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TETRAPLOID SOMATIC HYBRIDS OF POTATO (*Solanum tuberosum* L.) OBTAINED FROM DIPLOID BREEDING LINES

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Abstract: Intraspecific somatic hybrids between 16 different diploid breeding lines of *Solanum tuberosum* L. were produced by PEG-induced fusion. Manually selected heterokaryons were cultured in a Millicells-CM using a post-fusion protoplast mixture. Plants were regenerated from calli derived from heterokaryons obtained from 10 out of 38 combinations of diploid lines. Of the tested putative somatic hybrids, 14.2% were diploid, 72.8% were tetraploid and 13% pentaploid. The DNA amplification pattern obtained with RAPD or semi-random primers confirmed that 6 fusion combinations were hybrids. In most cases, the morphological traits were intermediate to those of the diploid fusion partners. About 23.0% of the tested somatic hybrids showed variation in their morphology. Of the tested somatic hybrids, 78.0% flowered and 86.0% tuberized. The cytoplasm of 9 diploid lines and 6 somatic hybrid combinations was analysed. Two of the diploid lines had W/S chloroplasts and α or ε mitochondria; the remainder contained T chloroplasts and β mitochondria. All the analysed somatic hybrids carried T chloroplasts and β mitochondria.

Key words: Cytoplasmic hybrids, Heterokaryons, Molecular markers, Protoplast fusion

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Abbreviations used: cpDNA - chloroplast DNA; CTAB - cetyltrimethyl ammonium bromide; FDA - fluorescein diacetate; mtDNA - mitochondrial DNA; NAA - naphthaleneaceic acid; PEG - polyethylene glicol; PVP - polyvinylpyrrolidone; RAPD - random amplification of polymorphic DNA; RFLP - restriction fragments length polymorphism

INTRODUCTION

Reducing the ploidy level of cultivated potato (from tetra- to diploid) facilitates crossing with the diploid wild *Solanum*, and makes intraspecific crossing and selection much simpler. Tetraploid plants can be recovered from selected diploid progeny by crossing (usually 2n x 4n), fusion of somatic cells 2n(+)2n, or chromosome doubling. The first two methods are more important as they combine traits from both components, leading to a higher degree of heterozygosity in the resultant tetraploid plant. Wenzel *et al.* [1] first proposed the use of different *in vitro* techniques for tetraploid potato plant generation. Tetraploid progeny formation after 2n x 4n crossing requires the diploid line to be able to form unreduced -2n gametes, which is not necessary in somatic hybridisation. A tetraploid progeny from crossing is genetically different from a tetraploid somatic hybrid. Nuclear genomes are recombined in crossing and combined in somatic hybrids. Cytoplasm is uniparentally transferred to sexual progeny, while somatic hybridisation can create a wide range of cytoplasmic rearrangements. Using either method may lead to the formation of two distinct classes of plants [review: 2].

Ramulu *et al.* [3] and Rasmussen *et al.* [4] presented results indicating high variation between potato hybrids derived from a single fusion combination, possibly due to somaclonal variation, aneuploidy and/or the influence of rearranged cytoplasm. Combining two genomes might induce a range of epigenetic changes, and this aspect, although it has not been analysed in somatic hybrids, should not be excluded. Several labs studied the use of somatic hybridisation to obtain tetraploid potato hybrids from diploid clones. Hybrids obtained by Mattheij and Puite [5] had a similar or higher tuber yield than cv. Bintje. Wenzel *et al.* obtained and evaluated tetraploid somatic hybrids for their tuber yield and starch content [6], and related the expression of these traits to the composition of the hybrid cytoplasm [7]. They also studied the fusion combining ability of dihaploid lines and its relationship to the type of cytoplasm [8] and the different cytoplasmic configurations present in somatic hybrids [9]. Somatic and sexual tetraploid hybrids obtained from two diploid lines were characterised by Cooper-Bland *et al.* [10], who found that late blight resistance in the hybrids was intermediate, while the tuber yield indicated hybrid vigour. Rasmussen *et al.* [4] found the foliage and tuber late blight resistance of hybrids derived from four different fusion combinations to be inherited independently, and the individual hybrids to vary from highly resistant to sensitive. Intermonoploid somatic hybrids were obtained by Johnson *et al.* [11].

We report on the somatic hybridization of selected diploid lines with several important traits (Tab.1). The methods of heterokaryon selection and regeneration of plants were established to suit their application in breeding programs. The obtained tetraploid somatic hybrids were verified using molecular markers. Chromosome numbers, type of organelles and selected phenotypic traits were described for the diploid lines and obtained somatic hybrids.

