Theor Appl Genet (2002) 104:539–546

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Evidence for tetrasomic inheritance in a tetraploid *Solanum commersonii* (+) *S. tuberosum* somatic hybrid through the use of molecular markers

Received: 23 July 2001 / Accepted: 9 August 2001

Abstract In order to assess the potential for interspecific recombination between the cultivated *Solanum tuberosum* (tbr) and the sexually isolated wild species *Solanum commersonii* (cmm), genetic analysis of a F_2 progeny obtained by selfing one tetraploid cmm (+) tbr somatic hybrid was performed through molecular markers. For this purpose, the extent of disomic and/or tetrasomic inheritance of species-specific RAPD and AFLP markers was determined by following their segregation in a 90-genotype progeny, and testing all the possible segregation ratios in a selfed tetraploid progeny. The RAPD analysis performed using 16 primers revealed that the cmm-specific RAPDs were mainly (93.7%) duplex markers and were equally distributed between loci with a disomic (46.7%) and tetrasomic (53.3%) inheritance. The AFLP analysis led to the identification of 272 (58%) informative AFLPs, which were either cmm- or tbr-specific markers. About 63% of cmm-specific AFLPs were duplex loci, most of which (92.6%) were inherited as tetrasomic loci. As regards the tbr-specific AFLPs, the percentage of simplex loci (52.9%) was higher than that of duplex loci (32.6%), and among the latter most (88.5%) were inherited as tetrasomic loci. Overall, 130 duplex markers were found, of which 53.1% were cmm-specific and 46.9% were tbr-specific. Out of 130 markers, 18 (13.8%) were inherited as disomic, and 112 (86.2%) as tetrasomic, loci. This implies that the majority of duplex markers were located on chromosomes which at meiosis tend to randomly pair as bivalents or to form tetravalents. The total number of simplex loci was 119, and most of them (82.3%) were tbr-specific loci. In some cases the observed segregation ratios even allowed us to clearly determine whether a random chromosome or chromatid segregation was detected. This was the case of three cmm-specific RAPDs, 19 cmm- and 25 tbr-specific AFLPs, which fit a 20.8:1 or 2.5:1 ratio, both cases for which a clear random chromatid segregation can be assumed, since they represent the limit of segregation expected when the distance between the locus and the centromere always leads to a cross-over event. The percentage of ascertained crossing-over events was around 37% out of the tetrasomically inherited loci clearly identified (128 loci), a value indicating that the flow of genes from the sexually isolated *S. commersonii* to the cultivated potato is possible, for at least a large proportion of genes.

Keywords *Solanum commersonii* · Interspecific recombination · Disomic/tetrasomic inheritance · RAPD · AFLP

Communicated by F. Salamini

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Introduction

The potential recombination between chromosomes of related species is a key point to transfer genes from the wild to the cultivated gene pool. The extent of recombination can be initially predicted on the basis of cytological observation of chromosome pairing at meiosis, even though this does not always involve crossing-over and recombination.

During the last 10 years species-specific molecular markers have provided a powerful tool to verify the occurrence of recombination between chromosomes of different species, since they allow the inheritance of chromosomal segments during the meiotic process to be monitored (McCoy et al. 1991; Williams et al. 1993;

McGrath et al. 1994; Novy and Helgeson 1994; Garriga-Calderé et al. 1997; Yamada et al. 1998).

In the genus *Solanum*, the occurrence of various wild relatives makes interspecific hybridization a valid approach for the genetic improvement of cultivated *Solanum tuberosum* (tbr). Indeed, the flow of genes from both congruent and incongruent species has been achieved through various breeding approaches (Hermsen 1994). In some cases, barriers to interspecific hybridization have been overcome, and both sexual (Singsit and Hanneman 1991; Louwes et al. 1992) and somatic hybrids (Mattheij et al. 1992; Austin e t al. 1993; Helgeson et al. 1993; Novy and Helgeson 1994; Yamada et al. 1997) between more or less distantly related species have been obtained.

