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Molecular, cytogenetic and morphological characterization of somatic hybrids of dihaploid *Solanum tuberosum* and diploid *S. brevidens*

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Summary. Fifty-eight somatic hybrid plants, produced both by chemical (11) and electrical fusion (47) of protoplasts of dihaploid *Solanum tuberosum* and *S. brevidens*, have been analysed by molecular, cytological and morphological methods. The potentially useful euploid plants constituted 34% of the total, of which 20% were tetraploid and 14% hexaploid; the remainder were aneuploid at the tetraploid, hexaploid and octoploid levels. Analysis of chloroplast DNA showed that 55% of hybrids contained chloroplasts from *S. brevidens* and 45% from *S. tuberosum*. Hexaploids, the products of three protoplasts fusing together, were analyzed with specific DNA probes, and this revealed that nuclear genome dosages could be either 2:1 *S. tuberosum*:*S. brevidens*, or vice-versa. Chloroplast types of hexaploids were not influenced by nuclear genome dosage, and all six possible combinations of genome dosage and chloroplast types were found amongst tetraploids and hexaploids. To examine the morphology of the hybrid population and its possible relation to the chromosome number and chloroplast DNA type, 18 morphological characteristics were measured on greenhouse-grown plants and analyzed by principal component and canonical variate analyses. Both analyses showed that nuclear ploidy has the most prominent influence on the overall morphology of the hybrids. Differential parental genome expression in the morphology of the hybrids is discussed. These results provide useful data on the range of genetic combinations that can be expected to occur amongst somatic hybrid plants.

Key words: Potato – *Solanum brevidens* – Somatic hybrids – Characterization (molecular, cytological, morphological)

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Introduction

The production of hybrids between parents with favourable characteristics is an essential feature of plant breeding. Recently, advances in plant tissue culture, including the development of protoplast regeneration systems in some major crops, have broadened opportunities for hybrid production. Using chemical or electrical procedures (Jones 1988), protoplasts from different donor plants can be fused together and somatic hybrids regenerated from the fusion products. Although one major attraction of this approach is that it bypasses sexual reproduction and, therefore, avoids crossing barriers, other characteristics render it useful for both compatible and incompatible species. In protoplast fusion, both nucleus and cytoplasm are combined. Moreover, since plastids from both parents are at least present initially in the same heterokaryon, opportunities are created for recombination between mitochondrial or chloroplast genomes. In addition, further genetic changes may result from somaclonal variation. As a result, protoplast fusion can lead to the production of a wide range of hybrid types, with respect to their nuclear and cytoplasmic constitutions. The applicability of this approach to the production of useful hybrids between incompatible species (Austin et al. 1985; Pental et al. 1986; Fish et al. 1987), as well as the reconstitution of hybrids that can be sexually produced (Sundberg et al. 1987; Sundberg and Glimelius 1986; Rosen et al. 1988), is already being explored.

To understand fully the potential of fusion in the production of novel hybrids, a sufficient population of fusion products is required to cover all the possible combinations of nuclear and cytoplasmic components that may occur. Over 60 different somatic hybrid combinations have already been reported (see Gleba and Sytnik 1984, for review), most of which are in the family *Solanaceae* (Schumann and Koblitz 1985). The number

characterized, however, has generally been limited and the analyses restricted in the number of techniques employed. The general morphology of somatic hybrids can vary (Austin et al. 1986; Handley et al. 1986; Fish et al. 1987, 1988 b) as can the chromosome number (Chupeau et al. 1978; Fish et al. 1987, 1988 a, b; Sundberg et al. 1987). In addition, assortment of chloroplasts clearly occurs, since normally each hybrid has been found to contain only one parental type, although both types may be present in the hybrid population (Barsby et al. 1984; Gressel et al. 1984; Gleddie et al. 1986; Sundberg et al. 1987). In contrast, examination of the mitochondria has shown that although both parental types can be present, recombination can occur (Belliard et al. 1979; Nagy et al. 1981; Ozias-Akins et al. 1987).

To date, detailed characterization in which analyses at the phenotypic, nuclear and cytoplasmic levels are compiled of a large number of hybrids has not been carried out. In our research programme on somatic hybridization within the *Solanum* species, such a study has been undertaken of hybrids between *Solanum brevidens* and dihaploid potato (*S. tuberosum*). *S. brevidens* is a wild diploid relative of potato that cannot be crossed directly with *S. tuberosum*. It is not tuber-bearing, has strong anthocyanin pigmentation and distinctive morphology and also carries resistance to two viruses – potato virus Y (Gibson et al. 1988) and potato leaf roll virus (Jones 1979). It has, therefore, been a popular candidate for protoplast fusion experiments (Austin et al. 1985; Barsby et al. 1984; Fish et al. 1987, 1988 a, b). Earlier studies have shown that somatic hybrids between *S. brevidens* and *S. tuberosum* express virus resistance from *S. brevidens*, but the level of resistance can vary in different genotypes from that of *S. brevidens* to that of the susceptible *S. tuberosum* parent (Austin et al. 1985; Gibson et al. 1988). In this study we present part of a detailed characterization of 58 somatic hybrids, 11 of which were produced from chemical fusion (Fish et al. 1987) and 47 from electrofusion (Fish et al. 1988 a). Each hybrid has been assessed on the basis of a range of morphological characters, of chromosome number and of chloroplast type. Furthermore, species-specific restriction fragment length polymorphisms (RFLPs) have been used to assess the parental genome dosage in hexaploid hybrids. The relationships between chromosome number, chloroplast constitution and morphological characters, including tuberization and pigmentation, have been examined. Further data on the mitochondrial genomes and on virus resistance of the 58 hybrids will be presented in later publications.