MATERIAL AND METHODS

Plant material

For this study, we obtained sixteen diploid lines (1A, 2B, 3C, 4D, 5E, 6F, 7G, 8H, 9I, 10J, 11K, 12L, 13M) from IHAR Mlochow, Poland. Their characteristics were described by Jakuczun *et al.* [12]. All of the lines are the result of interspecific hybridization. Tab. 1 presents selected traits of the lines, and the

Tab. 1. The characteristics of the diploid lines obtained from IHAR Mlochow and used in this study. The second column refers to the wild species that are the pedigree of the diploid lines.

Line No.	Wild *Solanum* species	Color of flowers	Resistant to:	Susceptible to:
1 A	tbr, chc	White	Y, X, E, Sy	M, S, L, Pi, EF
2 B	tbr, chc, phu	White	Y, X, L, E, Sy, N	M, PVS, EF
3 C	tbr, chc, grl, yun	Purple	Y, X, Sy, N, E	M, S, L, Pi, EF
4 D	tbr, grl, ver	-	Y, M, X, S, L, Sy, N	Pi
5 E	tbr, chc, yun	White	Y, X, Sy	M, S, L, Pi, E, EF
6 E	tbr, chc, yun	White	Y, X, EF, Sy	M, S, L, E,
7 G	tbr, chc, yun	White	Y, X, E, Sy	M, S, Pi, N
8 H	tbr, chc, yun	White	Y, X, Pi, E, Sy	M, S, N
9 J	tbr, chc, grl	White	Y, M, X, E, Sy	S, L, Pi, N
10 J	tbr, chc, phu, yun	White	Y, X, S, Sy	L, E, EF
11 K	tbr, chc, grl, mcd, phu, ver, yun	White	Y, Pi	X, M, L, N, E, EF, Sy
12 L	tbr, chc, grl, phu, yun	White	Y, M, S, L, Sy, E, EF	N
13 M	tbr, acl, chc, grl, sto, yun	White	Y, X	M, S, L, Sy, E, EF
14 N	tbr, chc, grl, phu, sto, yun	White	Y, X, L, N, E	M, Sy, EF
15 O	tbr, acl, chc, grl, mcd, sto, ver, yun	White	Y, M, L, X, Sy, E	S, Pi
16 P	tbr, acl, chc, grl, mcd, ver, yun	White	X, M, Sy	S

Y, M, X, S, L – potato viruses Y, M, X, S, potato leaf roll virus, respectively.
acl – *S. asaule*; chc – *S. chacoense*; E - *Erwinia* spp.; EF – *Erwinia* spp. + *Fusarium* sp.; grl – *S. gourlayi*; mcd – *S. microdontum*; N – nematodes; phu – *S. phureja*; Pi – *Phytophthora infestans*; sto – *S. stoloniferum*; Sy – *Synchytrium endobioticum*; tbr – *Solanum tuberosum* (dihaploids *S. tuberosum*), ver – *S. verrucosum*; yun – *S. yungasense*

wild *Solanum* species present in their pedigree. Protoplast donor plants and plants regenerated from protoplasts were maintained as aseptic shoots cultured on 1/2 MS [13] at 22ºC with a 16-h photoperiod under fluorescent daylight illumination (100 $\mu E \cdot m^{-2} \cdot s^{-1}$). Bleached, chlorophyll-free plants were obtained on MS medium [13] supplemented with 2 mg/l (0.007 mM) norflurazon SAN9789. Norflurazon is a herbicide inhibiting carotenoid synthesis, which leads to photooxidation of chlorophyll and bleaching of the leaves. The process is reversible. Transfer of bleached shoots onto norflurazon-free medium leads to the growth of green tissues.

Protoplast isolation

Protoplasts were isolated from the mesophyll of young leaves that were pre-treated according to Haberlach *et al.* [14]. Excised leaves were placed in floatation medium ($CaCl_2$ and NH_4NO_3 1 mM each, NAA 10.7 μM, NAA 4.4 μM) at 20ºC in the dark. After 48 hours, the medium was replaced by a preconditioning solution: MS salts and vitamins [13] 1/10 strength. Leaves were cut into small fragments, incubated for 24 hours at 4ºC, and then incubated in enzyme solution. Alternatively, the leaves were collected from plants and kept in the dark for 24 hours prior to protoplast isolation. Then, they were placed in a preplasmolysis solution (3 hours, 1/1000 strength of K3 macroelements [15], mannitol 0.45 M) and cut into small fragments, or had their lower epidermis injured with a brush. Three enzyme mixtures (10 ml per 0.5 g of leaf tissue) were tested: EZ-1 (Cellulisin 0.5%, Macerase 0.1%), EZ-2 (Cellulisin 1%, Macerase 0.1%) or EZ-3 (Cellulase 1%, Pectolyase Y-23 0.1%) prepared in K3 medium [15].