Among wild *Solanum* species, *Solanum commersonii* (cmm, 2n=2*x*=24) is one of the most interesting in spite of its sexual isolation from the cultivated *S. tuberosum* (tbr, 2n=4*x*=48), having been the genetic source of many useful traits, such as resistance to pests and diseases (Hanneman and Bamberg 1986), heat (Palta et al. 1981) and frost tolerance (Vega and Bamberg 1995), and high tuber specific gravity (Ehlenfeldt and Hanneman 1988). Various technological approaches, such as protoplast fusion (Cardi 2001) and the use of ploidy bridges (Novy and Hanneman 1991; Carputo et al. 1997), allowed the crossing barriers to interspecific hybridization between cmm and tbr to be overcome. In both cases, the hybrids produced were fertile, and backcross and/or selfed progenies were able to be generated (Barone et al. 2001; Cardi et al. 2001).

Cytological analysis and the use of species-specific molecular markers have already given preliminary indications as to the occurrence of recombination between homoeologous chromosomes in these cmm-tbr hybrids, since the former identified multivalent formation in both somatic and sexual hybrids (Conicella et al. 1997; Barone et al. 1999) and the latter provided evidence for some recombination events on five different chromosomes (Barone et al. 2001). In any case, the introgression of useful genes from the wild to the cultivated species requires that homoeologous recombination occurs to a great extent. Hence, a high degree of overall sequence homology between the two parental genomes is required in the hybrid. Unfortunately, only limited information (Hosaka et al. 1984; Matsubayashi 1991) is available on the phylogenetic relationships between *S. commersonii* and other *Solanum* species, including *S. tuberosum*, even though various studies with nuclear or chloroplast molecular markers have contributed to new insights into the phylogenetic relationships within this genus (Debener et al. 1990; Spooner and Sytsma 1992; Spooner et al, 1996; Kardolus et al. 1998; Bryan et al. 1999).

In order to better assess the potential for interspecific recombination between cmm and tbr, genetic analysis of a F_2 progeny obtained by selfing one tetraploid cmm (+) tbr somatic hybrid was performed using molecular markers. RAPD markers (Williams et al. 1990) were chosen for a preliminary analysis, and then AFLPs were selected as the primary marker system (Vos et al. 1995) since they allow several segregating markers to be monitored for each experiment.

The objectives of the present work were to estimate (1) the number of duplex and simplex loci present in both cmm and tbr parental genotypes, (2) the number of these loci which followed a disomic or a tetrasomic inheritance in the somatic tetraploid hybrid, (3) the degree of chromosome and chromatid segregation in a number of tetrasomic loci.

Materials and methods

Plant material

A fertile somatic hybrid (SH9A, 2n=4*x*=48) was previously obtained by protoplast fusion between accession PI 243503 of the diploid (2n=2*x*=24) wild species *S. commersonii* and the *S. tuberosum* haploid (2n=2*x*=24) DH 81–7-1463 (Cardi et al. 1993). By selfing the somatic hybrid a large tetraploid progeny has been obtained (Cardi et al. 2001) and an F_2 sample of 90 genotypes was used in the present work for genetic analyses. Leaves from the selfed genotypes, the somatic hybrid SH9A and the two parents, were collected from plants grown in a greenhouse. Total DNA was extracted from frozen leaves using the DNeasy Plant Mini Kit commercially provided by QIAGEN Gmbh (Hilden, Germany).

RAPD analysis

DNA from each genotype was amplified by using the PCR reaction conditions described by Williams et al. (1990). Each reaction consisted of 1× buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 0.001% gelatin), 0.1 mM of each dNTP, 0.2 µM of primer, 1.0 unit of *Taq* DNA polymerase and 20 ng of genomic DNA. Amplification was performed in a Perkin-Elmer/Cetus DNA thermal cycler programmed for 45 cycles of 1 min at 94°C, 1 min at 35°C, 2 min at 72°C, followed by 7 min at 72°C. Amplification products were separated by electrophoresis on a 1.5% agarose gel in 1×TAE buffer. Out of 34 cmm-specific RAPDs previously selected (Barone et al. 2001), 16 were used to amplify the DNA of the cmm and tbr parental genotypes, the somatic hybrid SH9A and the 90 F_2 genotypes.

