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Irregular meiosis in a somatic hybrid between *S. bulbocastanum* and *S. tuberosum* detected by species-specific PCR markers and cytological analysis

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Abstract A system of randomly amplified polymorphic DNA (RAPD) markers was developed to facilitate the transfer of *S. bulbocastanum* (*blb*) genes into the *S. tuberosum* (*tbr*) genome by hybridization and backcrossing. DNA from *tbr*, *blb* and the hexaploid hybrid was used as a template for polymerase chain reaction (PCR) amplification. Polymorphic RAPD products, originating from 10-mer primers, specific for *blb* were cloned and sequenced at their ends to allow the synthesis of 18-mer primers. The 18-mer primers allowed a more reproducible assay than the corresponding RAPDs. Of eight 18-mer primer pairs, four amplified the expected products specific for *blb*. However, the stringency of the primer annealing conditions needed to be carefully optimized to avoid amplification of the homeologous *tbr* product, suggesting that the original RAPD polymorphisms were due to single base-pair changes rather than deletions or insertions. Two primers used for amplification of backcross 2 progeny segregated in a 1:1 (presence:absence) ratio; the other two were unexpectedly absent. The most likely explanation for the loss of these markers is irregular meiosis in the original hexaploid hybrid and subsequent elimination of chromosomes. Cytological analysis of the meiosis in the hybrid demonstrated widespread irregular pairing and the presence of lagging univalents. In addition, the first backcross individual used as the parent for the second backcross had 54 chromosomes instead of the predicted 60. In conclusion, our results demonstrate that PCR technology can be used for the efficient isolation of taxon-specific markers in *Solanum*. Furthermore, by the use of these markers we detected the loss of chromosomes that was subsequently shown by cytological analysis to be caused by irregular meiosis of the somatic hybrid.

Key words *Solanum* · Potato · RAPD · Interspecific hybrids · SCAR

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Introduction

The *Solanum* wild tuber-bearing species related to the cultivated potato, *Solanum tuberosum*, represent an important reservoir of genetic diversity (Hawkes 1990). Resistance to different diseases has been introduced into *S. tuberosum* from wild species by wide hybridization and backcrossing. However, the introduction of genes into cultivated varieties is not straightforward. The first step, hybridization, must overcome sexual barriers and, often, ploidy barriers. The second step, the transfer of the target gene into the recipient genome, must take place with the concurrent exclusion of genes responsible for undesirable characters (Watanabe 1994).

Solanum bulbocastanum, a tuber-bearing, diploid wild species endemic to Mexico and a relative of cultivated tetraploid potato is resistant to several nematode pests. Our goal is the transfer of these resistance genes from *S. bulbocastanum* to cultivated potato. Because crossability barriers prevent the production of sexual hybrids (Hermsen and Ramanna 1976), Austin et al. (1993) generated a somatic hybrid by protoplast fusion. The hybrid, expected to have a hexaploid genome, was backcrossed to *S. tuberosum* to produce plants that should have the normal tetraploid genome of *S. tuberosum* and the haploid genome from *S. bulbocastanum*, and thus should be pentaploid. By repetitive backcrossing with *S. tuberosum*, portions of the *S. bulbocastanum* genomes

should be progressively lost by chromosome loss, possibly combined with homeologous recombination. Molecular markers that identify the *S. bulbocastanum* genome would facilitate the analysis of backcross progeny.