Materials and methods

Plant material

The 58 somatic fusion products of dihaploid ($2n = 2x = 24$) *S. tuberosum* (PDH 40) and diploid *S. brevidens* were produced by chemical and electrical fusion experiments (Fish et al. 1987, 1988 a) in which all the products following fusion were cultured, and the hybrids were initially selected on the basis of the intermediate morphology of regenerated shoots and on isozyme patterns. Confirmation of hybridity was then obtained for 21 of the plants by DNA hybridization (Fish et al. 1987, 1988 a). All 58 plants have been maintained as shoot cultures on MS 20 medium (with 2% sucrose) or on RBTM medium (MS 20 medium containing 0.05 mg/l NAA).

Analysis of the nuclear DNA

DNA was extracted from 2 g fresh weight of frozen leaf tissue from glasshouse-grown plants using a modification of the method of Dellaporta et al. (1983) that involved a 2-h RNAse digestion between the two precipitation steps. The total DNA (7–10 µg) was restricted by HindIII (BRL) according to supplier's instructions and separated by agarose gel (0.8%) electrophoresis in the TRIS-Borate EDTA (TBE, Maniatis et al. 1982). Transfer to Biodyne A membranes was accomplished by electroblotting in a 'Transblot Cell' (Biorad) in 25 m*M* sodium phosphate, pH 6.5, for 3.5 h at 200 mA followed by 1.5 h at a current of 1 A. DNA was then bound to the membrane by baking at 80 °C for 2 h. The 5' HindIII-BamHI fragment of pGM01, a cDNA clone of patatin (Mignery et al. 1984), and a random cDNA clone (SS12) (isolated by R. Potter, Rothamsted) from a library in pUC 9 were used to probe the filters. The probe was labelled to high specific activity by oligolabelling (Feinberg and Vogelstein 1983) with ^{32}P-ATP (Amersham International). Filters were hybridized according to Fish et al. (1988 a) or following the method of Church and Gilbert (1984). Final washes were carried out at 60 °C in 0.5 × SSC and the filter was wrapped in cling film. The filters were exposed to Fuji X-ray film at –80 °C with two intensifying screens for 1–7 days. The relative intensities of the parental bands on the autoradiograms of the euploid-hexaploid hybrids were quantified by densitometry (Ultroscan XL Enhanced Laser Densitometer, LKB) and used to determine the parental nuclear dosage.

RFLP analysis of chloroplast DNA

Chloroplast DNA was extracted from glasshouse-grown leaf material by a modification of the method described by Hosaka (1986). The main advantage of the modification was that 2–4 g less tissue was required for the analysis. Two to four grams of fresh leaf material was homogenized by three 3-S pulses (high speed) with a Waring blender (cup size 37 ml) in the presence of 15–30 ml of extraction buffer. The homogenate was filtered through four layers of cheesecloth and two layers of miracloth (Calbiochem) and centrifuged at 1000 × g for 10 min at 4 °C. The pellet was suspended gently in 20 ml of 50% sucrose solution with the aid of a soft paintbrush and centrifuged at 2500 × g for 20 min at 4 °C. The pellet was suspended in 1 ml of 50 m*M* TRIS 20 m*M* EDTA and lysed by addition of 200 µl of 10% sodium lauryl sarcisonate solution. The lysate was transferred to two Eppendorf tubes and in each extracted with 0.5 ml phenol/tube followed by extraction with 0.5 ml of chloroform. The DNA was precipitated by addition of 75 µl of Na-acetate and 0.7 ml of isopropanol. After the DNA pellet was washed with 70% ethanol, it was resuspended in 40 µl of TE.

Chloroplast DNA was restricted by BamHI (BRL) according to supplier's instructions. The DNA fragments were separated by agarose gel (0.8%) electrophoresis in TBE buffer (Maniatis et al. 1982), visualized by staining with ethidium bromide and photographed on a UV transilluminator.

Chromosome analysis

Chromosomes were counted from rooted shoot cultures as described by Karp et al. (1982).

