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Fertile somatic hybrids of *Solanum etuberosum* (+) dihaploid *Solanum tuberosum* and their backcrossing progenies: relationships of genome dosage with tuber development and resistance to potato virus Y

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Summary

The wild non-tuberous species *Solanum etuberosum* is resistant to biotic and abiotic stresses, but is very difficult to cross with cultivated potato. Therefore, interspecific somatic hybrids between a dihaploid clone of potato *S. tuberosum* (2n=2x=24, AA genome) and the diploid species *S. etuberosum* (2n=2x=24, EE genome) were produced by protoplast fusion. Among the 7 fertile fusion hybrids analysed by genomic *in situ* hybridisation (GISH), three groups of plants were found with the genomic constitution of AAEE, AAEEEE and AAAAEE. Four fusion hybrids had exactly the expected chromosome composition, while each of the three aneuploid hybrids had lost two chromosomes of *S. etuberosum*. Two backcross progenies were developed, and GISH analysis was applied to analyse transmission of the parental chromosomes into the sexual generations. BC_1 hybrids derived from the crosses of the hexaploid somatic hybrids with tetraploid potato were pentaploid with the theoretically expected genomic composition or with slight deviation from this expectation. In the three BC_2 hybrids analysed by GISH seven to 12 chromosomes of *S. etuberosum* were detected in the predominant *S. tuberosum* background. No recombinant chromosomes in the hybrids were detected. Genome dosage affects tuber formation in hybrids and their progenies, but has less effect on resistance to potato virus Y (PVY) in fusion hybrids. Several genotypes of the fusion hybrids and BC_1 progeny did not show viral infection even in the grafting experiments.

Introduction

To date transgenic approaches are the most-widely recognised new strategies for crop improvement. However, many agronomically important traits can not be transferred to crops by genetic engineering alone because they are controlled by polygenes, or the genetic control of the desirable traits is still unknown, or their genes are not isolated and cloned yet. Therefore, wide hybridisation between distantly related species remains attractive. More recently protoplast fusion approach has been applied to broaden the genetic basis of resistance to biotic and abiotic stresses in crop plants (Chen et al., 1999; Naess et al., 2000; McGrath et al., 2002).

Solanum etuberosum Lindl. is a wild non-tuberous diploid (2n=2x=24) species with higher levels of resistance to the most important viral diseases (Valkonen et al., 1992a) and to aphids (Valkonen et al., 1992b). *S. etuberosum* and cultivated potato *S. tuberosum* are reproductively isolated species due to the differences in endosperm balance numbers (EBN) (Johnston & Hanneman, 1982). Despite the barriers of incompatibility some interspecific hybrids between *S. etuberosum*

and cultivated potato *S. tuberosum* have been obtained using embryo culture (Watanabe et al., 1995) and *via* protoplast fusion (Novy & Helgeson, 1994a), but no backcross progenies were reported so far.

Fertile interspecific hybrids and backcross progenies were obtained by using the combination of *S. etuberosum* with two or more different tuber-bearing potato species both *via* sexual bridge crosses (Ramanna & Hermsen, 1981; Chavez et al., 1988) and *via* protoplast fusion (Novy & Helgeson, 1994a). Transfer of resistance to green peach aphid (Novy et al., 2002) and resistance to viral diseases from *S. etuberosum* to tuber-bearing potatoes were reported (Chavez et al., 1988; Novy & Helgeson, 1994b; Novy et al., 2002); however no genotypes with extreme resistance were described among these hybrids or their BC progenies.

Thieme & Thieme (1998) had identified an accession of *S. etuberosum* which has extreme resistance to potato virus Y (PVY^N, PVY^O). Different species of aphids showed high mortality on these plants suggesting that the resistance to PVY was due to unsuitability of the host for the vectors. Genetic control of the extreme resistance to viral diseases of exotic germplasm of *S. etuberosum* species as well as mechanisms of virus resistance are not yet fully understood. In order to transfer the new genetic material to potato interspecific hybrids between *S. etuberosum* and a diploid line of potato have been produced *via* protoplast fusion (Thieme et al., 1999). The objectives of the present study were to obtain sexual progenies of these fusion hybrids with cultivated potato and to study by GISH analysis the transmission of alien chromosomes of the E-genome (Ramanna & Hermsen, 1981) of *S. etuberosum* to the BC_1 and BC_2 generations. We also report the transfer of PVY resistance from *S. etuberosum* to backcross progenies of interspecific potato hybrids.