AFLP analysis

AFLP analysis was performed using the method described by Vos et al. (1995) and the commercially available AFLP kit and protocol (Gibco-BRL AFLP analysis System I, Life Technologies, Gaithersburg, Md.), which employs *Eco*RI and *Mse*I as restriction enzymes. For selective amplification, nine previously selected primer combinations (Barone et al. 2001) were used (*Eco*RI+AAC and *Mse*I+CAG, *Eco*RI+ACC and *Mse*I+CAA, *Eco*RI+ACT and *Mse*I+CAC, *Eco*RI+ACT and *Mse*I+CAG, *Eco*RI+ACT and *Mse*I+CAT, *Eco*RI+AGC and *Mse*I+CAA, *Eco*RI+AGC and *Mse*I+CTA, *Eco*RI+AGG and *Mse*I+CAA, *Eco*RI+AGG and *Mse*I+CAG) and the fragments obtained were resolved on a 6% denaturing polyacrylamide gel run at constant power of 50 W for 3 h. Gels were transferred to Whatmann 3 MM paper and dried for 2 h at 80°C on a gel dryer (BioRad). Subsequently, autoradiograms (X-ray films) were exposed to the gel for 1–2 days to visualize AFLP fragments.

Statistical analysis

For each marker only clear and unambiguous bands were scored, and numbered according to the molecular weight. Each informative band was scored independently as 1 for “presence” and 0 for

Table 1 Expected phenotypic segregation ratios[a] after selfing a tetraploid hybrid and informativeness of segregating ratios to distinguish tetrasomic vs disomic inheritance in the F_2 progeny. Modified from R.W. Allard (1999), Principles of Plant Breeding, edited by J. Wiley and Sons, New York, USA

Genotype	Disomic inheritance	Tetrasomic inheritance		Informative tetrasomic vs disomic segregation ratio
		Random chromosome segregation	Random chromatid segregation	
AAAa	1A	1A	738A:1a	NO[b]
AAaa	1A	35A:1a	20.8A:1a	Completely
Aaaa	3A:1a	3A:1a	2.5A:1a	Partially

[a] Allele A represents the presence of the band
[b] Not informative in the 90-genotype F_2 progeny analyzed

Table 2 Genotype of the locus A and the type of heredity proposed on the basis of all A:a segregation ratios that can be theoretically obtained in a 90-genotype population. Each segregation ratio was tested for all the values expected following the selfing of a tetraploid genotype

A:a segregation ratio	Segregation ratio with non- significant $\chi 2$ value[a]	Genotype in the tetraploid somatic hybrid	Type of heredity	Marker class[b]
90:0	1:0	Duplex	Disomic	A
From 89:1 to 85:5	35:1 and 20.8:1	Duplex	Tetrasomic	A
From 84:6 to 82:8	20.8:1	Duplex	Tetrasomic	A
From 81:9 to 76:14	–	Duplex or simplex	–	B
From 75:15 to 73:17	3:1	Simplex	Disomic or tetrasomic	C
From 72:18 to 60:30	3:1 and 2.5:1	Simplex	Disomic or tetrasomic	C
From 59:31 to 56:34	2.5:1	Simplex	Tetrasomic	A
From 55:35 to 1:89	–	Simplex	–	D

[a] $P>0.05$
[b] A=informative markers; B=unassigned markers; C=non-informative markers; D=distorted segregating markers

"absence", and classified as cmm-specific or tbr-specific markers when it was only present in either of the two parents, and as common markers when present in both parents. The presence of each species-specific band was also checked in the somatic hybrid SH9A. In the F_2 progeny only the segregation ratios of species-specific markers were analyzed, since those with common markers (triplex and quadruplex loci) are not informative enough to distinguish tetrasomic or disomic inheritance (Table 1). Following the assumption that each species-specific segregating marker could be present in the somatic hybrid as a duplex or a simplex locus, in an F_2 progeny the expected segregation ratios vary depending on the occurrence of disomic or tetrasomic inheritance. In the latter case, the ratios also vary depending on the presence or absence of crossing-over between the locus and the centromere. When disomic inheritance always acts as a direct consequence of pairing only between homologous chromosomes, the 1:0 segregation ratio is typical of a duplex locus and the 3:1 of a simplex locus. In the case of tetrasomic inheritance, which implies pairing between homoeologous chromosomes, the expected segregation ratios are 35:1 and 20.8:1 for a duplex and 3:1 and 2.5:1 for a simplex locus, when random chromosome or chromatid segregation acts, respectively. The goodness of fit of the expected segregation ratios was tested by χ^2 analysis, and following this analysis a tetrasomic or disomic inheritance was hypothesized for each locus (Table 2).