After a 16-h incubation in the dark at 22ºC with gentle mixing, a crude suspension of macerated leaf tissues was mixed (1:1 vol/vol) with 0.4, 0.44 or 0.47 M sucrose dissolved in a 1/10 CPW salt solution [16], filtered through a 100-μm mesh and centrifuged for 25 min at 62×g. Fluorescein diacetate (FDA) was added to enzyme mixed with crude protoplasts from chlorophyll-free leaves, i.e. leaves collected from plants grown on norflurazon-containing medium. FDA (final concentration 0.4 mM) was added 15-20 min prior to mixing with the sucrose/CPW solution. Floating protoplasts were collected, mixed with an equal volume of W5 solution [17] and centrifuged for 6 min at 84×g. The pellet was resuspended in W5 solution.

Protoplast fusion, selection and culture of heterokaryons

Protoplast fusion was induced with polyethylene glycol (PEG) MW 6000 via a modified procedure based on Glimelius *et al.* [18]. PEG-treated protoplasts were covered with 2 ml of SKM/A medium [19, 20]. Heterokaryons, i.e. protoplasts with double fluorescence (red from chlorophyll and yellow/green from FDA), were selected with a glass capillary from SKM/A medium and placed with this medium directly on the bottom membrane of a Millicells (Millicell-CM, 0.4 μm Culture Plate Insert, 12 mm Diameter). The Millicells were put in a 10-cm plate with post-fusion protoplast mixture. The Millicell

bottom membrane permitted diffusion between the medium inside the Millicell (with heterokaryons) and the outside nurse medium. After 48 hours, the medium with protoplasts suspended in Millicell was solidified by mixing it with an equal volume of 0.75% Sea Plaque agarose. Protoplasts were cultured at 26ºC in the dark. After 7 days, they were illuminated: light intensity 80 $\mu E \cdot m^{-2} \cdot s^{-1}$, 16-h photoperiod. At this point of the culture, dividing cells were counted. Protoplast-derived colonies were transferred onto C medium [14]. The number of minicalli (counted after two weeks) was expressed as the percentage of the selected putative heterokaryon cells forming minicalli. Calli 2-3 mm in diameter were transferred onto regeneration medium D and calli regenerating shoots were transferred onto elongation medium E (respectively, MSR 1 and 2 of Szczerbakowa *et al.* [21] modified by the replacement of *trans*-Zeatin with Zeatin riboside), and cultured in the same conditions as for the axenic shoot culture. Excised regenerated shoots (1-2 cm long) were rooted on MS [13] hormone-free medium and cultured as above. Stem cuttings were rooted and stored *in vitro* or transferred to soil for the phenotype assessment.

Chromosome number

The number of chromosomes was established as described by Schreiter [22]. Roots were collected from *in vitro* grown plants, treated with an aqueous solution of 8-hydroxyquinoline (0.029%) for 6 h at 18ºC, rinsed with tap water, and fixed in ethanol-acetic acid (3:1, v/v) for at least 4 h. Root tips of about 1-2 mm were excised from the fixed material and were placed on microscope slides, squashed in a drop of 1.5% acetocarmine and covered. The chromosomes were counted under immersion and magnification 1200x.

Molecular analysis of the regenerated plants

Total genomic DNA was extracted from fresh leaf tissue according to the modified CTAB method of Murry and Thompson [23]. The PCR amplification conditions and the nucleotide sequences of random 10-mers (RAPD) and 12- and 15-nucleotide semi-random primers were described in detail by Przetakiewicz *et al.* [24]. The types of mitochondria were identified according to Lössl *et al.* (1999). Total DNA, digested with *Eco*RI, *Bam*HI or *Hin*dIII, separated on 0.8% agarose, and blotted to nylon membranes (Nytran 0.45 μm), was hybridised with DIG-labelled mitochondrial probes. m79, m80, m93 and m112 were the clones of potato mitochondrial DNA used as templates for probe labelling [9]. Chloroplast types were identified based on the PCR fragment length amplified with ALC1 and ALC3 (cpDNA-specific primers) [9, 25].