Several DNA-based molecular markers have been developed in plants: restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD) (Williams et al. 1990), microsatellites and cleaved amplified polymorphic sequences (CAPs) (Akkaya et al. 1992). While RFLPs have been used to construct the genetic map of potato (Bonierbale et al. 1988; Gebhardt et al. 1989) and of other crop species (reviewed in O'Brien 1993), RAPDs promise to be a more cost-effective diagnostic technology for plant breeding. Because of its simplicity, RAPD analysis could be done in a moderately equipped laboratory, and since only a small amount of tissue is required, plants in a breeding program could be screened at the seedling stage. Furthermore, RAPD-based bulk segregant analysis is a very effective way to obtain markers linked to resistance genes (Michelmore et al. 1991; Barua et al. 1993; Penner et al. 1993). However, RAPD analysis is sensitive to minor changes in the amplification procedure because it uses short (10-mer) primers, and therefore reproducibility is a weak point of this technology. Paran and Michelmore (1993) have proposed the sequencing of RAPD products and their conversions to normal PCR products by the use of longer primers. These markers constitute a type of Sequence Tagged Site (STS, Olson et al. 1989) and have been termed Sequence Characterized Amplified Regions (SCARs; Paran and Michelmore 1993).

The objective of this study was to test the suitability of RAPD and SCAR markers to monitor the introgression of the *S. bulbocastanum* genome into the cultivated potato genome. We show here that RAPD and SCAR markers specific for *S. bulbocastanum* can be generated quickly and efficiently. Furthermore, by the use of these markers we discovered the loss of genetic material during meiosis of the somatic hybrid.

Materials and methods

Plant material

The parental species and the somatic hybrid (CBP) between *S. tuberosum* (R4) and *S. bulbocastanum* (SB22) were used. *S. bulbocastanum* (*blb*) is diploid (2n = 2x = 24) and *S. tuberosum* (*tbr*) is tetraploid (2n = 4x = 48). The interspecific hybrid (CBP) is hexaploid (2n = 6x = 72) and was obtained by protoplast fusion (Austin et al. 1993). The first backcross (BC_1) was derived from the cross CBP × *tbr* (A 84118.3) and the second (BC_2) backcross from the cross DG17 × *tbr* (A 84118.3); DG17 is one BC_1 individual. In all cases, the parental female is noted first.

DNA amplification

Total genomic DNA was isolated from leaf material by the method of Dellaporta et al. (1983), with two additional steps involving RNAase digestion and Sephacryl S-300 size-exclusion chromatography.

Genomic DNA from *tbr*, *blb* and CBP was used as a template for polymerase chain reaction (PCR) amplification. Either a single decameric primer or two different decameric primers from Operon Technologies (Alameda, Calif.) were used in each PCR reaction. Between 30 and 40 ng of DNA was used as a template in a 50-µl reaction volume that contained 10 m*M* Tris-HCl pH 8.0, 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 0.001% gelatin, 0.01% Tween-20, 0.01% Triton X-100, 100 µ*M* of each dNTP, 0.2 µ*M* primer and 1 unit of *Taq* polymerase (Promega, Madison, Wis.). The thermocycler (PTC-100, MJ Research, Watertown, Mass.) was programmed for an initial denaturation step of 93 °C for 3 min and then 45 cycles of 94 °C for 15 s, 35 °C for 30 s and 72 °C for 2 min. The 45 cycles were followed by a 2-min final extension at 72 °C. Amplification products were resolved by electrophoresis at 5 V/cm for 2 h in a 1.2% agarose gel.

Cloning and sequencing

The amplified products present only in *blb* and CBP were isolated by agarose gel electrophoresis and electroelution of the excised DNA using a unidirectional electroeluter (International Biotechnologies, New Haven, Conn.). The isolated DNA was reamplified using the same primer used in the original reaction and ligated into the pCR™ II vector from the Invitrogen TA Cloning System kit (Invitrogen, San Diego, Calif.). To screen putative clones, we amplified the cloned fragment with the M13 forward and reverse primers and then compared the size of the amplified fragment with the original one. The relationship of the cloned fragment to all the RAPD products from the original amplification was determined by hybridization. The cloned *blb*-specific products were used to probe the Southern transfer of a gel loaded with all the products amplified from *blb*, *tbr* and CBP by the same primer.

The ends of the clones were sequenced by the dideoxy-chain termination method using Sequence according to the protocol of the manufacturer (U.S.B., Cleveland, Ohio). For each cloned *blb*-specific product, two new oligonucleotide primers were designed by lengthening the original 10-mer by 8 or 7 bases.