Results

RAPD analysis

All the 16 primers tested on the F_2 progeny amplified one cmm-specific band, with a molecular weight ranging from 500 bp (OPH17) to 1,700 bp (OPAN1 and OPH6) (Table 3). Various tbr-specific bands were also amplified but they were not so clearly detectable as the cmm-specific ones: therefore, they were not considered in the segregation analyses. Out of the 16 cmm-specific RAPDs, seven (43.8%) were always present in the F_2 progeny, thus indicating no segregation (1:0). Among the other cmm-specific markers, only one (OPAN16) showed the 3:1 segregation ratio of a simplex locus (6.2%), but no assumption could be made on the type of inheritance (disomic or tetrasomic) since in both cases after selfing a simplex locus would segregate following a 3:1 ratio. For the last eight markers (50%) tetrasomic inheritance of a duplex locus was always assumed, and in some cases (OPH5, OPH6, OPH17) it was also possible to clearly assign the 20.8:1 segregation ratio to the typical random chromatid segregation of a selfed duplex locus.

AFLP analysis

AFLP analysis performed on the parental (cmm, tbr and SH9A) and on the 90 F_2 genotypes allowed cmm-specific, tbr-specific and common bands to be detected (Table 4). Except for ten cmm-specific and 38 tbr-specific bands, all cmm- and tbr-specific AFLPs were present in the cmm (+) tbr somatic hybrid. Moreover, 29 new AFLPs were also

Table 3 Cmm-specific RAPDs: for each marker the segregation ratio, the locus genotype, and the type of heredity are reported

Primers[a]	Molecular weight (bp)	No. of genotypes			Segregation ratio[b]	Locus genotype	Type of heredity
		Analyzed	With the band	W/out the band			
OPAN1	1700	86	85	1	35:1/20.8:1	Duplex	Tetrasomic
OPAN5	1300	89	89	0	1:0	Duplex	Disomic
OPAN16	750	87	72	15	3:1	Simplex	n.a.[c]
OPAN18	1300	83	83	0	1:0	Duplex	Disomic
OPH3	800	80	80	0	1:0	Duplex	Disomic
OPH5	750	88	80	8	20.8:1	Duplex	Tetrasomic
OPH6	1700	86	80	6	20.8:1	Duplex	Tetrasomic
OPH12	800	85	85	0	1:0	Duplex	Disomic
OPH15	1600	82	81	1	35:1/20.8:1	Duplex	Tetrasomic
OPH17	500	84	78	6	20.8:1	Duplex	Tetrasomic
P35	600	85	85	0	1:0	Duplex	Disomic
UBC8	1300	85	85	0	1:0	Duplex	Disomic
UBC12	1000	86	84	2	35:1/20.8:1	Duplex	Tetrasomic
UBC24	1250	90	77	3	35:1/20.8:1	Duplex	Tetrasomic
UBC54	1500	84	81	3	35:1/20.8:1	Duplex	Tetrasomic
UBC89	1200	85	85	0	1:0	Duplex	Disomic

[a] All the primers are commercially available (primers coded OPAN and OPH from the Operon Technologies, Alameda, Calif., and primers coded UBC from University of British Columbia Biotechnology Lab.), except for P35 which was randomly designed (sequence 5′-GCTATTGGCG-3′)

[b] The 35:1, 20.8:1, 3:1 and 2.5:1 segregation ratios were tested by χ^2 analysis. For each marker only the segregation ratios which exhibited non-significant χ^2 values ($P>0.05$) are reported

[c] n.a.=not assessable

Table 4 AFLP analysis: for each primer combination the total scorable, the number of common AFLPs, the total number of cmm- and tbr-specific AFLPs, are reported, together with the number of AFLPs which fit different segregation ratios (based on $\chi 2$ analysis, $P>0.05$)

