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Somatic hybrids between the cultivated potato *Solanum tuberosum* L. and the 1EBN wild species *Solanum pinnatisectum* Dun.: morphological and molecular characterization

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Abstract Interspecific somatic hybrids between the 1EBN-wild species *Solanum pinnatisectum* (*S. pnt*) and four different diploid breeding lines of *Solanum tuberosum* (*S. tbr*) were produced by electrofusion. *S. pnt* exhibits resistance to *Phytophthora infestans* and *Erwinia* blackleg. Somatic hybrids were identified by RFLP analysis using the oligonucleotide $(GATA)_4$ as a probe. In three of four combinations all regenerates obtained were somatic hybrids. All 86 somatic hybrids between the breeding line H256/1 and *S. pnt* were analyzed in detail with respect to morphological and molecular characters; 50% of the somatic hybrids showed normal intermediate leaf morphology. Tubers of somatic hybrid plants grown in the greenhouse as well as in the field were evenly shaped and remarkably similar to those of the *S. tbr* breeding line. Analysis of relative DNA content by flow cytometry revealed that 75% of the somatic hybrids were tetraploid, some were hypotetraploid and others polyploid or mixoploid. Slot-blot and RFLP analyses were carried out using repetitive and some single-copy DNA probes. The genome portion of the *S. tbr* breeding line was determined by slot-blot analysis using the species-specific repetitive probe pSA287. Obviously, most somatic hybrids contain the complete genomes of both fusion partners. In some of the somatic hybrids, a significantly lower intensity of the *S. pnt*-specific hybridization signal indicated a certain degree of asymmetry.

Key words Flow cytometry · Protoplast fusion · RFLP · Species-specific repetitive elements

Dedicated to Prof. Melchers on the occasion of his 90th birthday

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Introduction

The genus *Solanum* consists of about 900 species, 235 of which form tubers (Hawkes 1990). However, the ability to form tubers is not an essential requirement for the functional value of a species in potato breeding. Potato, therefore, is a crop plant with an enormously wide gene pool (Ross 1985). Resistance genes of several wild species have been introduced into the cultivated potato, *S. tuberosum* (*S. tbr*), either by taking advantage of non-reduced gametes of the wild species or by crossing the species with *S. demissum* (Ross 1986). In general, however, there exist several pre- and post-zygotic barriers against the sexual hybridization of different *Solanum* species with each other (Ross 1986).

In particular, sources of resistance to infection with *Phytophthora infestans* (late blight) are required. They do exist in several Mexican wild species; but it turned out to be very difficult or even impossible to cross these species with *S. tbr*. In addition to conventional methods for overcoming crossing barriers, like selecting genotypes recessive for certain barriers or carrying out large-scale and bulk pollinations under various environmental conditions (see Hermsen 1994 for a review), unconventional methods have recently gained increasing importance. Prezygotic barriers have been overcome by mechanical means such as cutting styles and direct pollination of the ovule (Swaminathan 1955) or by using genetically marked mentor pollen (Adiwilaga and Brown 1991). The most frequent postzygotic barrier to hybridization is the degeneration of the endosperm nourishing the embryo (Hermsen 1994). Johnston et al. (1980), therefore, developed the theory of the endosperm balance number (EBN) in order to enable the breeder to predict the success of a specific cross. But even though crossing partners had the same EBN, several species could not be successfully combined. 1 EBN species proved es-

pecially difficult to hybridize. In some cases inter-EBN hybridizations were successful in cases where the embryo had been rescued on a synthetic culture medium (Adiwilaga and Brown 1991; Singsit and Hannemann 1991). Often the breeder is confronted with a combination of several different barriers.

Somatic protoplast fusion has turned out to be a suitable method to overcome pre- and post-zygotic barriers. Those *Solanum* wild species which could not be sexually hybridized, or could only be indirectly sexually hybridized via bridging species, were combined with the germ plasm of *S. tbr* by somatic hybridization techniques. New sources of resistance to biotic and abiotic stresses, such as the resistance of *S. brevidens* and *S. etuberosum* to infection by viruses (Austin et al. 1986; Novy and Helgeson 1994), the resistance of *S. berthaultii* to insects (Serraf et al. 1991) or of *S. commersonii* to frost (Cardi et al. 1993), have been introduced by somatic hybridization into the potato genetic backround.

The Mexican wild species *S. pinnatisectum* (*S. pnt*, 2x, 1 EBN) exhibits field resistance to *P. infestans* and to *Erwinia carotovora* (blackleg). In contrast to several other Mexican wild species, such as *S. demissum* (6x, 4 EBN; Ross 1986), *S. stoloniferum* (4x, 2 EBN; Singsit and Hannemann 1991), *S. flenderi*, *S. hjertingii*, *S. papita* and *S. polytrichon* (each 4x, 2 EBN; Adiwilaga and Brown 1991), it has been impossible until now to obtain fertile progeny from a cross of *S. tbr* and *S. pnt*. Therefore, it was decided to combine *S. pnt* with four diploid breeding lines of *S. tbr* by protoplast fusion and to analyze the fusion products with respect to morphological and molecular characters.