Cytological analysis

Meiotic analyses were performed on flower buds fixed for 48 h in a solution of ethanol-acetic acid (3:1) at room temperature and stored in 70% ethanol at 4 °C. Anther squashes were stained with a 1% acetocarmine solution. Chromosome number was determined in root tips of young seedlings pre-treated with 8-hydroxiquinoline solution (0.29 g/l) for 4 h at room temperature and then fixed in an ethanol: acetic acid (3:1) solution for at least 24 h. Roots tips were hydrolized for 10 min in 1 *N* HCl at 60 °C, stained with leucobasic fuchsin solution (Coleman 1938) for 1 h and squashed in 45% acetic acid.

Results

Single and double primer RAPD amplifications

DNA from *S. tuberosum* (*tbr*), *S. bulbocastanum* (*blb*) and the somatic hybrid (CBP) obtained by protoplast fusion (Austin et al. 1993) was used as a template for PCR amplification with 62 decameric primers each used individually. Each primer amplified an average of 4.32, 4.69 and 4.21 products of *tbr*, CBP and *blb*, respectively. The product sizes ranged from 300 to 3000 base pairs (bp), most of them falling in the 500- and 2000-bp range (Fig. 1A). Twenty-four percent of the primers did not yield any product. 60% of the primiers were polymorphic and generated 38 products present in both *blb* and

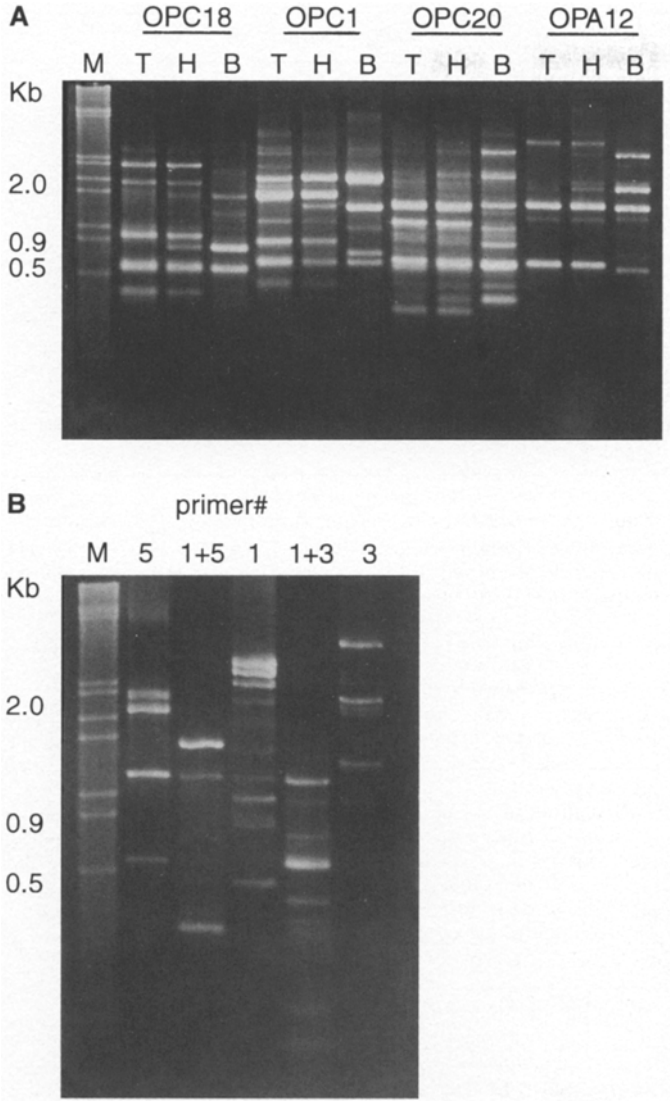

Fig. 1A,B RAPD analysis of *S. tuberosum* (T), *S. bulbocastanum* (B) and CBP-hybrid (H) DNAs. **A** Single primer amplification with four different primers. **B** Two primer amplification with primer OPA 1, 3 and 5 used separately and in combination on *S. tuberosum* DNA. (M) molecular size markers, bacteriophae lambda digested with *Hin*dIII and *Eco*RI