Primer combination	Total scorable AFLPs (no.)	Common AFLPs (no.)	cmm-specific AFLPs (no.)									tbr-specific AFLPs (no.)								
			Total	Segregating								Total	Segregating							
				1:0	35:1	20.8:1	35:1/ 20.8:1	3:1	2.5:1	3:1/ 2.5:1	Other		1:0	35:1	20.8:1	35:1/ 20.8:1	3:1	2.5:1	3:1/ 2.5:1	Other
E-AAC/M-CAG	53	19	8	0	0	1	3	0	1	1	2	26	0	0	3	5	2	2	7	7
E-ACC/M-CAA	60	28	14	0	0	1	11	0	1	1	0	18	0	0	2	4	0	0	8	4
E-ACT/M-CAC	48	11	10	0	0	0	6	0	0	3	1	27	1	0	3	4	2	1	15	1
E-ACT/M-CAG	51	17	10	0	0	5	3	0	0	1	1	24	0	0	0	6	2	0	13	3
E-ACT/M-CAT	75	30	12	1	0	1	4	0	4	2	0	33	3	0	2	8	2	2	13	3
E-AGC/M-CAA	47	22	11	2	0	3	2	1	0	1	2	14	2	0	3	2	0	1	4	2
E-AGC/M-CTA	41	26	1	0	0	0	0	0	0	0	1	14	0	0	1	3	0	0	5	5
E-AGG/M-CAA	62	27	13	0	0	2	6	0	0	1	4	22	0	0	1	5	1	3	10	2
E-AGG/M-CAG	32	17	6	1	0	0	2	1	0	2	0	9	1	0	0	2	0	1	4	1
Total	469	197	85	4	0	13	37	2	6	12	11	187	7	0	15	39	9	10	79	28
Percentage			100	4.7	0.0	15.3	43.5	2.4	7.1	14.1	12.9	100	3.7	0.0	8.0	20.9	4.8	5.4	42.3	15.0

observed in the somatic hybrid which were not detected either in the cmm or in the tbr parental genotype. These AFLPs were not further evaluated.

The number of scorable bands/primer combination varied from 32 to 75. Among the total 469 AFLPs scored, 85 (18.1%) were cmm-specific, 187 (39.9%) were tbr-specific, and 197 (42.0%) were common bands. Thus there were 272 (58%) informative AFLPs for our genetic analysis. The segregation of each cmm- and tbr- specific AFLP was followed on the 90 F_2 genotypes (Fig. 1), and all the possible segregation ratios (Table 2) were tested. Results grouped for each primer combination are reported in Table 4, which shows the number of AFLPs which fit different segregation ratios after the χ^2 analysis. In all cases the presence of the band represented the dominant allele. Only 11 (4.0% of total markers) cmm-specific and 28 (10.3%) tbr-specific markers did not fit any tested segregation ratio. The other 233 (85.7%) markers fit one or two expected segregation ratios ($P>0.05$). Four out of 85 cmm-specific AFLPs, as well as seven out of 187 tbr-specific AFLPs, did not segregate at all. In these 11 cases (4.0% of both species-specific markers) a disomic inheritance was assumed. Among the segregating AFLPs, in some cases the observed segregation ratios even allowed us to clearly determine whether a random chromosome or chromatid segregation was detected. This was the case of