The actual method for successful somatic hybridization between different diploid *S. tbr* breeding lines and *S. pnt* will be reported elsewhere (Schilde-Rentschler et al., in preparation). In the present paper, the results of the morphological and molecular analysis of the somatic hybrids are shown. Morphological analysis included a detailed chracterization of leaves and tubers of plants grown in the greenhouse and in the field. In the molecular analysis, somatic hybrids were identified by the restriction fragment length polymorphism (RFLP) technique with the oligonucleotide $(GATA)_4$ (Epplen 1988; Schilde-Rentschler et al. 1993). Measurement of DNA content by flow cytometry in combination with the hybridization of species-specific repetitive elements was applied to obtain information about the dosage and percentage of the genome of each fusion partner in the somatic hybrids. Selected somatic hybrids were analyzed in more detail by slot-blot and RFLP techniques using repetitive DNA probes (Hemleben 1993; Stelzer et al. 1994; Zanke et al. 1995) and chromosome-specific TG clones (Bonierbale et al. 1988).

Materials and methods

Plant material

Seeds of the wild species *S. pinnatisectum* Dun. (accession BGRC 008168) (*S. pnt*) were obtained from the Dutch-German potato gene bank (FAL Braunschweig). This accession was selected because evaluation data revealed high resistance to *P. infestans* and *E. carotovora* (blackleg). After surface sterilization, seeds were germinated on MS medium (Murashige-Skoog 1962) containing 2% saccharose and 0.25% Gelrite. One seedling was selected and propagated further.

In vitro plants of the following diploid *S. tuberosum* L. ssp. *tuberosum* breeding lines were used: line H256/1 (in Fig. 2 and Fig. 3 named "B15") was obtained from the Bayerische Landesanstalt für Bodenkultur und Pflanzenbau (LBP), Freising, Germany; line 89/2, line "BP15" (official name B15) and line 1076/1 were all obtained from Nordkartoffel Zuchtgesellschaft GmbH, Ebstorf, Germany.

Protoplast isolation, fusion and cultivation

Two-step protoplast isolation from in vitro plant material was performed according to Schilde-Rentschler et al. (1988). Fusion and cultivation of protoplasts and regeneration to plants were carried out according to Schilde-Rentschler et al. (1988). Each colony starting shoot regeneration was numbered and transferred to MS-medium (Murashige and Skoog 1962).

Hybrid identification by RFLP analysis

DNA was extracted from plant material as described by Saghai-Maroof et al. (1984), digested with the appropriate restriction endonucleases, separated on 1% agarose gels and Southern transferred to a nylon membrane (Hybond N, Amersham). For signal detection, non-radioactive labelling of the probe with digoxigenin (Boehringer, Mannheim, Germany) was applied. Southern hybridization with oligonucleotide $(GATA)_4$ and rDNA probes was carried out non-radioactively in accordance with the method of Kreike et al. (1990). Hybridization temperatures were 30°C for $(GATA)_4$ and 68°C for rDNA probes. For single-copy probes (TG clones) the method was slightly modified: the concentration of the hybridization probe was reduced to 3–6 ng of labelled DNA; the hybridization temperature was reduced to 62.5°C and, after hybridization, the membrane was washed twice for 15 min in 0.5×SSC/0.5% SDS at 62.5°C.

Measurement of DNA content by flow cytometry

Somatic hybrids were analyzed using media supplied by Partec GmbH, Münster, Germany. To release nuclei, the youngest leaf of each somatic hybrid plant was chopped with a razor blade in a droplet of 'DAPI/Partec-Standard-Meßlösung'. After filtration through a 40-µm nylon gauze, 2 ml of 'DAPI/Partec-Standard-Meßlösung' were added, and the suspension was incubated for 10 min at room temperature. The DNA content of the nuclei was determined with a Partec CA II cell analyzer and DPAC 2.0 software. In all cases, the fluorescence intensity of the breeding line H256/1 was used as a diploid (2x) standard.

Hybridization probes

The following *repetitive clones* were used: clone pRZ52, containing 25*S*, ITS2 (internal transcribed spacer 2) and 5.8*S* rDNA sequences (unpublished), and clone pRZ83, containing 18*S* rDNA sequences of *Cucurbita pepo* (squash), described by Torres and Hemleben (1994); clone pSA287 (accession no. X87235) described by Schweizer et al. (1988); and oligonucleotide 28-mer Oligo1, containing a *S. pnt*-specific 28-bp sequence of the 5*S* rDNA spacer, described by Zanke et al. (1995).

The following tomato genomic *single-copy* clones were used: TG 63 and TG 65 (mapped on potato chromosomes 10 and 4, respectively; Bonierbale et al. 1988). These probes were supplied by courtesy of Dr. S. D. Tanksley (Department of Plant Breeding and Biometry, Cornell University, Ithaca, New York 14853, U.S.A.).

Plasmids were isolated by the method of Holmes and Quigley (1981). The inserts in each clone were excised from the vector by

the appropriate restriction enzyme and eluted after separation on a 1.2% agarose gel by use of Gelase (BIOzym diagnostik, Hameln, Germany). DNA inserts of pRZ52, pRZ83, TG 63, and TG 65 were non-radioactively labelled with digoxigenin-dUTP (Boehringer, Mannheim, Germany) by the random priming method (Feinberg and Vogelstein 1983). [α-^{32}P]-dCTP was used for labelling of the insert of pSA287, whereas the 28-mer Oligo1 (Zanke et al. 1995) was end-labelled with [γ-^{32}P]-dATP and T4 polynucleotide kinase (Promega, Serva, Heidelberg, Germany) according to Sambrook et al. (1989).