Morphological analysis

The fusion parents and 56 somatic hybrids were established in the greenhouse from in vitro-grown shoots. Three separate sets, containing two replicates of each hybrid, were established at 2-week intervals. The plants were grown in S-compost (S-Products), in 20-cm pots, under 16-h day length maintained by supplemental lights of 150 μEin/m intensity. The minimum day and night temperatures were maintained at 18° and 13 °C, respectively. At the vegetative stage (plant height ranging from 20–30 cm), 18 morphological characteristics were measured to characterize the following: canopy and leaf structure, growth habit, foliage colour and glossiness, and anthocyanin pigmentation. The scoring was based on NIAB guidelines for identifying potato varieties (NIAB 1975). At flowering, anthocyanin pigmentation, vigour and plant height assessments were repeated. In addition, data on the number of days to budding and flowering as well as inflorescence and flower morphology were recorded. Pollen viability was determined by staining with 1% acetocarmine. The hybrids were divided into three maturity classes and harvested at senescence (70% yellowing of the leaves), and the presence of tubers/swollen stolons was recorded. Where tubers were produced, tuber yield, shape and average tuber weight were measured.

All data analyses were done using the Genstat 5 Program (Genstat 5 Committee 1987). The 18 characteristics recorded at the vegetative stage of all three data sets were subjected to principal component analysis (PCA) of the covariance matrix, to examine the 'spontaneous' grouping and variation of grouping between the sets. The three sets were then analyzed by canonical variate analyis (CVA). This analysis showed that the within-set variation was greater than the between-set variation and, therefore, we selected the earliest established set, which was the most complete, for further analysis. Using the previously described chromosome number classes (euploid tetraploids, euploid hexaploids and aneuploids at the tetraploid, hexaploid and octoploid levels) to define five classes, the data were analyzed by CVA to find the character combination which would minimize the variation within the chromosome number classes and maximize it between the classes. The canonic variable loadings were examined to determine the relative weights of the morphological characteristics to find the character combination which would have the greatest between-group variation relative to their within-group variability.

As only one-third of the hybrids flowered and produced tubers, the data on floral and tuber characteristics were not analyzed statistically, but correlations with the chromosome number, cpDNA type and parental nuclear genome dosage were examined.

Results

Molecular analysis

Hybridization of the patatin sequence probe, pGM01, to total DNA from hybrids showed that 50 plants exhibited all the patatin bands present in both nuclear genomes, 6 had varying numbers of bands from the *S. brevidens* in addition to *S. tuberosum* bands and 2 contained bands apparently only of *S. brevidens* DNA (Fig. 1). As all the latter 8 plants were aneuploid, and thus could have lost the chromosome(s) carrying the patatin loci, another probe was used to assess their nuclear DNA type. Probing with SS12, a random cDNA clone, produced clear hybrid RFLP patterns for 7 plants, whilst the bands in the 8th were almost entirely of the *S. brevidens* type (Fig. 2).

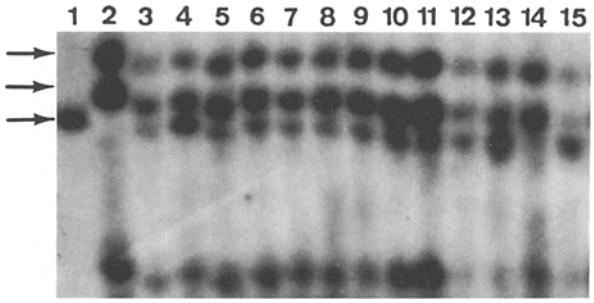

Fig. 1. Verification of nuclear hybridity and parental genome dosage in the somatic hybrids by hybridization of a patatin probe to extracted, restricted DNA by Southern blotting. *Lane 1: S. brevidens, lane 2: S. tuberosum, lanes 3–15:* putative somatic hybrids. *lanes 5–9:* hexaploids with two doses of *S. tuberosum* genome, *lane 13:* hexaploid with two doses of *S. brevidens* genome

Chloroplast analysis

Chloroplast DNA (cpDNA) types of the parental species could be readily distinguished by the RFLP patterns produced by BamHI restriction analyis (Fig. 3). Of the 58 hybrids analyzed, 55% had *S. brevidens* cpDNA and 45% *S. tuberosum* cpDNA. No mixed or recombinant cpDNA types were identified. The hybrid plants were obtained from four separate experiments, two of which involved chemical fusion and two electrical fusion. The cpDNA type frequencies in hybrids from the different fusion experiments are presented in Table 1.

A cytological study of the hybrid plants revealed that 34% were euploid, but only two-thirds of these had the tetraploid chromosome number of 48 (i.e. $2n=24+2n=24$), the other one-third being hexaploid ($2n=6x=72$). The remaining 56% of the hybrids were aneuploid at the tetra-, hexa-, and octoploid levels (Fig. 4). There were considerable differences in the frequences of hybrids in the five chromosome number classes from different fusion experiments (Table 1). The genome dosage of eight hexaploid hybrids was studied further. The relative intensity of the species-specific patatin bands revealed that half of the eight had two nuclear doses of *S. tuberosum* and one *S. brevidens* genome, whilst the remaining four contained the alternative combination (nuclear genome dosage: two- *S. brevidens*, one- *S. tuberosum*) (Fig. 1).