Materials and methods

Plant material

Figure 1 shows the fusion and crossing scheme used in the present study. Interspecific somatic hybrids were obtained *via* protoplast fusion (Thieme et al., 1999) between the dihaploid *S. tuberosum* line T67 (BAZ-GL-6.090 004-86N, the Institute for Agricultural Crops Gross Lüsewitz) and *S. etuberosum* (k-9141, VIR). All hybrids were identified by isozyme or by microsatellite analysis (Thieme et al., 1999). A highly fertile tetraploid breeding clone of potato, susceptible to PVY, (3.14104-90N NORIKA GmbH, Groß Lüsewitz) was used as the male parent in backcrosses.

Greenhouse and field experiments

All somatic hybrids and parental clones were propagated *in vitro* and transferred to the greenhouse where each genotype was represented by six plants. All flowering somatic hybrids were used in crossing experiments. Berries of the hybrids were harvested between five and eight weeks after pollination. Seeds and embryos were germinated *in vitro* on the MS-medium. Hybrids were planted with 40–50 replicates in the field where tuber development was analysed.

GISH analysis

Genomic DNA of *S. tuberosum* and *S. etuberosum*, was extracted from young leaves of greenhouse-growing plants. Probe DNA (DNA of *S. tuberosum* or rarely DNA of *S. etuberosum*) was sonicated until the fragments attained the size of 1–5 kb and furthermore direct labelled with FITC-12-dUTP using a nick translation mix (Boehringer Mannheim). Blocking DNA (DNA of *S. etuberosum* or sometimes DNA of *S. tuberosum*) was obtained by autoclaving total genomic DNA for 5 min yielding fragments of 100–500 bp in size. Mitotic metaphase chromosome spreads were prepared according to Zhong et al. (1996). The post-treatment and *in situ* hybridisation were performed according to Schwarzacher & Heslop-Harrison (1994) with modifications described in our previous studies (Gavrilenko et al., 2002). Slides were observed with an OLYMPUS BX-FLA 50 microscope using appropriate filters for FITC and DAPI. Digital images were recorded using a Digital Camera: Color View-8 analysed with Software: Analysis 3.0 by Soft Imaging System GmbH. A total of 10–20 well-spread metaphase cells of each plant were examined.

Flow cytometric analysis

DNA histograms were measured using a Cell Analyser CA II equipped for UV excitation and blue light emission (Partec GmbH, Münster, Germany) as described earlier (Gavrilenko et al., 1999).