Slot-blot analysis

Total DNA of the fusion partners and the somatic hybrids were isolated according to Saghai-Maroof et al. (1984); the RNA was removed by RNase A. DNA concentration was measured photometrically at 260 nm. After denaturation, identical amounts of DNA of each somatic hybrid (1250 ng, 250 ng, and 50 ng) were loaded per each slot to a nylon filter (Hybond N$^+$, Amersham) under vacuum using a Minifold II Slot-Blot apparatus as described by the manufacturer (Schleicher and Schuell, Dassel, Germany). After air-drying of the membrane, DNA was fixed by incubation in 4 N NaOH for 30 min and neutralized by incubation in 2xSSC at room temperature.

Hybridization of the DNA on the slot-blot membrane with the radioactively labelled probes pSA287 and 28-mer Oligo1, respectively, was performed at 68°C using standard conditions (Sambrook et al. 1989). The radioactivity per slot was either measured directly (for 3 min per slot) using a radioactivity scanner (Tracemaster 20, Berthold, Germany) or else was quantified indirectly by densitometric measurement of the blackening on X-ray film with an "elscript 400" densitometer (Hirschmann, Esslingen, Germany) and two-dimensional evaluation of the signal intensity (HD2 software, version 2.7).

The strength of hybridization with the 28-mer Oligo1 (in cpm) or the spot intensity of DNA with the clone pSA287 from each somatic hybrid was multiplied with its ploidy level to enable a comparison of the values of the hybrids with those of the fusion partners and with each other. For pSA287, the value of H256/1 was set at 100% and the values of the hybrids were divided by the H256/1 value to estimate the portion of its genome in the hybrid nuclei. In the case of Oligo1 the value of *S. pnt* was set at 100% and the values of the hybrids were divided by the *S. pnt* value.

Results

Success of somatic hybridization

Protoplasts of the wild species *S. pnt* were fused with four diploid *S. tbr* breeding lines (H256/1, 89/2, BP15, 1076/1). The time for regeneration of shoots was very short: the first shoots developed within 6 (combination H256/1+*S. pnt*) to 14 weeks (combination with 1076/1) after fusion (Table 1). Only one fusion experiment was carried out per fusion combination.

Using *Dra*I as a restriction enzyme in most cases and the digoxigenin-labelled oligonucleotide $(GATA)_4$ as a hybridization probe, RFLP analysis gave very clear polymorphisms which allowed us to identify and pre-select the hybrids (Fig. 1). As shown in Table 1, a large number of somatic hybrids obtained from fusions of the different diploid breeding lines with *S. pnt* were isolated. Surprisingly, in most combinations all regenerates obtained were hybrids. However, in the combination 1076/1+*S. pnt*, only 45% of the regenerates turned out to be hybrids. No regenerates from protoplasts of *S. pnt* alone were found among the regenerates analyzed.

Because of the large number of somatic hybrids, it was decided to characterize in more detail the fusion combination H256/1+*S. pnt* with the shortest regeneration time. In what follows, the data presented are mainly for somatic hybrids of this combination.

Morphological characterization of somatic hybrids

Leaf morphology

In terms of morphology, i.e. leaf shape and size, somatic hybrids were generally intermediate between both partners, the hybrid leaves being larger than those of the wild species *S. pnt* (Fig. 2). All 86 somatic hybrids between H256/1 and *S. pnt* were characterized by analyzing the leaf branches, interstitial leaflets, and general habitus of the plants. Some of the somatic hybrids exhibited wrinkled leaves or abnormalities in their leaf branches often correlated with an abnormal habitus. However, more important, approximately 50% of the hybrids showed normal leaf morphology.

Tuber morphology

Somatic hybrid plants of all combinations, greenhouse- as well as field-grown, formed evenly shaped tubers. In Fig. 3, tubers of a somatic hybrid are shown in comparison to those of the fusion partners *S. pnt* and *S. tbr* breeding line H256/1. Tuber sizes were rather similar to those of H256/1; tuber shape, however, showed a tendency to be more circular compared to the oval shape of the breeding line. The tuber flesh colour of the hybrids was intermediate between the yellow and white colour of H256/1 and *S. pnt*, respectively.

Table 1 Success of somatic protoplast fusions of different diploid *S. tbr* breeding lines with *S. pnt*

Fusion combination	Regeneration time of shoots (in weeks)	Number of regenerated plants	Number of somatic hybrid plants	Portion of somatic hybrid plants
H256/1 + *S. pnt*	6–8	86	86	100%
BP15 + *S. pnt*	8–12	44	44	100%
89/2 + *S. pnt*	8	6	6	100%
1076/1 + *S. pnt*	14–23	42	19	45%