Among the 58 hybrids analyzed, all possible nuclear cpDNA combinations at the euploid tetra- and

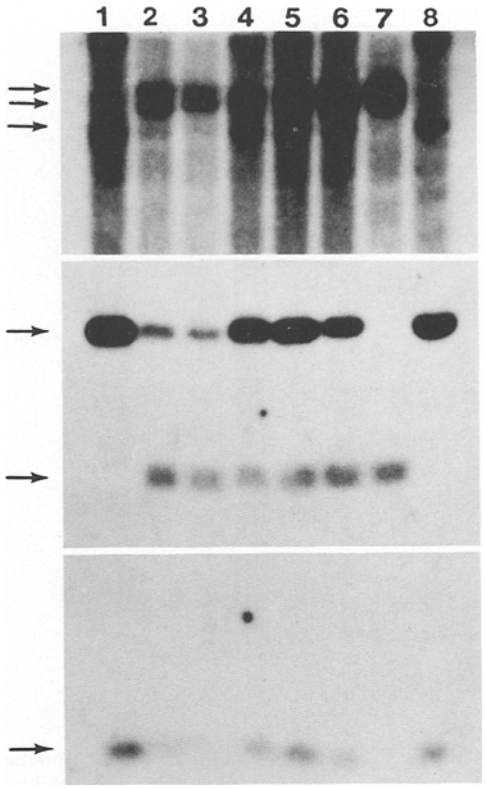

Fig. 2. Verification of nuclear dosage using the cDNA clone, SS12, as a probe for Southern analysis. *Lanes 1–6:* putative somatic hybrids, *lane 7: S. tuberosum, lane 8: S. brevidens. Lane 1:* a possible homokaryon of *S. brevidens, lanes 2–3:* hybrids showing partial genome transfer of *S. brevidens* to *S. tuberosum. Arrows* indicate bands specific to the parental species (all from one gel in which featureless segments were removed to save space)

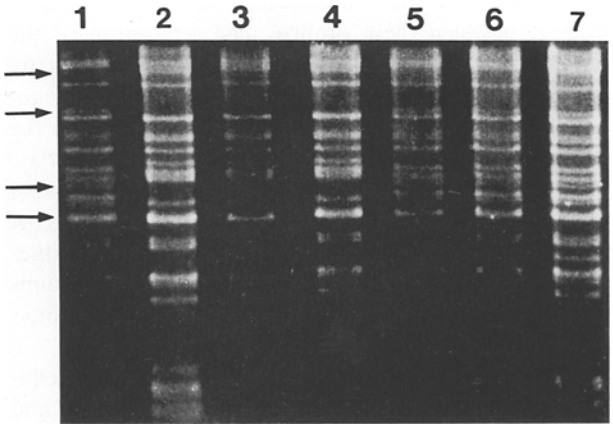

Fig. 3. Restriction patterns of cpDNA after digestion with Bam HI. *Lane 1: S. brevidens, lane 2: S. tuberosum, lanes 3–7:* somatic hybrids. *Lanes 3–4: S. tuberosum* chloroplasts, *lanes 5–7: S. brevidens* chloroplasts. *Arrows* indicate bands specific to either parental chloroplasts

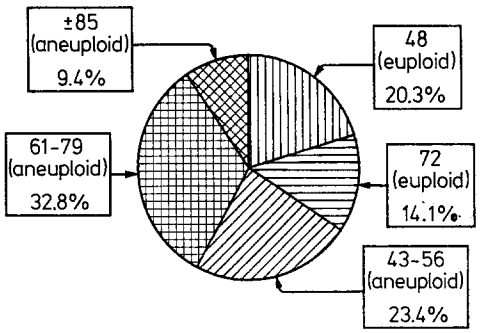

Fig. 4. Percentages of different chromosome number classes among the somatic hybrids

Table 1. Chromosome number and chloroplast DNA type of somatic hybrids from different electrofusion and chemical fusion experiments (euploids – tetraploid, 4x; hexaploid, 6x; aneuploids – tetraploid, 4x±; hexaploid, 6x±; octoploid, 8x±)

	4x	6x	4x±	6x±	8x±	*S. tub.*	*S. brev.*
Chemical fusion							
Experiment 1	2	–	2	1	1	–	5
Experiment 2	2	–	2	–	–	4	–
Electro fusion							
Experiment 1	1	7	7	10	3	12	14
Experiment 2	8	2	4	12	2	12	14
Total	13	9	15	23	7	28	35

hexaploid levels were identified and are summarized in Fig. 5.