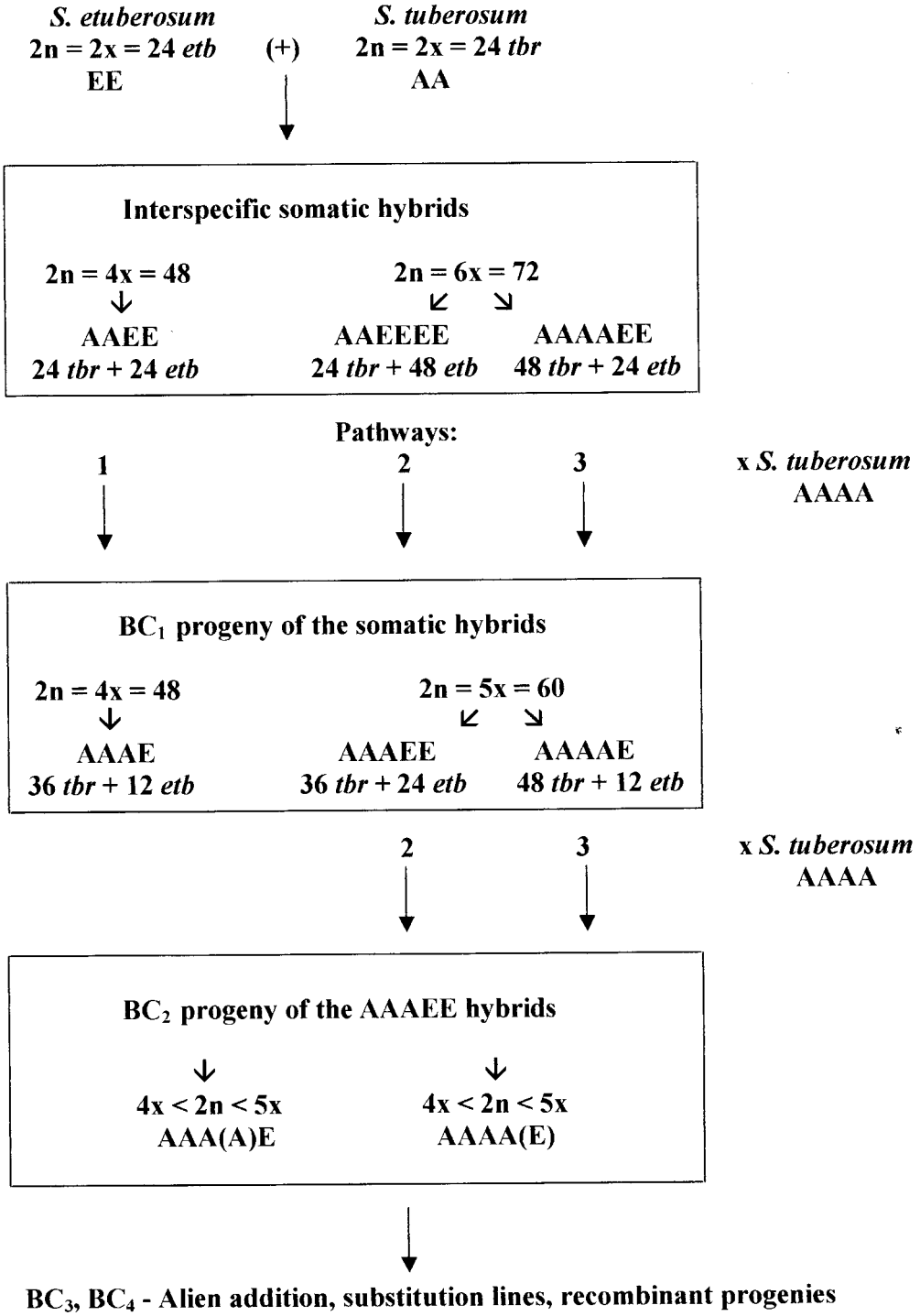

Figure 1. Fusion and crossing scheme for introgression of genetic material of *S. etuberosum* (E, haploid genome) into potato *S. tuberosum* (A, haploid genome). Various alternative pathways and the theoretically expected genomic constitutions of the hybrids are illustrated. In this scheme, the expected chromosome numbers are based on the hypothesis that intergenomic recombination by crossing over is completely absent. An incomplete chromosomal set is indicated in brackets as (A) or (E).

Virus test

PVY^N isolate Amigo N150/1 obtained from H. Weidemann (FBRC, Braunschweig) was maintained in *Nicotiana tabacum* cv. 'Xanthi'. Parental lines, somatic hybrids and BC plants were screened for resistance to PVY^N by mechanical inoculation of *in vitro* and greenhouse grown plants using carborundum and sap extracted from tobacco plants infected with PVY^N (Thieme & Thieme, 1998). After mechanical inoculation plants were kept for three weeks at 25 °C and day length of 16 h. 20 to 30 replicates per genotype were assayed for the presence of PVY in their leaf tissue using an enzyme-linked immunosorbent assay (ELISA) test. For grafting experiments greenhouse grown tobacco plants and *in vitro* plants (10 to 20 replicates per genotype) were used as PVY-infected recipient and scion, respectively. Newly developed plant parts of the scions were sampled for ELISA four weeks after greenhouse cultivation at 20 °C. Selected genotypes and parental lines as well as control varieties were cultivated in the field with natural and artificial virus infection pressure. After a storage period of three months the harvested tubers, if present, were planted in greenhouse and the sprouts were used for ELISA tests.

Results

Crossing experiments and fertility of the fusion hybrids

Interspecific fusion hybrids *S. etuberosum* (+) *S. tuberosum* showed a high variation in plant vigour, flowering ability and fertility. Among 67 hybrids grown under greenhouse conditions 23 did not produce flowers, 34 had only a few flowers and the 10 most vigorous fusion hybrids had intensive flowering. Seven of the 10 vigorous hybrids were able to produce berries in the crosses with a tetraploid clone of cultivated potato. These seven fertile hybrids were selected for use in the present study.

According to flow cytometry analysis, three of the seven selected hybrids were tetraploid and four were hexaploid (Table 1). Tetraploid hybrids produced 19 berries with 68 seeds, but only 15 seeds (22%) were able to germinate *in vitro* (Table 1). Based on the fertility characters hexaploid hybrids could be divided into two groups. The first group (hybrids: 8/1/2/1, 27/2/14/1 and 6/1/2/1) had relatively low numbers of seeds per berry. They produced a total of 63 seeds, but only 11 (18%) of them germinated (Table 1). The second group was represented by one hybrid, 27/2/12/1, which had the highest rate of fertility with 155 seeds per berry and 128 (83%) of seeds germinated (Table 1). Overall, out of 271 pollinated flowers of the fusion hybrids, a total of 154 BC_1 seedlings (57% seedling set) was obtained.