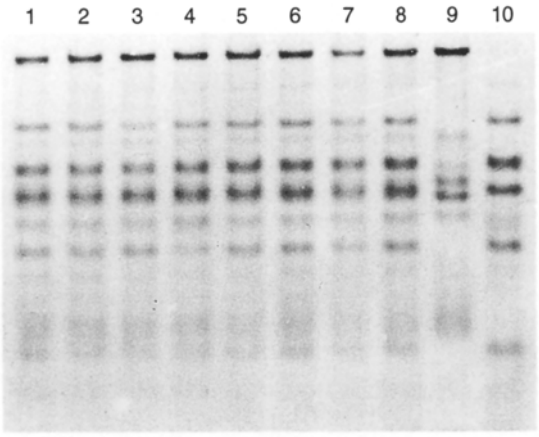

Fig. 1 RFLP banding pattern of H256/1 (*lane 9*), *S. pnt* (*lane 10*), and selected somatic hybrids after Southern hybridization with the oligonucleotide $(GATA)_4$

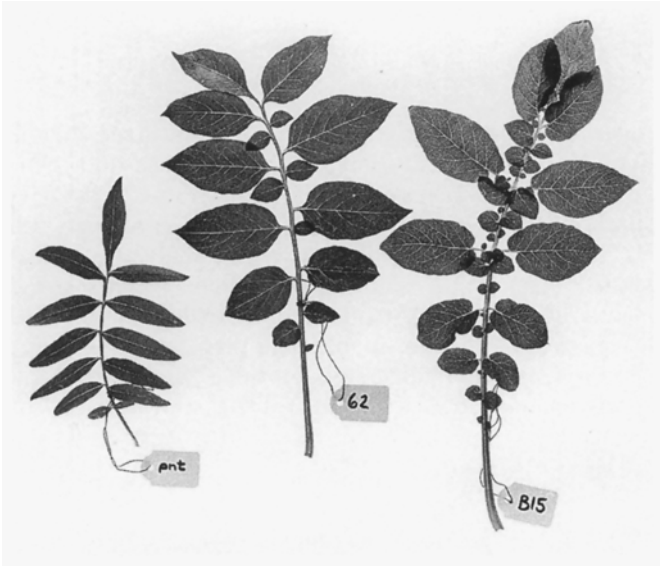

Fig. 2 Leaves of the the wild species *S. pnt* (*pnt*), a somatic hybrid (*no. 62*) and the *Solanum tuberosum* breeding line H256/1 (*B15*)

Evaluation of the relative DNA content

Flow cytometric analysis revealed a slight difference between the DNA content of the *S. pnt* and the *S. tbr* nuclei (Figs. 4a and b). While the 2n-peak of *S. pnt* correlated with channel 21–22 and the S-phase peak with channel 43, the peak of the *S. tbr*-partner H256/1 coincided with channels 25 and 50, respectively. In the first measurement 2 months after regeneration, the main fluorescence intensity peaks of most somatic hybrids were found in channels 46–47, which is the sum of the values of the fusion partners as expected for a complete addition of the genomes (Fig. 4c). The rest of the somatic hybrids were either hypoploid, hypertetraploid, or mixoploid. The percentage of somatic hybrids of each ploidy level is indicated in Fig. 5.

Flow cytometric diagrams showing more than one 1C-peak (Fig. 4d) could often be correlated to abnormal leaf

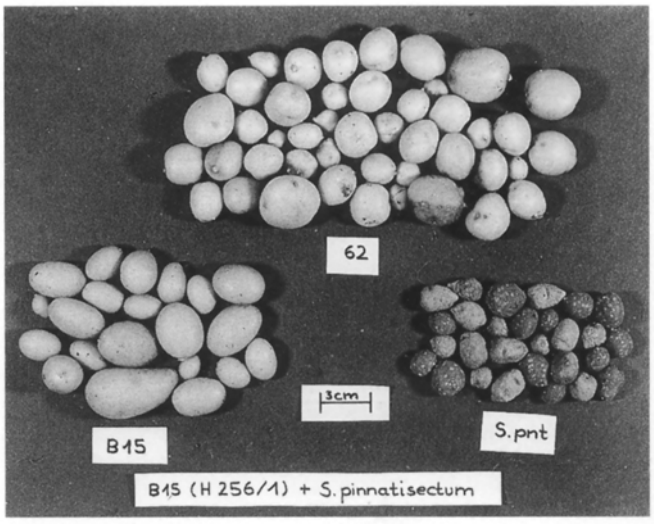

Fig. 3 Tubers of the *S. tbr* breeding line H256/1 (*B15*), a somatic hybrid (*no. 62*), and the wild species *S. pnt* (*pnt*)

morphology and general habitus. Interestingly, somatic hybrids nos. 34, 61, 69 and 72 which had less DNA than would correspond to the tetraploid level exhibited no abnormalities with respect to morphological characters when grown either in the greenhouse or in the field. In order to analyze this phenomenon, the relative DNA content values of the triploid and hypotetraploid somatic hybrids 34, 61, 69 and 72, as well as of some hybrids with mixed ploidy levels (mostly plants with ploidy levels higher than 4x) were measured first in cells of shoot cultures 2 months after regeneration, and subsequently the DNA content was measured a second time 7 months later. In addition, the ploidy level of these hybrids was determined after their cultivation in the greenhouse. Interestingly, most of those shoot cultures with an initial ploidy level below tetraploid showed a tendency to upregulate their DNA content to the tetraploid level (see somatic hybrids 61, 69, and 72; Table 2).

The same phenomenon was observed (data not shown) with the plants grown in the greenhouse. On the other hand, most of those hybrids which initially had a mixed ploidy level showed a tendency to reach a uniform ploidy level (see somatic hybrids 47, 85, and 89; Table 2). Remarkably, all of the hybrids which in the early stages were tetraploid remained stable in their ploidy level.