the CBP. The remaining 16% were monomorphic or yielded products specific for the *tbr* genome. Each polymorphic primer generated from 1 to 3 polymorphic products specific for the *blb* genome, but most primers (20/28) produced only 1 polymorphic band.

It should be possible to obtain additional products by using a combination of primers. To test this, we ran PCR reactions combining two different primers. We tested eight of the possible combinations (in a 1:1 ratio) of 7 different primers, each previously used in a single primer amplification. All of the reactions with 2 primers yielded new products different from the products made by each primer alone (Fig. 1B). Each combination generated from 3 to 8 amplification products whose sizes ranged from 100 to 2300 bp, usually (40%) with sizes from 100 to 500 bp. Six of the eight combinations produced 2 to 3 products each specific for the *blb* genome.

Out of 181 *blb* products analysed, 36% were specific for the *blb* genome and 64% were common between *blb* and *tbr*. In general, the hybrid had a combination of both *tbr* and *blb* banding patterns. However, 26 products present in one or another parent were not present in the hybrid; similarly, 11 hybrid products were not present in the patterns of the parents.

Cloning and sequencing of species-specific RAPD bands

We cloned 20 different RAPD products that were putatively specific for the *blb* genome. To verify that the cloned RAPD products were specific to the *blb* and the CBP genomes, we used each clone to probe RAPD Southern blots. A *blb*-specific product should hybridize to the corresponding band in both the *blb* and the CBP lanes. However, it should not hybridize to the *tbr* products of corresponding size. Out of 20 primers, 18 satisfied this species-specific criterion, but 2 cloned RAPDs hybridized to *tbr* even though no visible DNA of the corresponding size was present in the gel.

The ends of 8 cloned *blb*-specific products were sequenced, and new primers were synthesized by adding 8 bases to the original 10-mer primers. All new primers were 18-mers, with the exception of $LPA8_{560-2}$, which is a 17-mer. Thus, for each cloned fragment 2 new primers were made (Table 1).

Amplification of genomic DNA with the 18-mer primers

Genomic DNA from *tbr*, CBP and *blb* was used as a template for PCR amplification with each pair of the 18-mer primers. All eight primer pairs produced multiple products rather than the expected single product for *blb*. Table 2 summarizes the results obtained when we amplified genomic DNAs from *tbr*, CBP and *blb* with the eight long primer pairs. At the annealing temperature of 55 °C, each of the primer pairs $LPA2_{900}$ and $LPC19_{1300}$ ($LPC19_{1300}$ also in Fig. 2) yielded the expected product for *blb* and CBP and small quantities of the product for *tbr*. When the annealing temperature was increased from 55 °C to 65 °C, unexpected products were eliminated and a single band of the same size as the original RAPD fragment was obtained for both *blb* and CBP.

At the annealing temperature of 50 °C in the presence of 5% DMSO, $LPA9_{900}$ yielded a single abundant product of the appropriate size in both *blb* and CBP, but not in *tbr*. $LPC20_{400}$ at 58 °C yielded the correct product from both *blb* and CBP, but other fragments were amplified in *tbr* and CBP. The remaining four primer pairs, $LPA7_{560}$, $LPA16_{500}$, $LPA8_{560}$ and $LPB17_{500}$, under the conditions that were tested, yielded the ex-

Table 1 Sequence of the 18-mer primers and the type of polymorphism detected by the amplification of *S. bulbocastanum*, *S. tuberosum* and the hybrid (CBP) genomic DNAs with the pair of primers