Assessment of morphological characters indicated wide phenotypic variation amongst the somatic hybrids which exceeded those of the parental phenotypes. In order to examine the relationships of the 18 characteristics measured, they were transformed to a set of uncorrelated variables called principal components. These new variables are linear combinations of the original characteristics and are derived in decreasing order of importance, so that the first principal component accounts for most of the variation. Through comparison of the character loadings presented in Table 3, it can be determined which of the characters in the original data set contribute most to the grouping of the genotypes. When all the 18 characteristics recorded at the vegetative stage were analyzed by PCA on the covariance matrix, it became evident that plant height dominated the first principal component, having a character loading of −0.962, i.e. it contained almost all the separation power of this component. It therefore overrode the information contained in the other characteristics and was omitted from subsequent analysis. When the remaining 17 characteristics were used,

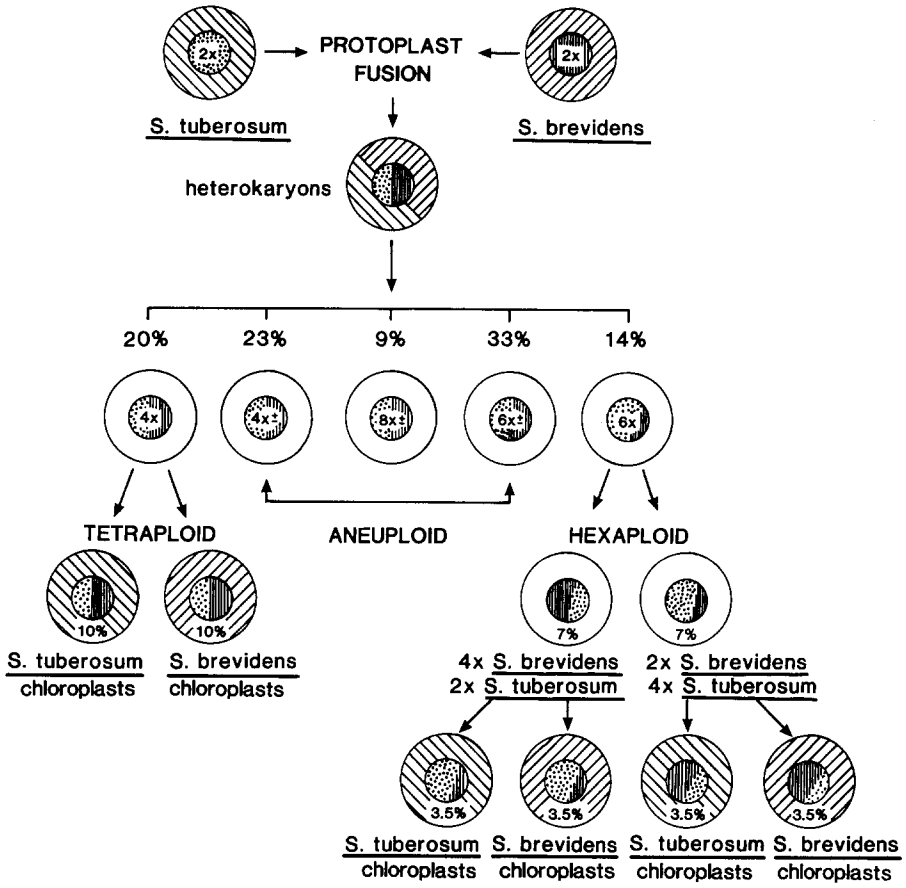

Fig. 5. Summary of the somatic hybrids regarding their ploidy, cpDNA type and parental genome dosage (for hexaploid hybrids)

only the first three principal components contributed significantly to the variation, the first four latent roots being 1476.1, 564.0, 421.4 and 274.8 and, when expressed as a percentage of the total grouping (trace) explained 36.7%, 14.0%, 10.5% and 6.8%, respectively, of the variation contributing to the grouping.

As shown in Fig. 6 (a and b), the PCA grouped hybrids that were tetraploid or aneuploid at the tetraploid level separately from hybrids that were hexaploid or aneuploid at the hexaploid level and, therefore, separated the hybrids according to their ploidy. Interestingly, the Mahalanobis distances (inter-group distances) between the aneuploid and euploid groups at both tetra- and hexaploid level were very similar (Table 4). Hybrids that were aneuploid at the octoploid level were few in number and did not group separately in this analysis. The characteristics which contributed most towards the first principal component, i.e. had the highest character loadings, were secondary leaflet number, structure of the compound leaf, lateral leaflet surface and plant habit (Table 3). The second principal component spread the genotypes of the two main ploidy levels into two groups, on both sides of the value 0 (Fig. 6 a and b). The three highest latent vector co efficients (Table 3) for this PC were characteristics of the anthocyanin pigmentation, a strong feature in *S. brevidens,* and which therefore indicates the proportionate expression of the parental genomes.

CVA minimizes the variation within preassigned groups (here chromosome number classes) and maximizes it between these groups. The separation of the chromosome number classes was accentuated (Fig. 6c and d). The first canonical variate separated the genotypes according to ploidy, as in PCA. In addition, for the aneuploid tetraploid class, the second canonical variate values were mostly greater than zero and the aneuploid octoploids were all clustered above the hexaploids on the second canonical variate. The hexaploids formed a rather heterogenous group, however, in which two subgroups were split along the CV 2. The canonical variate loadings are presented in Table 3.