Molecular characterization: analysis of the portion of the fusion partner genomes in the somatic hybrids

On the basis of the flow cytometric data, 14 somatic hybrids between H256/1 and *S. pnt* were selected for a detailed analysis at the molecular level. To determine whether the genomes of both fusion partners are completely represented in the nuclei of the somatic hybrids, two experimental approaches were applied.

Firstly, a slot-blot analysis of the somatic hybrids with species-specific repetitive probes was carried out to determine the genome portion of the fusion partners in the nu-

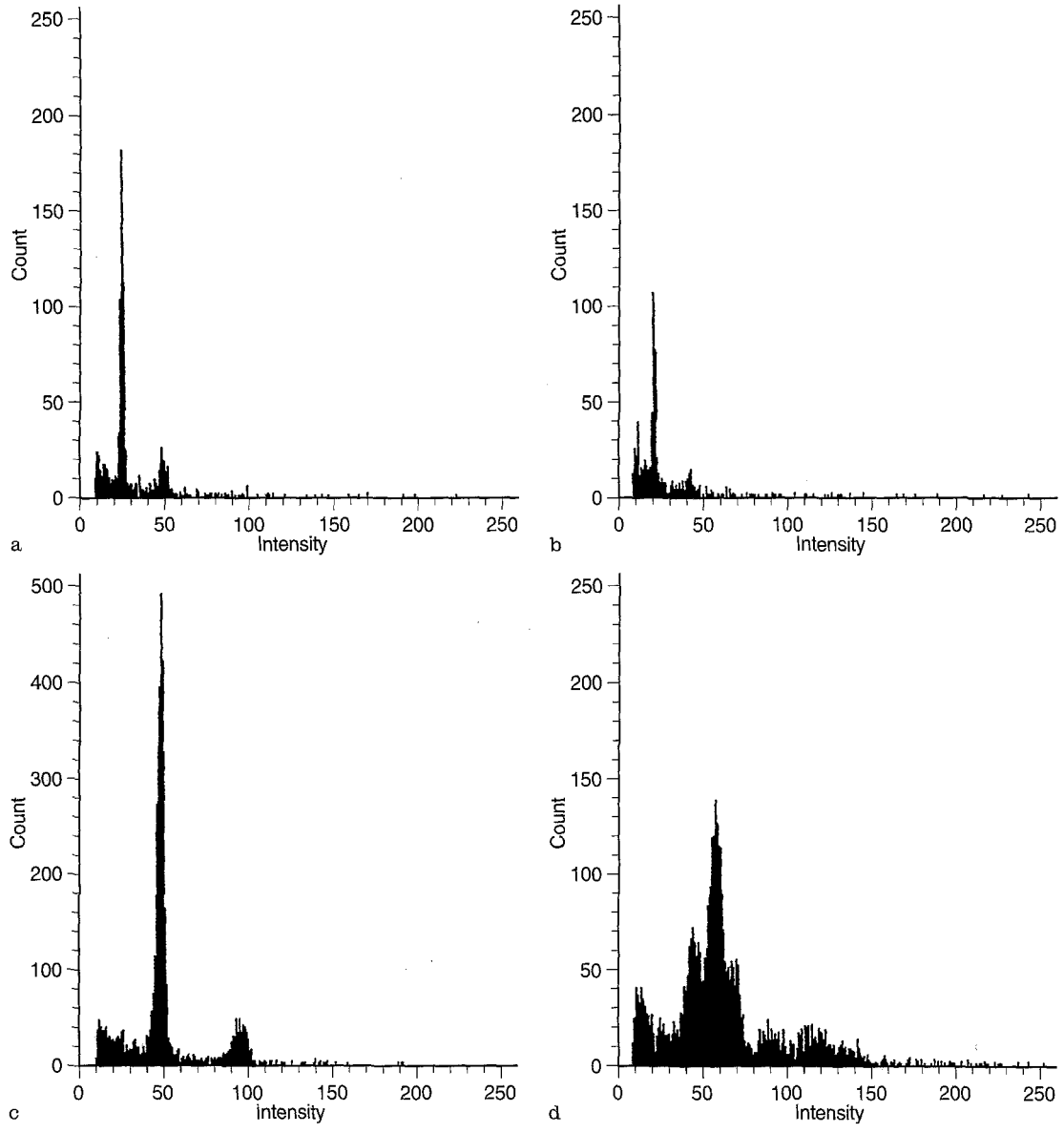

Fig. 4 Histograms of flow cytometric measurements of the diploid *Solanum tuberosum* breeding line H256/1 (**a**, main peak: channel no. 25), the diploid wild species *Solanum pinnatisectum* (**b**, main peak: channel no. 22), the tetraploid somatic hybrid no. 68 (**c**, main peak: channel no. 47) and the mixoploid somatic hybrid no. 89 (**d**, main peaks: channel nos. 47/63/73)

