew poorly during the winter of 1977/78. In the spring a considerable number of them were grafted on tomato stock var. Supravite for growth improvement. Some of these grafts were transferred to Copenhagen on May 17, 1978 for the analysis of RuBPCase. As can be seen from Figures 2 to 6 the hybrids differ in habitus and vigour. They are clearly different in leaf shape and flower morphology from the potato and tomato stocks used for protoplast fusion. Hybrid 1b/2h in Figures 2a and 2b grows on its own roots. The flowers (Figure 6 – inset) are filled and their colour is a highly diluted purple (from potato?) and a weak yellow (from tomato?). The plant has fibrous roots and a slightly thickened rhizome (Figure 2b). A shoot with filled flowers has grown from the rhizome and a flower bud has developed under ground. In Figure 3 the hybrid 6a/4z is represented by four plants, the large one growing as a scion on tomato stock, the small ones on their own roots. Figure 4 depicts hybrid 6b/1x/2b with probably parthenocarpic fruits and Figure 5 the poorly growing, possibly hexaploid hybrid 7a/20e at the right and the hybrid 6b/1x/2a at the left. The latter plant has developed flowers with purple, yellow and white stripes (Figure 6).

To what extent the various characters are due to modifications caused by differences in callus transfer, shoot isolation, transfer to soil or differences in time of grafting cannot be decided at present. Most likely the differences are due to different chromosome constitutions, especially hyperploidy. It is also conceivable that some of the plants are chimeras containing tissues with different chromosome numbers.

4. DISCUSSION

The isoelectric focusing of RuBPCase provided phenotypic markers of the chloroplast and nuclear genomes of tomato and potato so that the products of protoplast fusion between these two species could be analyzed. It is clear that the number of polypeptides of different isoelectric behaviour from each subunit could be increased by post-translational modification, by artefacts introduced during cell rupture and enzyme purification or during reduction and alkylation of the protein. Some of these problems have been discussed previously (12) and in the present study care was taken to prevent polyphenol modification of the protein and artefacts arising during reduction and alkylation. With these limitations in mind it can be stated that the isoelectric focusing behaviour shows that the products of both tomato and potato nuclear genomes are present in the RuBPCase oligomer from the hybrids investigated. In addition, the hybrids 1b/2h, 6a/4z and 6b/1x contain the tomato large subunit and hence the tomato chloroplast DNA is functional, whereas in the hybrid 7a/20e the potato RuBPCase large subunit and therefore the potato chloroplast DNA is present. With this analysis it is impossible to exclude completely the presence of the alternate large subunit as a small percentage of the total. Further analysis of the protein by N-terminal sequencing or peptide mapping will decide these issues.

At the time of fusion the potato protoplasts contained proplastids and the tomato protoplasts fully differentiated chloroplasts. It is an intriguing question if the strong representation of tomato chloroplast DNA in three of the hybrids is due to the differentiated state of the tomato chloroplast at the time of fusion. The fourth hybrid, 7a/20e with its dominating presence of potato chloroplast DNA may have arisen as pointed out in section 3.3 from a triple fusion product and thus from a cell with twice the number of potato plastids than in the case of the other hybrids.

A sexual hybrid of Nicotiana tabacum and Nicotiana knightiana was considered previously not feasible. In connection with the production of somatic hybrids from these two species MALIGA et al. (25) found that this hybrid can be produced sexually at least in the direction N. knightiana ♀ × N. tabacum ♂. Also in the case of the somatic Datura hybrids of SCHIEDER (37) sexual hybrids can be obtained with some aid. We suspect that also for the hybrids of

Lycopersicon esculentum and Solanum tuberosum described in this report means will be found to produce them sexually. ZENKTELER (personal communication) has tried in vitro fertilization, but has so far been unsuccessful. This is perhaps due to the high activity of phenol oxidases in connection with the experimental manipulation.

It is thus possible but not proven that the somatic hybrids described here are the first ones which cannot be produced sexually. If all potato-tomato hybrids, also those with 48 chromosomes, should prove to be sterile their use in plant breeding research would be limited. It is on the other hand likely that the four plants investigated so far are aneuploids and that the true amphidiploid is either already present in the material now available in Tübingen or will be found in new fusion experiments. The fact that both partners are self-fertile gives a good chance that the true amphidiploid is also fertile. Since one now knows what a tomato-potato hybrid plant looks like it will be easier to select them in larger numbers and to identify the true amphidiploid. To what extent practically useful new combinations of genes from the rich assortments of wild, primitive and cultured forms of tomato and potato can be derived by somatic hybridization remains to be seen. In both species monoploid to tetraploid types are known and it is quite possible that combinations of other chromosome types will yield not only viable potato-tomato hybrids by the fusion of protoplasts but also more vigorous hybrids.

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