GISH analysis of the fusion hybrids

Seven fertile fusion hybrids were analysed by using GISH, and their genome composition corresponded to the theoretically expected scheme (Figure 1). Two tetraploid hybrids (31/1/2/1, 29/2/1/1) were euploids (2n=4x=48, AAEE) with the complete chromosomal set of both parental lines (24 chromosomes of *S. tuberosum* and 24 chromosomes of *S. etuberosum,* AAEE, Figure 2a). A hypotetraploid hybrid, 10/1/1/1, had lost two *S. etuberosum* chromosomes (Table 1, Figure 2b).

Two groups of the hexaploid (2n=6x) fusion hybrids were identified by GISH. The euhexaploid hybrid, 27/2/12/1, had the AAAAEE genomic constitution (2n=6x=72) with 48 chromosomes of potato and 24 chromosomes of *S. etuberosum* (Figure 2c, Table 1). Three hexaploid hybrids (8/1/2/1, 27/2/14/1 and 6/1/2/1) possessed the AAEEEE genomic composition. Euhexaploid hybrid, 6/1/2/1, had 24 chromosomes of potato and 48 chromosomes of *S. etuberosum.* Two remaining AAEEEE hybrids (8/1/2/1 and 27/2/14/1) were hypohexaploids (2n=6x=70), each of them had lost two chromosomes of *S. etuberosum* (Table 1, Figure 2d).

Virus resistance tests and tuber performance of the fusion hybrids

Three tetraploid hybrids (31/1/2/1, 10/1/1/1 and 29/2/1/1) had no virus infection after mechanical inoculation under greenhouse and *in vitro* conditions as well after grafting (Table 2). Three hexaploid hybrids (8/1/2/1, 27/2/14/1, 6/1/2/1) did also not show virus incidence after mechanical inoculation and grafting. In contrast, the 27/2/12/1 hexaploid hybrid was susceptible to virus infection (Table 2).

Tetraploid hybrids developed elongate tubers in the field (Table 2). Hexaploid fusion hybrids: 8/1/2/1, 27/2/14/1 and 6/1/2/1 did not form tubers under the field conditions. The hexaploid hybrid 27/2/12/1 developed larger tubers elliptic in shape (Table 2).