Primer	Sequence[a]	Polymorphism[b]
$LPA2_{900\text{-}1}$	**TGCCGAGCTG**GCTGCATC	Dominant at 65 °C
$LPA2_{900\text{-}2}$	**TGCCGAGCTG**AAGGAATT	
$LPA7_{600\text{-}1}$	**GAAACGGGTG**ATCTTCAG	None detected
$LPA7_{600\text{-}2}$	**GAAACGGGTG**CGCCATGC	
$LPA16_{600\text{-}1}$	**AGCCAGCGAA**TTGTTGAG	None detected
$LPA16_{600\text{-}2}$	**AGCCAGCGAA**CAGTAGCG	
$LPC19_{1300\text{-}1}$	**GTTGCCAGCC**TTCCTCCT	Dominant at 65 °C
$LPC19_{1300\text{-}2}$	**GTTGCCAGCC**ATGATGAG	
$LPA9_{1000\text{-}1}$	**GGGTAACGCC**ATTCATAG	Dominant at 50 °C
$LPA9_{1000\text{-}2}$	**GGGTAACGCC**AAATCTACG	
$LPC20_{400\text{-}1}$	**ACTTCGCCAC**TACTTCAC	Dominant at 58 °C
$LPC20_{400\text{-}2}$	**ACTTCGCCAC**TACTTCGT	
$LPB17_{560\text{-}1}$	**AGGGAACGAG**GACAGTTG	None detected
$LPB17_{560\text{-}2}$	**AGGGAACGAG**CATAAAAC	
$LPA8_{560\text{-}1}$	**GTGAACGTAGG**CCCAAACA	None detected
$LPA8_{560\text{-}2}$	**GTGACGTAGG**GAGCTGG	

[a] The bold characters represent the sequence of the original 10-mer primer
[b] Polymorphism detected at specific annealing temperature for each pair of primers; dominant refers to the presence of a product in *S. bulbocastanum* and its absence in *S. tuberosum*

Table 2 Specificity of 18-mer primers for parental genotypes. Amplification of *tbr* (T), CBP-hybrid (H) and *blb* (B) DNAs with primer pairs that were derived from *blb*-specific RAPDs (see results). The appearance of amplification products in low (+) or high (++) yields or the lack of amplification products (−) was tested at different temperatures

Primers	Pcr Conditions																	
	50[a]			55			58			60			62			65		
	T	H	B	T	H	B	T	H	B	T	H	B	T	H	B	T	H	B
LPC_{1300}	nd	nd	nd[b]	+	++	++	nd	nd	nd	+	++	++	+	++	++	−	++	++
$LPC20_{400}$	nd	nd	nd	+	+	+	−	++	++	nd	nd	nd	nd	nd	nd	nd	nd	nd
$LPA9_{900}$	−	++	++[c]	−	+	+	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
$LPA2_{900}$	nd	nd	nd	+	++	++	nd	nd	nd	+	++	++	nd	nd	nd	−	++	++
$LPC7_{560}$	nd	nd	nd	++	++	++	nd	nd	nd	+	+	++	nd	nd	nd	nd	nd	nd
$LPA16_{500}$	nd	nd	nd	++	++	++	nd	nd	nd	++	+	+	+	−	−	nd	nd	nd
$LPA8_{560}$	nd	nd	nd	++	++	++	nd	nd	nd	++	++	++	++	++	++	+	+	+
$LPA17_{500}$	++	++	+	++	++	++	+	+	+	+	+	+	−	−	−	nd	nd	nd

[a] Annealing temperature in degrees centigrade
[b] nd, not determined
[c] With the addition of 5% dimethyl sulfoxide