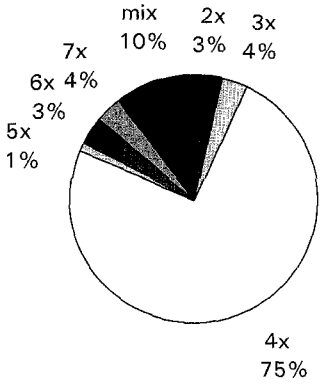

Fig. 5 Portion of each ploidy level from all somatic hybrids between *S. pnt* and H256/1 derived from flow cytometric measurements of the DNA content

cleus of the hybrids. The repetitive element pSA287 (Schweizer et al. 1988) is a 183-bp satellite DNA repeat. Hybridization of the radioactively labelled insert of pSA287 to genomic DNA of *S. pnt* resulted in only very weak signals after long exposure times (Stadler et al. 1995). In contrast, the DNA of the *S. tbr.* breeding line H256/1 gave a very strong hybridization signal with pSA287, a characteristic repetitive element for H256/1 (Schweizer et al. 1993; Zanke et al. 1995). Furthermore, this repetitive element has been mapped at 11 sites in nine linkage groups of the potato by RFLP linkage mapping (Gebhardt et al. 1995).

Thus, pSA287 is a suitable probe for a one-step-analysis of the H256/1-genome portion in the nuclei of the somatic hybrids. DNA (1250, 250, and 50 ng, respectively) of the selected somatic hybrids was blotted onto a filter and hybridized with the insert of pSA287. Saturation hybridization occurred with 1250 ng of DNA. After calculation using these data (for details see Materials and meth-

Table 2 Flow cytometric (relative fluorescence intensity) estimation of DNA content and the derived ploidy level of selected hybrids in the first (2 month after regeneration) and the second (7 months later) determination. / (example 4x/6x): more than one 1C-peak with its corresponding 2C-peak is observed in the histogram. The channel numbers and the ploidy levels are listed in order of decreasing height of the fluorescence peak belonging to it

Somatic hybrid no.	Peak channel no.: (first determination)	Peak channel no.: (7 months later)	Ploidy level (first determination)	Ploidy level (7 months later)	Change in ploidy level
H256/1	25	25	2x	2x	No
S. pnt	22	21–22	2x	2x	No
34	40.4[a]	40	3x(+)	3x(+)	No
47	61.4/28.1	62 (/46)	5x/2.5x mix	5x	Unmixed
50	49.4	44	4x	4x	No
61	42.3	46	4x(–)	4x	Raised up to 4x
62	49.1	46	4x	4x	No
68	47.8	45.5	4x	4x	No
69	40.4	47.5	3x(+)	4x	Raised up to 4x
70	78.5	66/49	6x(+)	6x(–)/4x mix	Mixed
72	34.9	46	3x	4x	Raised up to 4x
75	63.5	64	5x(+)	5x(+)	No
76	56.3/62.5/89.5	55/79	5x(–)/5x(+)/8x(–) mix	5x(–)/7x(–) mix	New mix
85	70.7/45.2	71	6x/4x mix	6x	Unmixed
89	46.8/62.8/73.4	54	5x(+)/4x/6x	5x(–)	Unmixed

(+) Clearly higher ploidy level
(–) Clearly lower ploidy level
[a] Broken values are the mean of several measurements of the same somatic hybrid

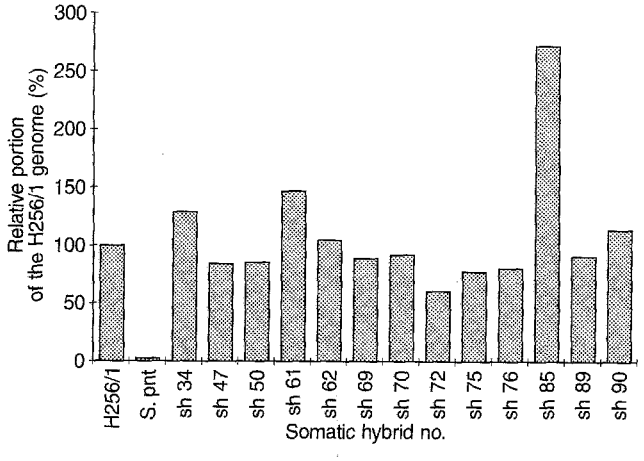

Fig. 6 Relative portion of the H256/1 genome in the nuclei of the fusion partners *S. tbr* breeding line H256/1 and *S. pnt* and selected somatic hybrids (shoot cultures) according to slot-blot hybridization with the radioactively labelled insert of pSA287

ods) it was possible to compare the values of the somatic hybrids and the fusion partner (Fig. 6). The results can be interpreted as follows: most of the somatic hybrids contain a more or less complete H256/1 genome, because their levels of intensity were around the value of 100%. Somatic hybrids nos. 34 and 61 (both hypotetraploid) obviously have a relatively higher genome portion of H256/1 in comparison to that of *S. pnt*.

As far as the characterization of *S. pnt* genome parts in the somatic hybrids is concerned, no species-specific satellite repeat which is able to hybridize to many chromosomes of *S. pnt* is yet known. Instead, the 28-mer Oligo1 (Zanke et al. 1995) was used to re-probe the slot-blot filters which had previously been hybridized with pSA287. The 28-mer Oligo1 is a 28-nucleotide sequence of the 5*S* rDNA spacer specific for *S. pnt* in comparison to H256/1. The 5*S* rDNA has been mapped on chromosome 1 in tomato (Lapitan et al 1990). Therefore, it provides information on the presence of chromosome 1 of *S. pnt* in the somatic hybrids. Actually, all somatic hybrids reacted with this probe. The putative hexaploid somatic hybrids nos. 70 and 89 showed a significantly stronger hybridization signal with Oligo1 (Table 3) indicating a higher genome dosage of *S. pnt* in these hybrids.