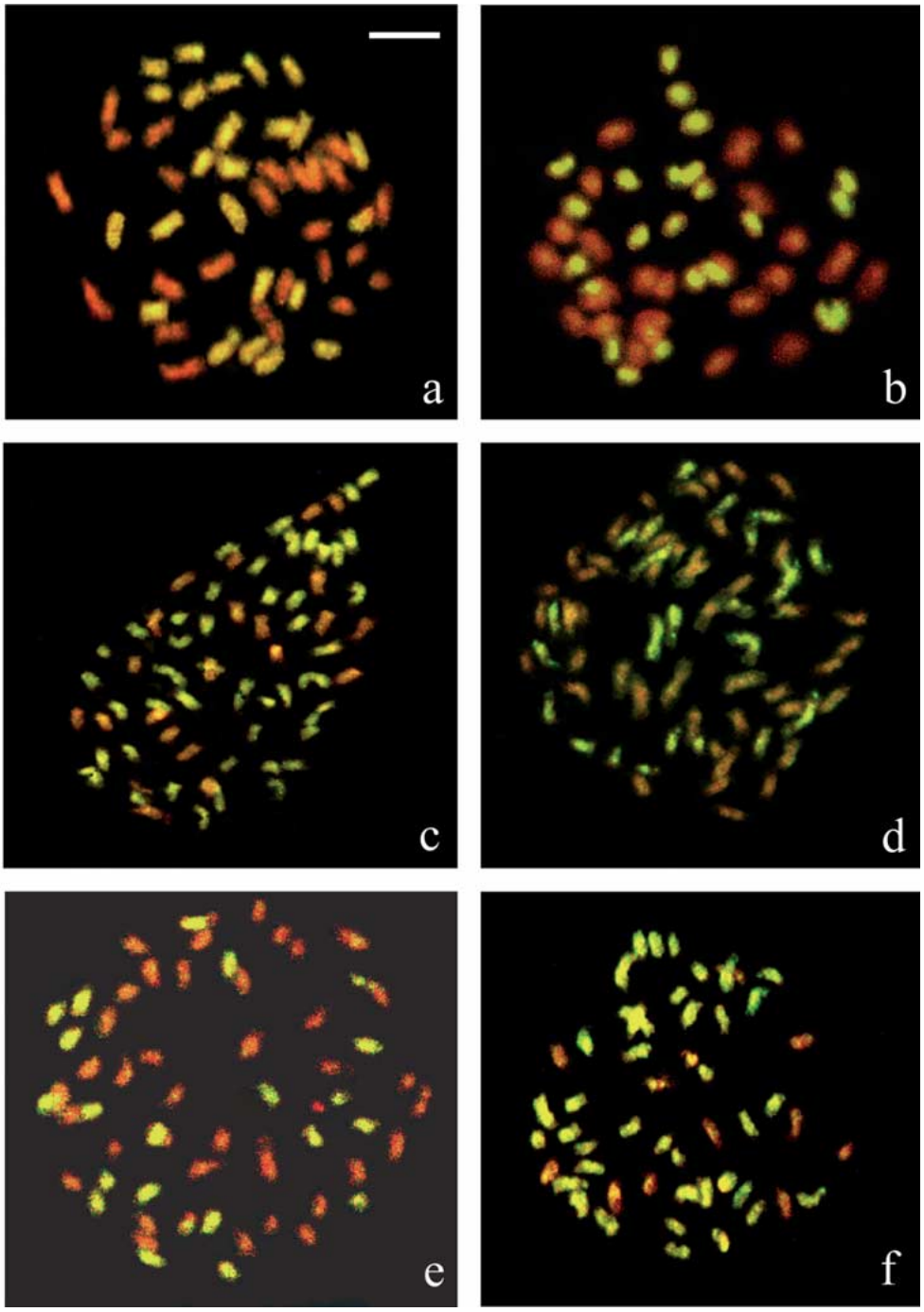

Figure 2. GISH of mitotic cells of interspecific somatic hybrids between *S. etuberosum* (EE, 2n=2x=24) and dihaploid cultivated potato (AA, 2n=2x=24). Total potato DNA labelled by FITC (c, d, f), the *S. etuberosum* chromatin fluoresces red following by counterstaining with propidium iodide (PI). *Vice versa* – total *S. etuberosum* DNA labelled by FITC (a, b, e), the potato chromatin fluoresces red following by counterstaining with PI. Root tip cells of the somatic hybrids: (a) 29/2/1/1 – AAEE, 2n=4x=48; 24 chromosomes of potato, 24 chromosomes of *S. etuberosum*; (b) 10/1/1/1 – AAEE, 2n=4x=46; 24 chromosomes of potato, 22 chromosomes of *S. etuberosum*; (c) 27/2/12/1 – AAAAEE, 2n=6x=72; 48 chromosomes of potato, 24 chromosomes of *S. etuberosum*; (d) 27/2/14/1 – AAEEEE, 2n=6x=70; 24 chromosomes of potato, 46 chromosomes of *S. etuberosum.* (e) BC_1 clone 64/10 (AAAEE; 2n=59; 38 chromosomes of potato, 21 chromosomes of *S. etuberosum*). (f) BC_2 clone 64/10/4/1 (2n=51; 39 chromosomes of potato, 12 chromosomes of *S. etuberosum*).

Table 1. Fertility and chromosomal composition of the somatic hybrids (SH) *S. etuberosum* (+) 2x *S. tuberosum* and their backcross progenies identified by GISH analysis