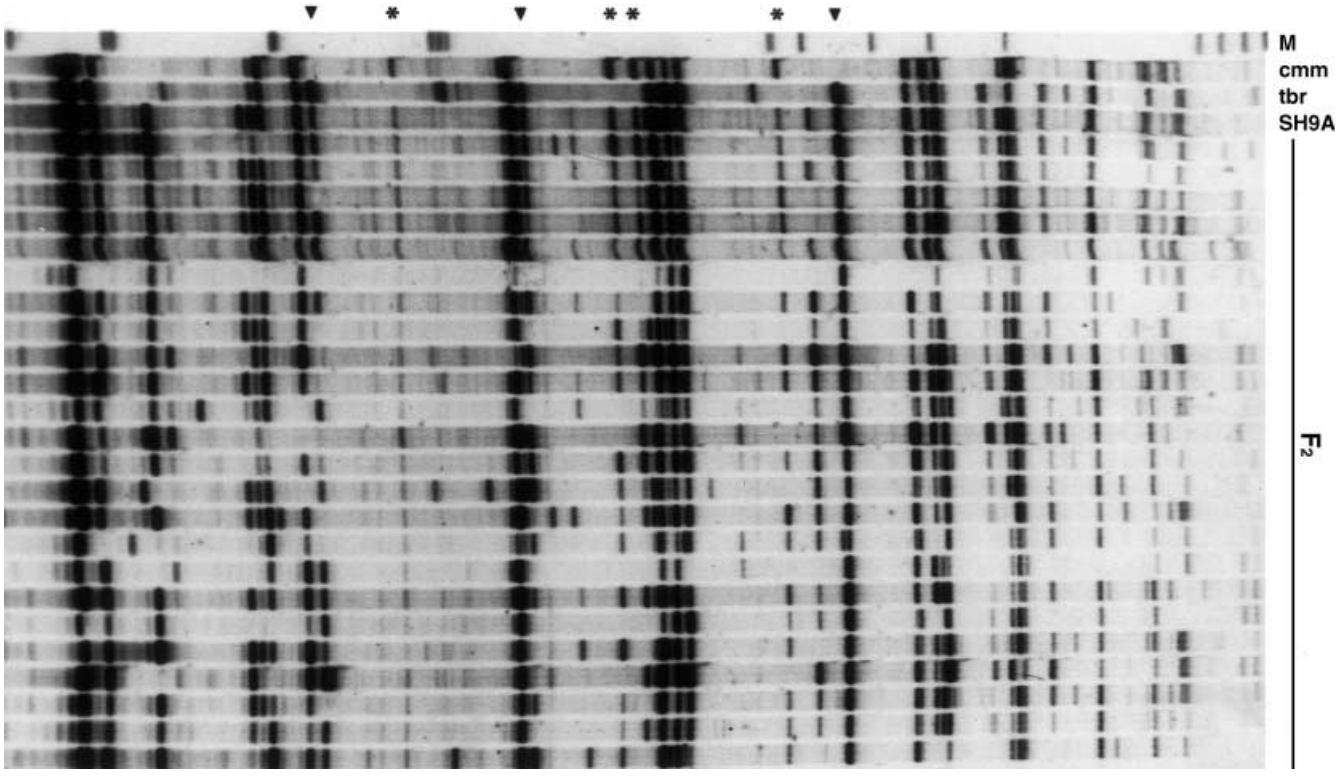

Fig. 1 AFLPs generated using the *Eco*RI-AGG+ *Mse*I-CAG primer combination. The segregation of cmm-specific (indicated by *asterisks*) and tbr-specific (indicated by *arrowheads*) AFLPs is shown for a sample of 24 F_2 genotypes. cmm=*S. commersonii*, tbr=*S. tuberosum*, SH9A=somatic hybrid, M=molecular-weight marker V (Boheringer Mannheim)

Table 5 Number and percentage (in parenthesis) of cmm-specific RAPDs, and cmm- and tbr-specific AFLPs which exhibit duplex or simplex genotypes. As far as duplex markers are concerned, both disomic and tetrasomic inheritance is also indicated

Species-specific markers	Analyzed loci no.	Type of segregation[a]				
		Duplex			Simplex no.	Distorted no.
		Total no.	Type of heredity			
			Disomic no.	Tetrasomic no.		
cmm-RAPD	16	15 (93.7)	7 (46.7)	8 (53.3)	1 (6.3)	0
cmm-AFLP	85	54 (63.5)	4 (7.4)	50 (92.6)	20 (23.5)	11 (12.9)
tbr-AFLP	187	61 (32.6)	7 (11.5)	54 (88.5)	98 (52.4)	28 (15.0)
Total	288	130	18	112	119	39

[a] The percentage of disomic and tetrasomic loci refers to the total number of duplex loci

the 13 cmm- and 15 tbr-specific AFLPs which fit a 20.8:1 ratio, and of six cmm- and ten tbr-specific AFLPs which fit a 2.5:1 ratio, for which a clear random chromatid segregation can be assumed in all cases. In most cases the distinction between random chromosome and chromatid segregation was not possible. Indeed, many AFLPs showed a segregation ratio which fit both the 35:1 and 20.8:1 (37 cmm- and 39 tbr-specific markers) ratios for duplex loci, and both the 3:1 and 2.5:1 (12 cmm- and 79 tbr-specific markers) ratios for simplex loci.

Table 5 summarizes the data related to both RAPD and AFLP analysis with respect to the type of segregation (duplex or simplex) and the type of heredity (disomic or tetrasomic), the latter in the case of duplex loci. The cmm-specific RAPDs were mainly (93.7%) duplex markers and were equally distributed between loci with a disomic (46.7%) and tetrasomic (53.3%) inheritance. By contrast, 92.6% of duplex cmm-specific AFLPs (63.5% of markers analyzed) were inherited as tetrasomic loci. As regards the tbr-specific AFLPs, the percentage of simplex loci (52.4%) was higher than that of duplex loci (32.6%), and among the latter most (88.5%) were inherited as tetrasomic loci. Overall, 130 duplex markers were found, of which 53.1% were cmm-specific and 46.9% were tbr-specific. Out of 130 markers, 18 (13.8%) were inherited as disomic and 112 (86.2%) as tetrasomic loci.