Phenotypic assessment

We recorded the plant height, stem colour, leaf length, colour, number and shape, flower colour, and tuber formation of mature diploid and somatic hybrid plants soil-grown in greenhouse conditions.

RESULTS

Protoplast fusion, culture and regeneration of heterokaryons and chromosome number

The conditions for plant in vitro culture supported the growth of all the diploid lines, though only 6 out of the 16 tested lines grew on a medium with norflurazon. Only plants kept in the dark for 24 h yielded viable protoplasts. Leaf pre-culture as per Haberlach *et al.* [14] was not effective. Of the three tested enzyme mixtures, viable protoplasts were obtained with EZ1 supplemented with 15% sucrose. The protoplast yield ranged from 0.6 to 1.8×10^6 per 1 g of leaf tissue.

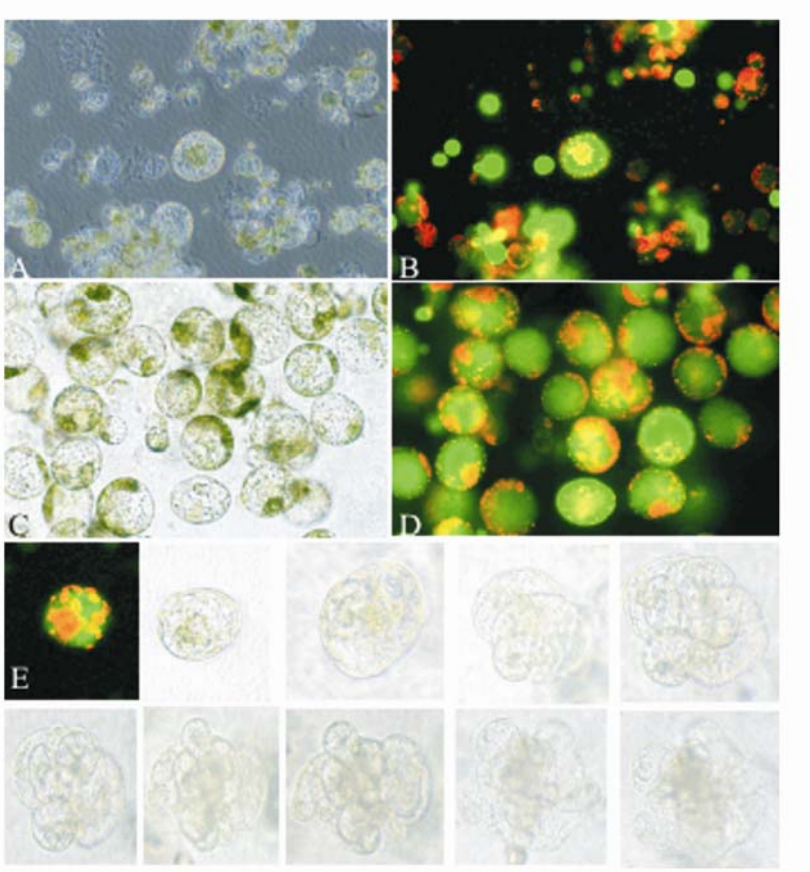

Fig. 1. Protoplast fusion and culture of selected heterokaryons. PEG fusion products under white (A) and blue light (λ 490 nm) (B). Selected heterokaryons in Millicel-CM under white (C) and blue light (D). Cell wall regeneration and subsequent divisions of selected heterokaryon (E).

Thirty eight fusion experiments were done using different combinations of the 16 diploid lines. In the post-fusion mixture, the percentage of protoplasts with double fluorescence, indicating putative hybrids (Fig. 1A and B) ranged from

0.8 to 21% (average 6%). Over 7560 putative heterokaryons were manually selected (Fig. 1C-1E) and cultured on SKM/A.The plating efficiency estimated after two weeks' culture in Millicells ranged from 1 to 50%. In total, 1071 minicalli were obtained from 7560 selected heterokaryons. Further culture on C medium supported the growth of all except 4 combinations: 6F(+)5E, 6F(+)3C, 9I(+)5E and 9I(+)3C. After a subsequent 2-3 weeks, bright green callus was formed. Of 412 calli cultured on the regeneration medium, 25 calli derived from combinations 2B(+)3C, 7G(+)3C, 4D(+)3C, 4D(+)5E, 4D(+)2B, 3C(+)2B, 3C(+)5E, 13M(+)15O, 13M(+)16P and 14N(+)16P were able to regenerate 479 shoots. Regenerated plants identified as somatic hybrids had 48 chromosomes, while the remainder (those not confirmed with RAPD or semirandom primers as somatic hybrids) had 24 or, in one case, 60 chromosomes (Tab. 2, Fig. 2).