The CVA procedure was also used to group the genotypes using parental cpDNA type to define classes, and the hybrids were separated on the basis of 17 morphological characteristics into the two cpDNA groups. Only two hybrids with *S. tuberosum*-type chloroplasts overlapped with the *S. brevidens* types. The loadings showed that the four characteristics which contributed most to

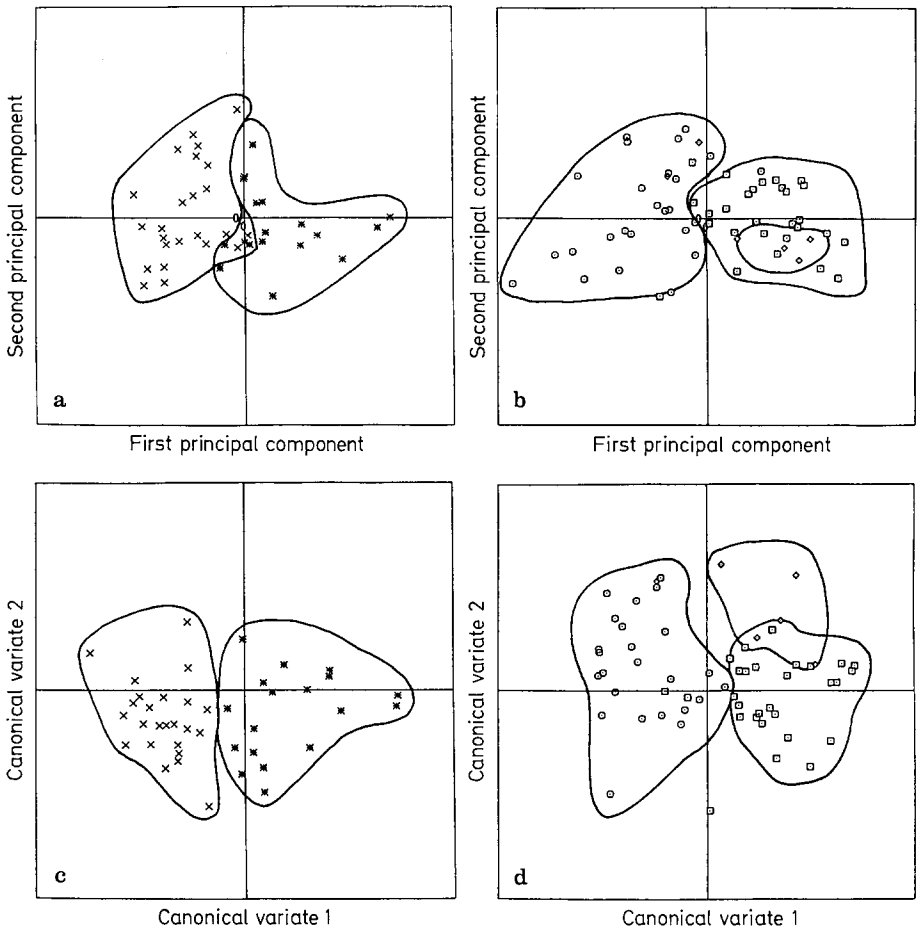

Fig. 6a–d. Principal component and canonical variate analysis of the somatic hybrids. **a** and **c** -euploid tetraploids (x) and hexaploids (*). **b** and **d** -aneuploid tetraploids (⊙) hexaploids (⊡) octoploids (◇)

Table 2. Tuberization frequency in the five chromosome number classes of the somatic hybrids differing in chloroplast DNA type (cpDNA) (*S.t.* = *Solanum tuberosum*, *S.b.* = *Solanum brevidens*)

cpDNA type	4x		6x		4x±		6x±		8x±	
	S.t.	*S.b.*	*S.t.*	*S.b.*	*S.t.*	*S.b.*	*S.t.*	*S.b.*	*S.t.*	*S.b.*
No. tuberizing	3	2	3	2	1	3	6	4	1	2
No. non-tuberizing	4	4	1	3	3	6	3	6	2	0
Total tuberizing	5		5		4		12		3	
Total non-tuberizing	8		4		11		11		3	

this grouping were terminal leaflet shape, anthocyanin pigmentation at leaflet axil, number of stems and glossiness of the leaves (Table 3).

In total, 18 hybrids produced flowers. Of these, 11 were euploids, of which 8 were tetraploids. The pollen stainabilities ranged from 0.05% to 3% among the hybrids. Of the genotypes that flowered, 11 had *S. brevidens* type cpDNA and two of the three hexaploids that flowered had two doses of *S. brevidens* nuclear genome.