The total number of simplex loci was 119, and most of them (82.3%) were tbr-specific loci.

Discussion

The production of *S. commersonii* (+) *S. tuberosum* somatic hybrids provided the basis for the transfer and incorporation of useful genes from the wild to the cultivated species (Cardi 2001). However, their genetic potential can only be exploited if meiotic recombination between homoeologous chromosomes occurs, which requires synapsis and crossing-over. Interspecific somatic hybrids between diploids can be considered as disomic polyploids (De Jong et al. 1993), but the relationship between genomes of the parental species greatly determines both chromosome pairing and recombination. Matsubayashi (1991), when studying the genome relationships among potato species on the basis of cytological analysis and cross compatibility, assigned the compatible taxonomic series to *S. commersonii* (AA) and *S. tuberosum* (AAA^tA^t). In addition, the phylogenetic classification of various *Solanum* species based on chloroplast DNA analysis (Hosaka et al. 1984) defined a very close relationship between the cmm and tbr genomes. By contrast, the high morphological diversity exhibited by these two species (Hawkes 1990), as well as their low similarity indexes estimated through a nuclear DNA analysis (Sebastiano et al. 1999), suggested that they could be more-distantly related. The discordance between the relationships established through nuclear and cpDNA analyses has already been reported in potato (Spooner et al. 1996) as well as in other plant groups (Rieseberg and Wendel 1993). Moreover, phenetic trees inferred by RFLP analysis carried out on wild and cultivated relatives of *S. tuberosum* were ambiguous in determining the phylogenetic relationships within closely related clusters of the genus *Solanum* (Debener et al. 1990). Therefore, a more-detailed taxonomic classification, based on the use of different molecular markers and a varied number of *Solanum* species, is required to better assess the phylogenetic relationship between these two species.

Previously, great variability in morphological, reproductive and productive traits was observed in a population derived by selfing the somatic hybrid SH9A (Cardi et al. 2001). However, in that study it was not possible to conclude whether the observed variation represented not only an intragenomic recombination and segregation of parental chromosomes, but also an intergenomic (i.e. between homoeologous chromosomes) recombination. In order to acquire new evidence in this respect, a similar F_2 population was analyzed with a set of molecular markers in the present study. In fact, the tetrasomic inheritance of duplex and simple loci is clear evidence for the mode of pairing, tetravalent formation and the occurrence of crossing-over between homoeologous chromosomes.

Type of inheritance

The first step of our molecular analysis aimed to classify the analyzed loci as duplex or simplex loci. All the homozygous loci (duplex) in either one or the other parent were informative to determine the type of inheritance (disomic or tetrasomic) in the cmm (+) tbr somatic hybrid, even though additional indications also came from some heterozygous (simplex) loci, which clearly segregated as tetrasomic loci (2.5:1).

When considering the cmm-specific RAPDs, approximately 50% of markers were inherited as disomic and 50% as tetrasomic. The AFLP markers exhibited a different behaviour, since more than 85% were inherited as tetrasomic loci, independent of their being cmm- or tbr-specific AFLPs. The difference between the percentage of disomic cmm-specific RAPDs and AFLPs could be explained on the basis of their possible different distribution on the potato chromosomes. In fact, the seven cmm-specific RAPDs that exhibited a disomic inheritance could map as cluster loci on the same few chromosomes, which could preferentially pair as disomics. Non-random distribution of RAPD markers was also observed in *S. bulbocastanum-S. tuberosum* hybrids, as well as in other crops (Naess et al. 2001). Indeed, the higher number of cmm-specific AFLPs analyzed justifies their more-uniform distribution on the 12 potato chromosomes, thus probably explaining the higher percentage of cmm-specific AFLPs that showed tetrasomic inheritance. The mapping of these species-specific markers is in progress and will clarify the different behaviour of the RAPD and AFLP markers. By summing the tbr- and cmm- AFLPs which showed tetrasomic inheritance (112 loci), it is possible to conclude that more than 90% of the duplex loci (130) analyzed are located on chromosomes which at meiosis tend to randomly pair as bivalents or to form tetravalents. This value (90%) of potential homoeologous pairing is very high if compared not only to that observed in the case of hexaploid somatic hybrids between the cultivated *S. tuberosum* and the distantly related wild species *Solanum brevidens* (Williams et al. 1993) and *Solanum bulbocastanum* (Masuelli et al. 1995), but also when compared to the values observed in the somatic hybrid between *S. tuberosum* and the more closely related species *Solanum acaule* (Yamada et al. 1998).