Tab. 2. Molecular and cytogenetic analysis of the putative somatic hybrids.

Putative somatic hybrids	Differentiating primers used to verify hybridity		Inheritance of the polymorphic fragments from the component lines	Chromo-some No.	No. of calli/ No. of regenerated plants
	RAPD	Semi-random			
2B(+)3C	#9580	ET 33/15	3C	24	4 / 38
3C(+)2B	#9580	ET 33/15	3C or 2B	24	1 / 9
3C (+)5E	#9448	ET 36/15	3C	24	2 / 18
7G(+)3C	#9432	ET 30/12	3C	60	2 / 60
4D(+)3C	#9442	ET 28/12	both components	48	6 / 20
4D(+)5E	#9448	ET 34/15	both components	48	2 / 40
4D(+)2B	#9448	ET 29/12	both components	48	1 / 11
13M(+)15O	#9622	ET 26/12	both components	48	1 / 25
13M(+)16P	#9624	ET 28/12	both components	48	5 /133
14N(+)16P	#9624	ET 28/12 ET 30/12	both components	48	1 / 25

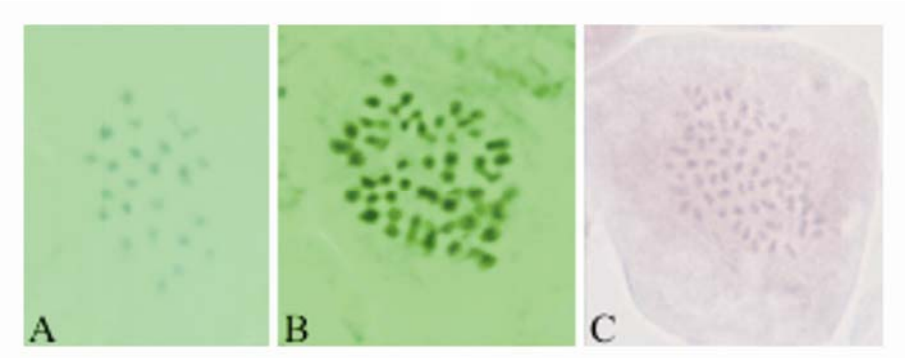

Fig. 2. Chromosome plates in the root tip cells of regenerated plants with 24 (A), 48 (B) and 60 (C) chromosomes.

Molecular analysis

The polymorphism in the diploid lines was revealed by at least 13 RAPDs and 12 semi-random primers (data not shown). The RAPD and semi-random primers used to verify the putative somatic hybrids are shown in Fig. 3A and 3B. 395 plants from 6 fusion combinations showed an amplification pattern of polymorphic fragments inherited from both fusion components, while 98 plants gave the remaining 4 combinations with an amplification pattern from only one component.

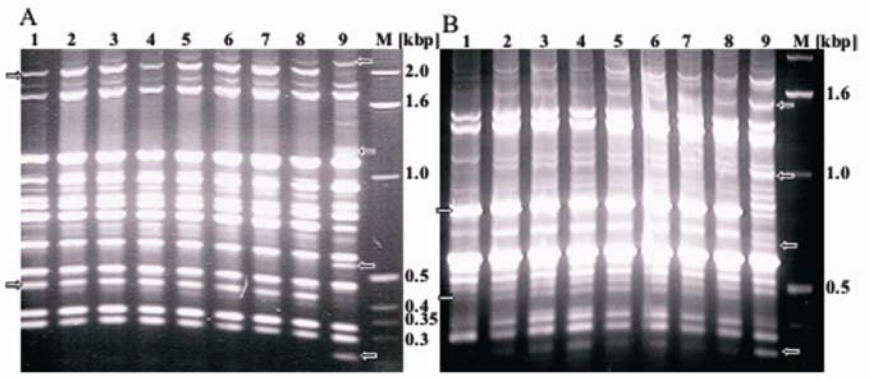

Fig. 3. Amplification patterns with semi-random primers ET 28/12 (A), ET 30/12 (B). Lane 1 – diploid 14N, lane 9 – diploid 16P, lanes 2-7 – putative hybrids. Unique bands from donor line 14N (white arrowheads with black outline) and line 16P (black arrowheads with white outline) are highlighted.