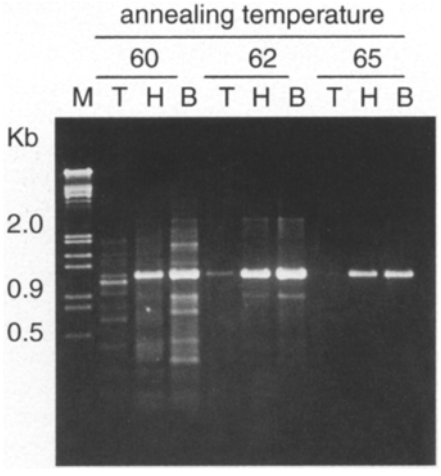

Fig. 2 Amplification of DNA from *S. tuberosum* (T), *S. bulbocastanum* (B) and CBP-hybrid (H) with the 18-mer primer pair $LPC191_{300}$, at three different annealing temperatures. (M) molecular size markers, bacteriophase lambda digested with *Hin*dIII and *Eco*RI

pected product from all three genomes. $LPA7_{560}$, $LPA16_{500}$ and $LPB17_{500}$ also amplified other fragments.

Segregation of the SCARs in BC_2 progeny

DNA from 25 plants of the BC_2 progeny were used for amplification with the 18-mer primer pairs (data not shown). $LPC19_{1300}$ and $LPC20_{400}$ yielded a product of the same size as the original RAPD markers from which they were derived, but $LPA2_{900}$ and $LPA9_{900}$ did not amplify any product. The segregation for $LPC19_{1300}$ and $LPC20_{400}$ in BC_2 progeny was 13:12 and 11:14 (present:absent), respectively. $LPA2_{900}$ and $LPA9_{900}$ were used for the amplification of DNA from 4 plants (DG17, DG5, DG3, DG15) of the BC_1 progeny. $LPA9_{900}$-generated products were present in 1 and $LPA2_{900}$ products in 2 out of 4 plants tested; neither $LPA9_{900}$ nor $LPA2_{900}$ were present in DG17, which was the parent used for the BC_2.

Hybridization analysis of longer primers' amplification products

The cloned DNA amplification products of $LPC19_{1300}$, $LPC20_{400}$, $LPA2_{900}$ and $LPA9_{900}$ were radiolabeled and hybridized to Southern blots of genomic DNA from