Genotype	Flowers*, (No. of berries)	Fertility, No. of seeds produced (Average No. of seeds per berry)	No. of seedlings obtained (%)**	Ploidy level (2n)	Genome composition	No. of chromosomes of parental species	
						S. tuberosum	*S. etuberosum*
Tetraploid somatic hybrids:							
31/1/2/1	7 (4)	14 (3.5)	6 (43)	4x (48)	AAEE	24	24
10/1/1/1	7 (3)	6 (2.0)	3 (50)	4x (46)	AAEE	24	22
29/2/1/1	23 (12)	48 (4.0)	6 (13)	4x (48)	AAEE	24	24
Total	37 (19)	68 (3.6)	15 (22)				
Hexaploid somatic hybrids:							
8/1/2/1	101 (2)	4 (2.0)	0	6x (70)	AAEEEE	24	46
27/2/14/1	76 (13)	47 (3.6)	10 (21)	6x (70)	AAEEEE	24	46
6/1/2/1	54 (17)	12 (0.7)	1 (8)	6x (72)	AAEEEE	24	48
Total	231 (32)	63 (1.8)	11 (18)				
Hexaploid somatic hybrids:							
27/2/12/1	3 (1)	155 (155)	128 (83)	6x (72)	AAAAEE	48	24
BC_1 progeny of 6x SH 27/2/14/1:							
64/10	91 (6)	36 (6.0)	24 (63)	5x (59)	AAAEE	38	21
64/6				5x (59)	AAAEE	37	22
BC_1 progeny of 6x SH 6/1/2/1:							
61/1				5x (60)	AAAEE	36	24
BC_1 progeny of 6x SH 27/2/12/1:							
69/2	52 (15)	255 (17.0)	140 (55)	5x (60)	AAAAE	48	12
BC_1 progeny of 4x SH 31/1/2/1:							
39/2	12 (2)	10 (5.0)	5 (50)	6x (73)	AAAAEE	48	25
34/1				6x (73)	AAAAEE	49	24
34/4	10 (7)	77 (11.0)	24 (31)	6x (74)	AAAAEE	50	24
BC_2 progeny of 5x BC_1 64/10:							
64/10/1/1				≈ 4x (52)	AAA(A)(E)***	42	10
64/10/4/1				≈ 4x (51)	AAA(A)(E)***	39	12
69/2/2/2				≈ 4x (50)	AAA(A)(E)***	43	7

* No. of pollinated flowers.
** % of germinated seeds.
*** not complete chromosomal set is indicated in brackets as (A) or (E).

Fertility of the BC_1 hybrids, GISH analysis and flow cytometry of the BC_1 hybrids

The BC_1 hybrids were involved as female in subsequent crosses with a tetraploid potato breeding line. Overall, out of 165 pollinated flowers of the BC_1 hybrids, a total of 193 BC_2 seedlings were obtained (Table 1).

All BC_1 hybrids, derived from crosses between hexaploid fusion hybrids and tetraploid potato (AAAA) had the expected 5x ploidy level as confirmed by flow cytometry (Figure 1). BC_1 line, 69/2, which had originated from the cross of the euhexaploid AAAAEE hybrid 27/2/12/1 and tetraploid *S. tuberosum* AAAA, had 48 chromosomes of potato and 12 chromosomes of *S. etuberosum* (AAAAE), as corresponded to our scheme (Figure 1, Table 1). BC_1 line 61/1, which was produced from the euhexaploid 6/1/2/1 fusion hybrid with reverse genomic constitution of AAEEEE, had the expected chromosome composition with 36 chromosomes of potato and 24 chromosomes of *S. etuberosum* (AAAEE). BC_1 lines 64/10 and 64/6 which had originated from the hypohexaploid 27/2/14/1 (AAEEEE) fusion hybrid, had the genomic composition of AAAEE. The lower than expected number of E-genome chromosomes in the BC_1 clones 64/10 and 64/6 might be explained by aneuploidy of the parental fusion hybrid 27/2/14/1. At

Table 2. PVY transmission (% of infected plants) and tuber formation in *S. tuberosum* (+) *S. etuberosum* somatic hybrids and BC_1 progenies after mechanical inoculation under *in vitro*, greenhouse, field and grafting conditions (n = 10–40)

Genotype	PVY transfer (%)				Tuber
	In vitro	Greenhouse	Field	Grafting	formation*
S. etuberosum	0	0	0	0	NT, NS
S. tuberosum T67	97	87	50	100	T (ET)
Somatic hybrid (AAEE)					
31/1/2/1	–	0	0	0	T (EST)
10/1/1/1	0	0	2	0	T (EST)
29/2/1/1	0	0	0	0	T (EST)
Somatic hybrid (AAEEEE)					
8/1/2/1	0	0	–	0	NT
27/2/14/1	0	0	–	0	NT
6/1/2/1	0	0	0	0	NT
Somatic hybrid (AAAAEE)					
27/2/12/1	70	24	33	41	T (ET)
BC_1 progeny of the hexaploid somatic hybrid 27/2/14/1:					
64/2	17	63	–	–	–
64/3	23	90	–	–	–
64/4	43	73	–	–	–
64/5	23	40	–	–	–
64/6	0	0	–	0	–
64/7	30	47	–	–	–
64/9	13	47	–	–	–
64/10	3	5	41	0	T (EST)
BC_1 progeny of the hexaploid somatic hybrid 6/1/2/1:					
61/1	0	0	0	0	T (EST)

* Tuber performance of the field grown plants: NT – no tubers; NS – no stolons; T – tuber formation; EST – elongate-shaped tubers; ET – elliptic tubers. '–' data have not been analysed yet.

the same time these BC_1 lines had gained one-two chromosomes of potato (Table 1, Figure 2e).