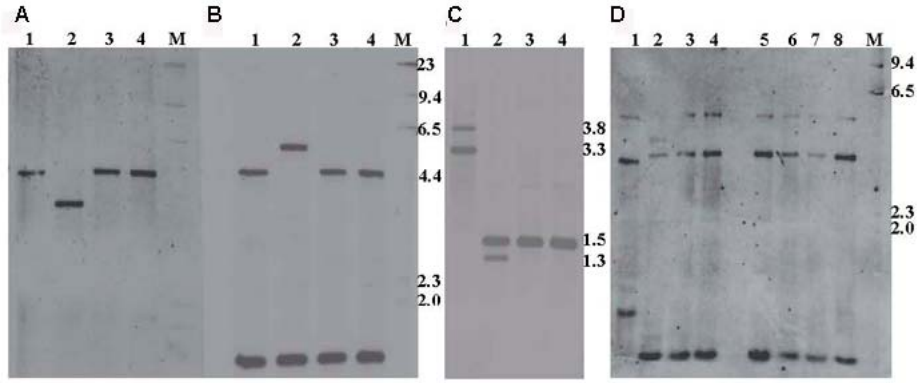

Fig. 4. RFLP of mtDNA from diploid lines hybridized with mitochondrial probes. DNA digested with *Eco*RI and probed with m79 (A), m93 (B) and m112 (D). DNA digested with *Hin*dIII and probed with m112 (C). Lanes: 1-2B, 2-3C, 3-4D, 4-5E, 5-13M, 6-14N, 7-15O, 8-16P.

Mitochondrial probes revealed different patterns of RFLP (Fig. 4A-4D), corresponding to those described by Lössl *et al.* [9] for mitochondrial classification. 2B, 4D and 5E hybridized with m79, m80, m93 and m112 with the same pattern, while the pattern was different to that for line 3C. Hybridisation of 2B, 3C, 4D, 5E, 13M, 14N, 15O and 16P with m112 diversified the mitochondria into two types: β or ε (Fig. 4C, 4D). Line 2B carried ε mitochondria, line 3C α mitochondria and all the remaining lines and somatic hybrids mitochondria β (data not shown).
The length of the PCR-amplified cpDNA fragment identified the chloroplast type in the diploid lines and somatic hybrids. The presence of a 380-bp fragment indicated type T (*S. tuberosum*) and a 622-bp fragment type W/S, present in wild *Solanum* [25]. Lines 2B and 3C had W/S chloroplasts. Lines 4D, 5E, 13M, 14N, 15O, 16P and all the somatic hybrids had type T. Tab. 3 shows the chloroplast and mitochondria types in the diploid lines and their somatic hybrids.

Tab. 3. Chloroplast and mitochondrion type in the diploid lines and somatic hybrids.

Diploid line A			Diploid line B			Somatic hybrid A (+) B			
	mt	cp		mt	cp		mt	cp	Number of plants regenerated / analyzed
2B	ε	W/S	4D	β	T	2B (+) 4D	β	T	11 / 9
3C	α	W/S				3C (+) 4D	β	T	120 / 23
5E	β	T				5E (+) 4D	β	T	40 / 25
15O	β	T	13M	β	T	15O (+) 13M	β	T	20 / 12
16P	β	T				16P (+) 13M	β	T	120 / 17
16P	β	T	14N	β	T	16P (+) 14N	β	T	84 / 12

Plant establishment and phenotypic assessment
Tab. 4 and Fig. 5 show selected phenotypic traits. The hybrid plants grown in soil in greenhouse conditions were of similar height to the parental forms. Only 4D(+)3C/1 and 4D(+)2B/1 were shorter than the original diploid component lines (Fig. 5). Leaf length was the most altered trait in the hybrids. Six hybrids had shorter leaves and fewer leaflets. The remainder had longer leaves than the diploid lines. In general, the leaf lengths and the leaflet number were relatively reduced. Hybrid plants 4D(+)3C/1 only had one leaflet. Hybrids with fewer irregular leaflets had shorter leaves. Plants 4D(+)5E/1 and /2, and 13M(+)16P/1 and /2 had an intermediate leaf shape. 13M(+)16P/3 and 4N(+)16P/1 had leaves similar to those of the parental diploid lines. The leaves were mostly dark green or green, but those of 13M(+)15O/1 were light green.
Diploid 4D plants formed blue flowers only after exogenous application of GA_3. The remaining components developed white flowers. Hybrids 4D(+)3C/1 and /2 were not able to develop flowers, 4D(+)3C/7 formed flower buds that did not develop further, and 4D(+)5E/1 and /2 developed complete flowers. Hybrids derived from blue and white flowering lines formed light blue flowers (Fig. 5).