Results of tuberization of different classes of somatic hybrids are presented in Table 2. In total, 40% of the hybrids showed varying degrees of tuberization. The total tuber yield/plant from the pots ranged from 155 g to 0.5 g. Four of the hybrids (all hexaploid) surpassed the yield of PDH40, one having three times the yield of the *S. tuberosum* parent. The frequencies of tuberizing hybrids in the different ploidy levels revealed that 50% of the euploid hexaploids tuberized. All of these had two doses of *S. tuberosum* and one dose of the *S. brevidens* nuclear genome. The other half of the hexaploids that did not tuberize had the alternative nuclear genome combination. Among the euploid tetraploid group, only one-third showed tuber formation. Field data on tuberization of hybrids will be presented in a subsequent publication.

Table 3. Vector loadings in the principal component and canonical variate analyses. Sec.leaflet no. – secondary leaflet number; Term.lflet no. – terminal leaflet number; Lat.lflet no. – lateral leaflet number; AC – anthocyanin

Character	Principal component			Canonical variate (chromosome classes			Canonical variate (cpDNA)
	1	2	3	1	2	3	
Vigour	0.11	0.02	0.11	0.06	0.58	0.63	0.18
Height	–	–	–	0.02	0.04	−0.01	–
Habit	0.20	−0.12	0.30	−0.11	−0.12	0.12	0.24
Density	−0.19	0.05	−0.16	0.33	−0.05	0.12	0.24
Stem no.	−0.04	0.17	0.09	−0.04	0.15	0.14	−0.52
Branch no.	0.02	0.10	−0.27	0.17	−0.08	−0.38	−0.07
Leaf colour	0.18	−0.17	−0.18	−0.45	−0.70	0.03	−0.23
Leaf gloss	0.18	−0.16	−0.04	0.16	0.45	−0.38	0.41
Leaf structure	0.54	−0.24	0.08	−0.28	0.02	0.07	0.28
Sec.leaflet no.	−0.61	−0.32	0.53	0.12	−0.10	0.06	−0.01
Leaflet margin	0.19	−0.09	0.36	0.13	−0.17	0.45	−0.18
Leaflet surface	0.27	−0.30	0.35	−0.32	0.16	−0.13	−0.20
Term.lflet shape	−0.07	0.01	0.10	−0.63	0.71	−1.20	0.71
Lat.lflet shape	−0.13	0.13	0.04	0.23	0.48	0.17	−0.22
AC stem	−0.01	−0.34	−0.20	−0.11	−0.04	−0.18	0.29
AC leaf axil	−0.10	−0.50	−0.30	0.18	0.11	0.01	−0.25
AC leaflet axil	−0.16	−0.39	−0.15	0.23	−0.08	0.42	0.54
AC rachides	−0.11	−0.31	−0.21	−0.03	0.04	−0.36	−0.21

Table 4. The Mahalanobis distance matrix between the chromosome number groups in the canonical variate analysis

	4x	6x	4x±	6x±	8x±
4x	0.00				
6x	2.98	0.00			
4x±	1.55	2.87	0.00		
6x±	3.40	1.54	3.26	0.00	
8x±	3.26	2.86	3.32	3.52	0.00

Discussion

Information is presented on characterization of 58 somatic hybrids of dihaploid *S. tuberosum* and diploid *S. brevidens* on their nuclear genomic properties and parental nuclear and cytoplasmic constitutions. Following analysis of parental nuclear genome doses in the hexaploids, all the possible combinations of nuclear and cpDNA components at tetra- and hexaploid levels have been identified.

The somatic hybrids were obtained from four fusion experiments, two using chemical fusion (calcium ions at high pH, Fish et al. 1987) and two employing electrofusion (Fish et al. 1988a). The latter approach was more effective, with 12.3% of regenerated shoots identified as hybrid, as opposed to 2.6% from the chemical fusion. As can be seen from Table 1, individual experiments yielded different frequencies of tetraploid, hexaploid and octoploid plants. Although the numbers from chemical fusion are small, there are relatively more tetraploids than are apparent from the electrofusion experiments. In addition, from one chemical fusion, experiment plants with only *S. brevidens* chloroplasts were obtained, whilst in the second chemical fusion, the reverse occurred and the hybrids had *S. tuberosum* chloroplasts. From the electrofusion experiments, with more hybrids to analyze, the chloroplast type distribution was about equal. These observations show that the methodology and conditions employed for fusion have a strong bearing on the genetic combinations found in resultant hybrids. Electrofusion products are influenced by protoplast density, alignment field conditions, length of alignment time, fusion pulse voltage and duration, and fusion medium composition (Tempelaar and Jones 1985a, b; Tempelaar et al. 1987; Jones 1988). The effects of varying these parameters (e.g. on the proportion of 1:1 or multiple fusions) has been well documented. Differences between experiments will, therefore, change the balance of fusion products obtained. Hybrids from chemical fusion in which PEG was not employed in this case yielded fewer multiple fusion products. However, this would not be expected when PEG is employed as a chemical fusogen, and similar (although less well-defined) variables will influence the balance of fusion products from chemical fusions.