Unexpected results were obtained with the BC_1 progeny derived from the tetraploid fusion hybrids (Figure 1). According to flow cytometry analysis, all 15 BC_1 hybrids from sexual crosses of the tetraploid AAEE fusion hybrids with the tetraploid potato (AAAA) were hexaploids (2n=6x). Three of them analysed by GISH had the genomic constitution of AAAAEE (Table 1).

Virus resistance tests of the BC_1 hybrids and their tuber performance

Among the eight normally developed BC_1 plants from a cross between the virus resistant AAEEEE 6x hybrid (27/2/14/1), and tetraploid *S. tuberosum* (AAAA), only 64/6 was resistant to PVY, revealing no virus after the tests. Another AAEEEE hexaploid fusion hybrid (6/1/2/1), gave rise to one BC_1 clone (61/1) that had no virus infection after mechanical inoculation and after grafting as well as in the field with natural and artificial virus infection pressure (Table 2). The pentaploid BC_1 hybrids with the AAAEE genomic composition developed elongate-shaped tubers in the field.

GISH analysis of the BC_2 hybrids

GISH analysis revealed that in the three BC_2 hybrids that had originated from the hexaploid fusion hybrids about 40 potato chromosomes and 7–12 alien chromosomes of *S. etuberosum* were preserved (Table 1, Figure 2f). Deviation from the expected chromosome composition of the BC_2 hybrids concerned loss or

gain of chromosomes from both parental genomes (Table 1). Both BC_1 and BC_2 hybrids analysed contained only intact parental chromosomes. No recombinant chromosomes were detected.

Discussion

The synthesis of interspecific fusion hybrids is the first step to transfer desirable alien genes into a crop plant. The essential next step is the backcrossing of the interspecific fusion hybrids with cultivated plants. A large number of somatic hybrids must be produced to search fertile genotypes. In our experiments ten vigorous and flowering genotypes were selected from 67 fusion hybrids, but only six of them were able to generate BC_1 seeds. In contrast, only a few tetraploid hybrids of the same *S. etuberosum* (+) 2x *S. tuberosum* combination were produced by Novy & Helgeson (1994a). All of their hybrids were unable to grow under field conditions and were characterised by poor vigour and sterility. Comparison of the present results with previous fusion hybrids *S. etuberosum* (+) 2x *S. tuberosum* (Novy & Helgeson, 1994a) indicate an effect of the parental genotype on hybrid vigour, fertility and tuberisation.

Based on the GISH analysis the fusion hybrids could be divided into the three groups indicated as AAEE, AAEEEE and AAAAEE. Crossability of the fusion hybrids with tetraploid potato did not follow EBN rules. Hybrids of each genomic type (AAEE, EBN = 3; AAEEEE, EBN = 4; AAAAEE, EBN = 5) were able to cross with tetraploid potato (AAAA, EBN = 4). The highest rate of seedling production was observed for the hexaploid hybrid 27/2/12/1 (AAAAEE, EBN = 5), although it formed only one berry. In general backcrossing of the fusion hybrids to the cultivated potato resulted in increasing seed set of the BC_1 plants.

GISH analysis of the most important basic hybrid material produced in this study showed that the chromosome composition was as theoretically expected and corresponded to our scheme. A few cases of missing E genome chromosomes of *S. etuberosum* were detected. Of the seven fusion hybrids analysed in this study, three had each lost two chromosomes of the E genome. The same tendency of the preferential loss of chromosomes of the E genome was observed in the related fusion combination of potato (AA) and *S. brevidens* (EE) (Gavrilenko et al., 2002). Probably, asynchrony in mitotic behaviour might play a role in the loss of alien chromosomes of the E genome. Delayed condensation of the rDNA loci of *S. brevidens* in the AAAAEE fusion hybrids supports this possibility (McGrath & Helgeson, 1998).