Tab. 4. Selected morphological traits of the diploid lines and somatic hybrids.

Tested lines and hybrids	Height [cm]	Stem colour	Leaf colour & length [mm]	Leaflet shape & number	Flower colour & number	Tuber formation
4D	20	green-grey	green, 15	elongated, 9	blue *	+
3C	20	green-purple	light green, 12	elongated, 9	purple, 12	+
5E	32	green-purple	dark green, 9	oval, 7	white, 3	+
2B	40	green-purple	dark green, 12	interm., 9	white, 4	+
13M	40	green-grey	light green, 15	elongated, 9	white, 8	+
14N	40	green-grey	green, 18	elongated, 8	white, 10	+
15O	42	green	light green, 18	elongated, 9	white, 8	+
16P	45	green-purple	dark green, 16	elongated, 9	white, 13	+
4D(+)3C	14-22	green-grey	dark green, 4-10	irregular, 1 - 9	- , buds *	-
4D(+)5E	30	green-purple	dark green, 8	interm., 5	blue-white, 12	+
4D(+)2B	18	green-purple	dark green, 7	irregular, 3	white, 9	+
13M(+)16P	45-80	green-grey	dark green, 7-20	interm., 5 - 12	white, 9 - 13	+
13M(+)15O	70	green	light green, 18	elongated, 9	white, 14	+
14N(+)16P	100	green-purple	dark green, 20	elongated and irregular, 9	white, 16	+

*Flowered after the application of GA_3

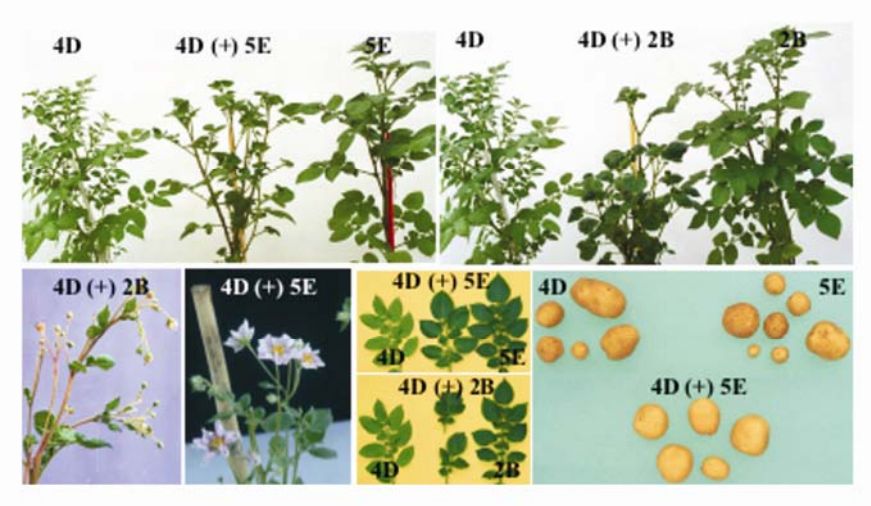

Fig. 5. Morphology of diploid and hybrid shoots, flowers, leaves and tubers.

All the hybrids except 4D(+)3C/1 and 4D(+)3C/2 developed tubers (Fig. 5). The tubers formed by *in vitro*-derived plants were misshapen, and the tubers formed by soil-grown plants were similar to those of the original lines. A higher phenotypic diversity (malformed leaves and tubers, lower plant height, a lack of flowers) was observed in hybrids derived from the lines carrying different types of mitochondria: 4D(+)2B and 4D(+)3C. Those obtained from lines with the same type of mitochondria (β) grew taller, had regular leaf shape, and also formed flowers and tubers.