In total, one-third of the hybrids were euploid, of which 20% were at the tetraploid level and 14% at the hexaploid level. These hybrids represent the potentially useful plants from a practical viewpoint, since useful characters in hexaploids can also be preserved by backcrossing with tetraploid *S. tuberosum*, to reduce the

ploidy to tetraploid whilst selecting for desired characters (J.P. Helgeson, personal communication). This figure is in general agreement with a number of earlier studies indicating the range of chromosome numbers found for somatic hybrid plants (Chupeau et al. 1978; Sundberg et al. 1987; Fish et al. 1987, 1988a).

The cpDNA types in the total hybrid population apparently assorted randomly, with a slight excess of hybrids showing the *S. brevidens* type (55%). This finding agrees with that of Sundberg et al. (1987). However, it differs from the only other study involving somatic hybrids of *S. tuberosum* and *S. brevidens* in which cpDNA was investigated, where all the hybrids examined (six) had *S. brevidens* chloroplasts (Barsby et al. 1984). However, as discussed above (Table 1), in the latter case the result was probably influenced by the small number of hybrids studied. Similar 1:1 sorting-out of chloroplasts has been demonstrated in somatic hybrids of *S. nigrum* and *S. tuberosum*. It has been reported that sorting-out follows rapidly after fusion (Morgan and Maliga 1987) and, therefore, the likelihood of finding complementary RFLPs at the plant level (incomplete assortment) or recombinants is low.

Closer examination of the possible association between ploidy and cpDNA type showed that nuclear DNA dosage did not affect the cpDNA frequencies, as at both the tetraploid and hexaploid levels the cpDNA percentages were the same as for the total hybrid population. The cpDNA transmission to the hybrids was also not affected by aneuploidy.

DNA hybridization using the patatin probe proved to be an excellent tool for identification of nuclear hybridity and, moreover, for studying parental nuclear DNA doses in the hexaploid hybrids. DNA hybridization techniques have previously been used in a few cases to study somatic hybrids (Fish et al. 1987, 1988a; Rosen et al. 1988; Borisjuk et al. 1988; Saul and Potrykus 1984; Uchimiya et al. 1983). It was evident in this study that if a relatively low copy sequence, such as patatin (Twell and Ooms 1988), is used for hybrid identification, it is important to repeat the analysis with additional probes to cover more of the genome, in order to detect hybrids which may have lost chromosome(s) carrying the particular sequence of the first probe. In conjunction with karyotyping techniques, the use of several probes could also be a step towards identification of chromosome-specific RFLPs.

Assessment of 18 morphological traits indicated a wide range of phenotypic variation among the somatic hybrid plants. Analysis of the variation by PCA and CVA using the chromosome number or chloroplast type classes resulted in grouping the hybrids based on their morphological traits. The most prominent feature of the variation when analyzed by PCA was the effect of ploidy level on the grouping of the hybrids in the first principal component. Similar correlations between ploidy level and various morphological characteristics of plants regenerated from tissue culture have been reported earlier (Pehu et al. 1987; Santos and Handro 1983; Sree Ramulu et al. 1976). The second most important dimension apparent in the second principal component separated the genotypes within the ploidy levels and seemed to result from the relative strengths of expression of the particular parental genomes, since the three highest loadings in the second principal component were characteristics of the anthocyanin pigmentation, which is a strong feature in *S. brevidens*.

The CVA also grouped the hybrids according to chromosome number classes, which confirmed the ploidy effect. In addition, it showed that the aneuploid tetraploids and octoploids differed from the euploid groups regarding terminal leaflet shape, leaf colour and plant vigour and were, therefore, clustered in more distinct gorups than in the PCA. The division of the hexaploids into two subgroups was probably due to the parental nuclear genome dosage, which resulted in stronger expression of the features of the parent with the higher dosage. This is in keeping with the observed relationship between nuclear dosage and tuber formation in the hexaploid hybrids, in which only hybrids with two *S. tuberosum* genomes produced tubers (Table 2). This confirms the earlier speculation of Fish et al. (1988b) and the results of Austin et al. (1986). In both PCA and CVA, the tetraploid group displayed a higher degree of heterogeneity than the hexaploids. Furthermore, the frequency of tuberizing and non-tuberizing hybrids amongst the euploid tetraploid plants was not 1:1 as might be expected, but instead there were more non-tuber-bearing genotypes. Both observations suggest that, at the tetraploid level, there are complex interactions between the parental genomes that are to some extent overridden by the dosage effect in the hexaploid hybrids. Interestingly, CVA with parental chloroplast type classes also resulted in separation of the hybrids on the basis of their morphological characters into the two chloroplast types. The significance of this finding is not yet clear. These results demonstrate the potential of protoplast fusion to produce a range of novel hybrids with distinct morphological characteristics related to their different combinations of nuclear and cytoplasmic constitutions. The significance of these classes with respect to their agronomic performance in field trials and to their resistance to PVY, PVX and PLRV will be described in forthcoming publications.

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