Crossing-over events

Since homoeologous pairing does not always imply recombination, some evidence of the occurrence of crossing-over is required, which also depends on the position of each locus with respect to the centromere. Multivalent formation can result in double reduction, which is clear evidence of crossing-over between non-sister chromatids and is indicated by the appearance of the recessive phenotype after selfing a triplex genotype. However, in our analysis it was not possible to identify triplex loci on the

basis of the segregation ratios, since a greater population would be necessary. We could only clearly distinguish the segregation ratios obtained after selfing duplex or simplex loci, and then, depending on the segregation ratios observed, we were able to hypothesize the occurrence, or otherwise, of cross-over events. Therefore, we determined how many duplex and simplex tetrasomic loci segregated according to a random chromatid segregation ratio (20.8:1 and 2.5:1 for duplex and simplex loci, respectively); that is, the limit case of segregation expected when the distance between the locus and the centromere always leads to a cross-over event.

Unfortunately, in most cases the segregation of both tbr- and cmm-specific markers fit more than one segregation ratio, and only in those cases for which clear 20.8:1 and 2.5:1 segregation ratios were observed could a cross-over event be assumed (three cmm-specific RAPDs, 19 cmm- and 25 tbr-specific AFLPs). The percentage of these events was around 37% if considering the RAPDs and AFLPs which fit these ratios, out of the tetrasomically inherited loci clearly identified (128 loci). Also the cases for which both the 35:1 and 20.8:1 or the 3:1 and 2.5:1 ratios were observed could potentially be the consequence of recombination events. In fact, random chromosome and random chromatid segregations represent the two limiting cases of segregation in a tetrasomic polyploid genotype. The first can only occur under the special circumstances of random pairing and absence of chiasmata between the centromere and the locus, whereas the second can occur only under conditions of random pairing but with a certain frequency of crossing-over between the centromere and the locus. So, these segregation expectancies are of limiting types and the true segregation will probably lie somewhere in between. This could justify the high number of cmm- and tbr-specific markers which fall in the loci classified as 35:1/20.8:1 and 3:1/2.5:1 segregating loci. Indeed, the reduced number of F_2 genotypes (90) could also explain the difficulty in clearly assigning the segregation ratio of these AFLPs to either one or the other type. Probably, the same analysis performed on a larger population could lead us to assign with a statistical significance more markers to one or the other class, as also reported when mapping RAPDs in the polysomic polyploid alfalfa (Yu and Pauls 1993). The reduced size of the population and the different genetic, physiological and environmental factors, which can cause distorted segregation ratios, could explain most cases of unclassified loci (classes B and D, Table 2).

Despite all the unresolved cases, we were able to achieve the objective of our molecular analysis, which was performed to verify to what extent the recombination between cmm and tbr chromosomes occurred in the tetraploid cmm (+) tbr somatic hybrid. Indeed, our data clearly suggest that the variability observed in an F_2 progeny previously analyzed for various morphological traits (Cardi et al. 2001) could be the result not only of segregation but also of recombination between the homoeologous cmm and tbr genomes. As a result, several F_2 plants with improved agronomic characteristics in comparison with the parental somatic hybrid could be identified. Accordingly, when an independent F_2 progeny from the same somatic hybrid was analyzed for frost tolerance and acclimation capacity, a large variability for both traits was observed, and plants were identified with a better frost tolerance and/or acclimation capacity than that of the somatic hybrid, and close to that of the parental wild species (Seppanen et al. 1998). Therefore, the combination of our molecular data with the observed morphological and physiological variability in various F_2 progenies from the same cmm (+) tbr somatic hybrid, clearly indicated that a flow of genes from the sexually isolated cmm to the cultivated potato is possible, for at least a large proportion of genes.

Acknowledgements Contribution no. 227 from the Research Institute for Vegetable and Ornamental Plant Breeding, CNR. The authors thank Dr. D. Carputo and Prof. F. Veronesi for critical reviewing of the manuscript, Dr. Mark Walters for editing the manuscript, and A. Cozzolino for her technical assistance. This research was partially financed by Mi.P.A.F in the framework of the Project "Miglioramento Genetico della Patata" and by FSE. The experiment complies with the current laws of Italy.

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