DISCUSSION

Half-strength MS medium for the culture of the donor plant, a 24-h dark period prior to isolation and the presence of PVP in the enzyme mixture were needed to obtain viable protoplasts. FDA did not diffuse from stained to non-stained protoplasts during co-culture, so the double fluorescence marked the results of protoplast heterofusion. Fluorescence lasted for about 4 days, so selection was possible during this period. Waara *et al.* [26], Puite *et al.* [27] and Mattheij and Puite [5] used FDA similarly. Only the SKM/A culture medium supported divisions of diploid potato protoplasts. Culturing the selected heterokaryons in Millicells with post-fusion protoplast mixture was the only effective way to induce sustained heterokaryon growth, yielding over a thousand putative hybrid calli (plating efficiency c. 14%). Culture on C medium, based on Cul medium by Haberlach *et al.* [14], supported hybrid growth from most of the tested combinations. Properly developing calli from 10 fusion combinations regenerated shoots when transferred onto medium D, based on SA4 by Austin *et al.* [28]. Putative somatic hybrids were regenerated even after the fusion of 4D, 13M, 15O and 16P, which were never able to regenerate shoots themselves. The regeneration efficiency of heterokaryon-derived calli ranged from 6 to 100%. Similar results were obtained by Szczerbakowa *et al.* [29], Rokka *et al.* [30] and Helgeson *et al.* [31].

Putative hybrids were verified with two PCR-based systems using RAPD and semi-random primers. Here, 395 plants obtained from 6 out of 10 tested combinations generated unique, dominant amplification products inherited from both parents. The remaining 84 plants regenerated from 4 combinations that showed polymorphic fragments from only one parent. We also used a PCR-based system with semi-random primers targeting intron-exon splice junctions, first proposed by Weining and Langride [32] and already used by Przetakiewicz *et al.* [24] to verify somatic hybrids. Analysis of the putative hybrids with the two methods showed that semi-random primers revealed a higher level of polymorphism than RAPD did, as found previously [24]. Besides the verification of hybridity, this method is also useful for detecting novel fragments absent in the parental lines. In one case, verification of hybridity required amplification with two semi-random primers.

Analysis of the diploid lines used showed that they had either α, β or ε mitochondria. It is interesting that type γ, though relatively frequent in cultivars

[9], was not detected among these lines. Type α, detected in *S. acaule*, *S. stoloniferum* and *S. gourlayi* [9], was found in line 3C; β, present in *S. tuberosum* and *S. berthaulthii* [9], was found in 4D, 5E, 13M, 14N, 15O and 16P; and ε, originally detected in several wild species and *S. phureja* [9], was found in 2B. All these lines had the corresponding wild species in their pedigree. Somatic hybridisation between lines carrying different types were of three different combinations: β(+)β, β(+)α and β(+)ε. All the regenerated hybrid plants had type β, so α and ε were eliminated during subsequent cell divisions in a process of non-random segregation.

Rearrangements of mtDNA, reported for many somatic hybrids, were detected in potato by Lössl *et al.* [9] but only with the use of the m100 probe. The hybridisation pattern obtained with all other probes did not show any changes. In our study, we did not use the m100 probe, and the results obtained with the m79, m80, m93 and m112 probes consistently indicated only one specific type of mtDNA, which was compatible with the results of Lössl *et al.* [9]. We did not detect any mixed patterns of parental types of mtDNA in the analysed hybrids. All the plants had mitochondria fitting to one of the parental mtDNA patterns.

According to the Hosaka and Hanneman chloroplast classification [33], two of our lines carried W/S chloroplasts, and the remainder had T chloroplasts. W/S chloroplasts were present in the diploid lines together with α or ε mitochondria and T chloroplasts were always with β mitochondria. In two fusion combinations where W/Sα or W/Sε cytoplasm was combined with Tβ, the resultant hybrids had Tβ cytoplasm. We did not obtain any hybrids with W/S chloroplasts. Lössl *et al.* [9] found a similar pattern, strongly skewed towards type T.

Fusion combinations 4D(+)2B and 4D(+)3C showed the highest phenotypic diversity (leaf and flower morphology). Plants regenerated from combination 4D(+)5E were very similar to diploid line 2B, with flower colour intermediate to the parental lines. The intermediate colour of somatic hybrid flowers was reported on by Rokka *et al.* [30]. Several somatic hybrids described here did not develop flowers or produce tubers. Hybrids that failed to flower or form tubers were described by Austin *et al.* [34] and Rokka *et al.* [30]. Both traits are important if the hybrids are to be useful to plant breeders. Analysed and confirmed tetraploid hybrids are being subjected to detailed field analysis of selected traits to assess their utility in breeding programmes.

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