The BC_1 plants derived from the two groups of the hexaploid fusion hybrids were pentaploid with genomic composition of AAAEE or AAAAE as theoretically expected from our scheme (Figure 1, pathways 2 and 3). Tetraploid fusion hybrids (AAEE) generated BC_1 progeny with unexpected to the theoretical scheme ploidy level (6x) and genome constitution (AAAAEE) (Figure 1, pathways 1). Most probably, our tetraploid AAEE fusion hybrids formed unreduced gametes that took part in production of their BC_1 plants. Unreduced gamete formation has been observed in somatic hybrids of potato (Wolters et al., 1994).

Comparison of the GISH results with the tuber formation of the field growing interspecific hybrids showed that the ratio of parental genomes in hybrids affected tuber development. Hexaploid fusion hybrids with genomic constitution AAAAEE (2A:1E) produced large elliptic tubers. Tetraploid fusion hybrids (AAEE; 1A:1E) tuberised under field conditions like 6x AAAAEE fusion hybrids, however they formed more elongate, misshaped and smaller tubers. Tuber development of the two field growing pentaploid BC_1 plants (AAAEE; 3A:2E) was similar to the tetraploid fusion hybrids. Hybrids with the highest dosage of alien E genome of non-tuberous wild species *S. etuberosum* (AAEEEE; 1A:2E) did not form tubers. An increased number of chromosomes of *S. etuberosum* can lead to a new type of allelic interactions resulting in differences in gene expression patterns.

In the present study extreme resistance to PVY^N from *S. etuberosum* has been recovered in a number of hybrids since virus was not detected even after grafting. Similarly, symmetric somatic hybrids derived from the close combination between dihaploids of potato and another E-genome species *S. brevidens* were extremely resistant to the virus strain PVY^O which suggests the dominance of the PVY resistance of the wild E-genome species over susceptibility of potato with the A genome (Valkonen et al., 1994). Resistance to PVY^O was reported for fusion hybrids [*S. etuberosum* (+) *S. tuberosum* × *S. berthaultii*] and their backcross progenies (Novy & Helgeson, 1994b; Novy et al., 2002), however, none was as resistant as the *S. etuberosum* parent (Novy & Helgeson, 1994b). It is possible that virus susceptible tuber-

ous potato parents may influence the inheritance of extreme resistance to PVY of *S. etuberosum*.

Six of seven tested fusion hybrids revealed extreme resistance to PVY^N. The ratio of parental genomes in hybrids may also have some effects on expression of virus resistance. All three hexaploid fusion hybrids with AAEEEE (1A:2E) genome composition had extreme resistance to PVY, while hexaploid plants with the reverse genomic constitution AAAAEE (2A:1E) were susceptible to virus infection. At the same time two pentaploid BC_1 plants (AAAEE, 3A:2E) were highly resistant to PVY and seven pentaploid BC_1 genotypes were virus infected following mechanical inoculation. Other genetic factors as distorted genetic segregation, and the frequency of the particular alleles, may have effect on expression of virus resistance in BC_1 plants. The genetic control of resistance to PVY of exotic germplasm of *S. etuberosum* needs further characterisation with larger backcrossing populations. The BC_2 and BC_3 hybrids will be useful for the localisation of the virus resistance (genes) on specific chromosomes of *S. etuberosum*.

Aneuploidy in the hybrids did not influence expression of virus resistance. Both aneuploid fusion hybrids (10/1//1/1, 8/1/2/1, 27/2/14/1) and aneuploid BC_1 clone 64/6 were extremely resistant to PVY although they had each lost one-two chromosomes of the E genome, suggesting that the specific chromosomes of *S. etuberosum* controlling resistance to PVY were not lost in the aneuploid hybrids.

Towards to the goal of transferring valuable traits of *S. etuberosum* into potato, intergenomic recombination is essential, but in the present study it was not possible to detect recombinant segments through GISH. Our data are in concordance with results of Dong et al. (1999) who demonstrated that somatic hybrids between *S. etuberosum* and tuberous potatoes (*S. tuberosum* × *S. berthaultii*) transmitted the intact chromosomes of the A and E genomes into their BC progenies. These results may be due to the intragenomic structural changes accumulated in the several linkage groups of the E-genome of *S. etuberosum* (Perez et al., 1999). It is also possible that recombination between chromosomes of A and E genomes had occurred but small recombinant segments might be difficult to differentiate with GISH alone, especially those localised in the distal euchromatin regions of potato chromosomes (Ramanna & Wagenvoort, 1976). Further cytogenetic and molecular markers analyses of the BC_2 and BC_3 progenies are required to determine the potential of introgression of virus and aphid resistances from *S. etuberosum* to cultivated potato.

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