Secondly, RFLP analysis with two ribosomal DNA probes (pRZ52 and pRZ83, markers for chromosome 2) and some single-copy probes was carried out. The results obtained for selected somatic hybrids with respect to the intensity of fusion partner-specific bands are summarized in Table 3 (see under 'presence of particular chromosomes'). The differences between the intensity values of the specific bands are pointed out with symbols (+ and – for significantly stronger or weaker band intensity, respectively). In all somatic hybrids an addition of the bands of the fusion partners was detected, and most of them showed an identical intensity of the fusion partner-specific bands. However, in a few cases significant differences in terms of intensity were found; for example, hybridization signals of somatic hybrid no. 61 were significantly lower for the *S. pnt*-specific bands with the rDNA probes for chromosome no. 2, whereas somatic hybrid no. 70 showed higher intensities for *S. pnt*-specific bands with the markers for chromosomes 1, 2, 4, and 10 and lower intensities for the *S. tbr*-specific bands (Table 3).

Combining the data obtained by slot-blot analysis with the respective probes, it appears that most of the hybrids

Table 3 Summary of RFLP and slot-blot experiments for selected hybrids. **P**: *Solanum pinnatisectum*-specific band is more (+) or less (–) intensive. **T**: *Solanum tuberosum*, breeding line H256/1-specific band is more intensive (+) or weaker (–). i.i.: fusion partner-specific bands have identical intensities

Item	Ploidy level	Genome portion		Presence of particular chromosomes					
Genome area	Entire genome	H256/1; *S. pnt* chromosomes	H256/1 chromosomes	H256/1; *S. pnt*			*S. pnt* chromosome	H256/1; *S. pnt* chromosome	H256; *S. pnt* chromosome
				Chromosome no. 2	Chromosome no. 2	Chromosome no. 2	no. 1	no. 4	no. 10
Method	FCM analysis	RFLP $(GATA)_4$	Slot-blot pSA287	RFLP pRZ52		RFLP pRZ83	Slot-blot 28-mer Oligo 1	RFLP TG 65	RFLP TG 63
Explanation Fusion partners and somatic hybrid *no.*	Relative fluorescence intensity at the time of the DNA isolation	*Dra*I digestion	Relative portion of the H256/1 genome in the hybrids (%)	*Dra*I/*Bam*HI double digestion	*Eco*RI digestion	*Eco*RI digestion	Relative presence of the chromosome 1 of *S. pnt* in the hybrids (%)	*Dra*I digestion	*Dra*I digestion
H256/1	25	i.i.	100	i.i.	i.i.	i.i.	0	i.i.	i.i.
S. pnt	22	i.i.	0	i.i.	i.i.	i.i.	100	i.i.	i.i.
34	40.4	i.i.	127 **T +**	i.i.	i.i.	i.i.	112	i.i.	i.i.
47	61.4/28.1 mix	i.i.	85	i.i.	i.i.	**T –**	178	i.i.	i.i.
50	49.4	i.i.	85	i.i.	i.i.	**T ++**	88	i.i.	i.i.
61	42.3	i.i.	147 **T +**	**P –**	**P –**	**P –**	72 **P –**	i.i.	i.i.
62	49.1	i.i.	104	i.i.	i.i.	i.i.	145 **P +**	i.i.	i.i.
69	40.4	i.i.	88	i.i.	i.i.	i.i.	71 **P –**	i.i.	i.i.
70	78.5	i.i.	91	**P +**	i.i.	**T –**	196 **P +**	**P +**	**P +**
72	34.9	i.i.	59 **T –**	i.i.	i.i.	i.i.	73 **P –**	**P –**	i.i.
75	63.5	**P +**	76	**P +**	i.i.	i.i.	186 **P +**	i.i.	i.i.
76	56.3/62.5/89.5 mix	i.i.	79	i.i.	**P –**	i.i.	115	i.i.	i.i.
85	70.7/45.2 mix	**T +**	271 **T +**	i.i.	**P –**	i.i.	119	**P –, T +**	**P –, T +**
89	46.8/62.8/73.4 mix	**P +**	89	**P +**	i.i.	i.i.	203 **P +**	**P +**	**P +** (1 Band)

are completely symmetric products. Some exceptions exhibited interesting features: e.g. hybrid no. 61 seemed to have a relatively small genome part of *S. pnt* whereas somatic hybrids 75 and 89 had a larger portion of *S. pnt* than of H256/1. For both somatic hybrids nos. 75 and 89, which are more or less hexaploid, it can be stated that they are fusion products of two *S. pnt* protoplasts and one H256/1 protoplast. Somatic hybrid no. 85 is presumably a fusion product of two H256/1 protoplasts and one *S. pnt* protoplast.

Discussion

This study contains a description of somatic hybrids derived from protoplast fusion experiments between four different diploid *S. tuberosum* breeding lines and the 1EBN wild species *S. pinnatisectum*. It was possible to regenerate a large number of somatic hybrid plants. Most plants regenerated within the short time of 6 to 12 weeks, and although no selective pressure was applied, nearly all of the regenerates were somatic hybrids as confirmed by RFLP analysis with the oligonucleotide probe $(GATA)_4$. Whereas in the combination with the breeding line 89/2 no more plants could be regenerated in this experiment, many more plants regenerated in the other two combinations, but were not further analyzed. Therefore, it can not be excluded that, for the latter, parental lines appear among the plants, regenerating later. Expression of hybrid vigor has been reported for the fusion of breeding lines (Debnath and Wenzel 1987; Deimling et al. 1988) as well as for combinations with wild species (Cardi et al. 1993; Schilde-Rentschler et al. 1993). In contrast Ward et al. (1994) previously reported the isolation of four somatic hybrid plants of *S. pnt* and a diploid *S. tbr* breeding line; they used selective media and the hybrids needed 1 year for regeneration. These four somatic hybrids were mixoploid plants.

Somatic hybrids between the *S. tbr* breeding line H256/1 and *S. pnt* were analyzed with respect to several morphological and molecular characters. Most of the 86 somatic hybrids were normal-looking plants with intermediate leaf phenotype both in the greenhouse and in the field. All somatic hybrids formed tubers of good shape and reasonably large size. Remarkably, the morphology of the tubers was not intermediate between both fusion partners but very similar to that of the diploid *S. tbr* fusion partner H256/1. Therefore the gene dosage of the diploid *S. tbr* is lower than that of the tetraploid *S. tbr* which was used as a counterpart to the wild species genome in other fusion experiments (Austin et al. 1986; Pehu et al. 1990; Austin et al. 1993). Thus, there is hope for the breeder that, with our somatic hybrids, less backcross generations are needed for yielding clones with good tuber size and form.

The DNA content of most somatic hybrids between H256/1 and *S. pnt*, determined first in shoot cultures, appeared to reach the sum of the ploidy levels of the fusion partners; 75% of them were stably tetraploid. The rest of the somatic hybrids was hypotetraploid [4x(–) down to 3x] and others were polyploid or mixoploid. This is probably the result of fusion events in which three or four protoplasts were fused, or neighboring colonies mixed, and which then developed into plants. While the hyper- and mixoploid somatic hybrids showed abnormalities in their morphological characters in the greenhouse, as expected, surprisingly greenhouse plants of the hypotetraploid somatic hybrids looked normal. To analyze this phenomenon, we re-examined the ploidy level of the shoot cultures, that formerly had been hypotetraploid, and of the greenhouse plants of the same somatic hybrid. As shown in Table 2, for the shoots propagated in vitro, as well as for the adult plants in the greenhouse, we found that in these somatic hybrids the DNA content grew to the normal tetraploid level within 9 months after the initial regeneration. This is probably the explanation for the absence of morphological abnormalities observed in these somatic hybrids. Further investigations, for example by the genomic in situ DNA hybridization technique (Parokonny et al 1994), would be necessary to determine the origin of the subsequently 'added' DNA. Similar to our observations, Sidorov et al. (1987) fused an albino mutant of a sexual hybrid of *S. tbr* × *Solanum phureja* with *S. pnt* and obtained normal-looking plants from initially abnormal shoots of somatic hybrids after propagation by nodes. In consequence, it is strongly indicated that greenhouse plants rather than shoot cultures of somatic hybrids should be analyzed; this is especially important if X-ray fusion is applied.

The genome portion of the breeding line H256/1 was measured by slot-blot experiments using a specific satellite repeat (pSA287; Schweizer et al. 1988) as a probe since this repeat is represented at the ends of most of the potato chromosomes (Gebhardt et al. 1995). It was demonstrated that most of the selected somatic hybrids carried the complete H256/1 genome in their nuclei. One hexaploid somatic hybrid was identified (hybrid 85) carrying two genomes of H256/1 and one of *S. pnt*, a typical result of a triple fusion event.

As determined by several repetitive and single-copy RFLP probes and by a species-specific probe for *S. pnt* we found that most hybrids also carried the complete *S. pnt* genome in their nuclei. However, although these experiments were not planned as asymmetric fusion experiments, as reported earlier by us (Schierbaum et al. 1993) and other groups (e.g. Xu et al. 1993), a certain degree of variation with respect to the portion of the *S. pnt* genome in the nuclei of some somatic hybrids was observed. Plants which after regeneration were initially hypotetraploid [4x(–) down to 2x] mostly had lost *S. pnt* genome parts during the regeneration process. In particular, the *S. pnt*-specific bands for RFLP markers of chromosome no. 2 were of lower intensity (e.g. in hybrid no. 61; Table 3). A similar result has been obtained by Pijnacker et al. (1989) in somatic hybrids between *S. tbr* and *S. phureja:* cytological investigation showed the preferential loss of chromosome no. 2 of *S. phureja* in these somatic hybrids.

In conclusion, we succeeded in combining the 1 EBN-*Solanum* wild species *S. pnt* with four *S. tbr* breeding lines. At least three of these turned out to be very suitable as fu-

sion partners as can be seen from the large number of regenerates and the high percentage of morphologically normal tetraploid somatic hybrids obtained. Detailed investigations are in progress to evaluate resistance to pathogens and the fertility of the somatic hybrids.

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