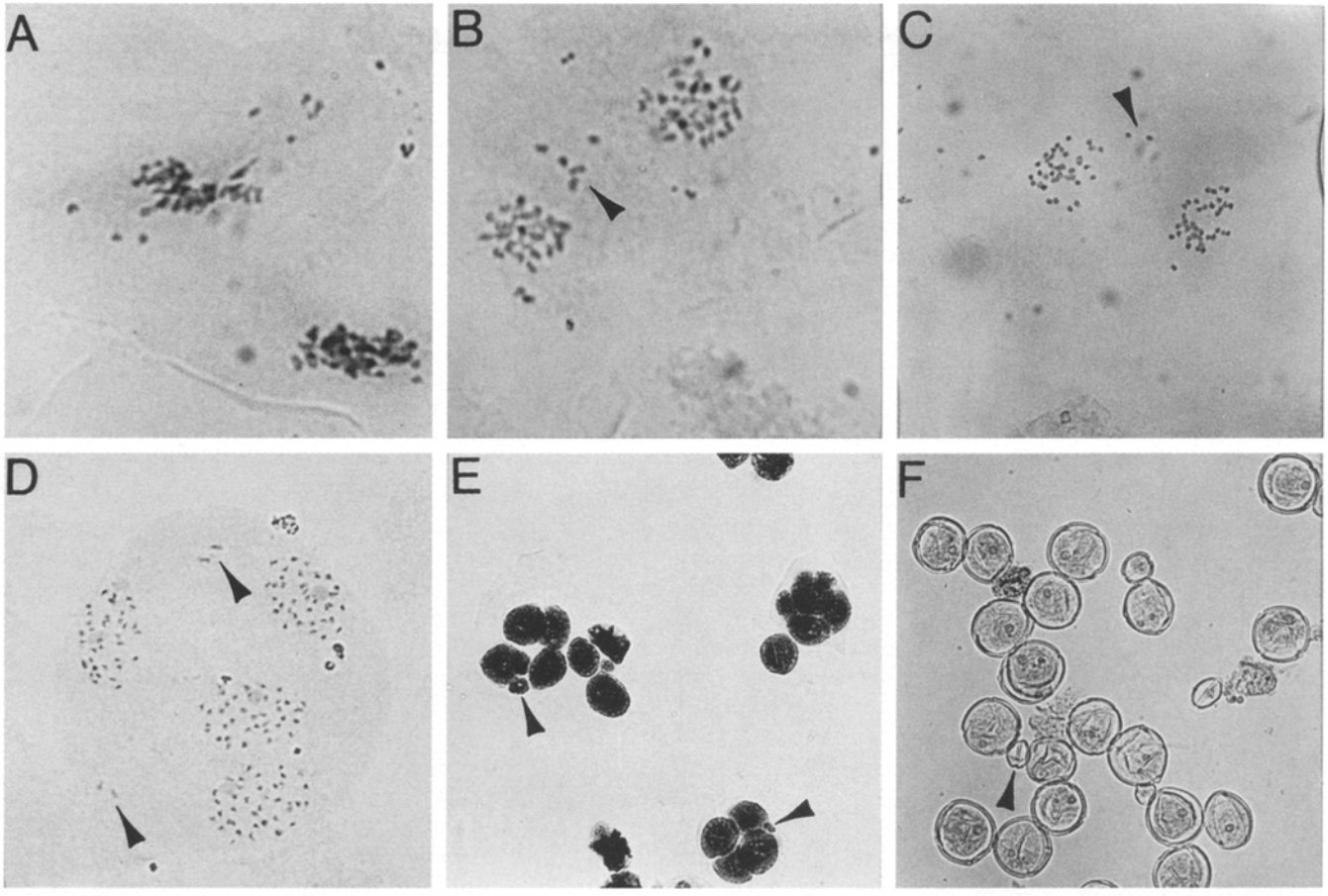

Fig. 3A–F Meiotic observations of CBP. **A** Metaphase I showing stickiness of chromosomes and early separation of univalents. **B** Six lagging chromosomes at telophase I (arrow). **C** Meiocyte at telophase I with 36 and 34 chromosomes in each pole and two lagging chromosomes (arrow). **D** Telophase II with four chromosomes not included in the nuclei (arrows). **E** Tetrads with micro-cells (arrows). **F** Pollen grains and micro-pollen (arrow)

tbr, *blb*, and CBP plants to determine their genomic organization. The fragment number and hybridization signals of the four probes suggested that they are present in genomic DNA in low copy number and may be useful as RFLP probes (data not shown).

Cytological observations

The analysis above showed that *blb*-specific markers originally present in CBP were present only in some of the individuals in the BC_1 progeny, all of which were expected to be hemizygous for these markers. As a result, in the BC_2 progeny *blb*-specific markers were either absent or segregated in the expected 1:1 ratio. To determine whether the markers were lost due to abnormalities during meiosis in the CBP hybrid, we examined chromosome behaviour in microsporogenesis. At metaphase I, the chromosomes showed a tendency to clump together in the equatorial plate making it difficult to observe the chromosomal configuration (Fig. 3A). Nevertheless, some chromosomal figures, especially univalents, could be scored. Further observations were made at diakinesis, where the average frequency of chromosomal figures for seven cells analyzed was 11.5 univalents, 24.25 bivalents and 4 trivalents. Many of the univalents observed at metaphse I remained as lagging chromosomes at anaphase I (Fig. 3B and C) and appeared to remain unassociated with any metaphase plate through the second meiotic division. At telophase II 84% of the cells observed had betweenn 1 and 6 dispersed chromosomes per cell (Fig. 3D). Forty-five percent of the tetrads had 1 or 2 micro-cells (Fig. 3E). Chromosome counts in root tips of 5 BC_1 plants (DG17, DG15, DG14, DG19 and DG6) showed that these plants had 54, 55, 53, 60 and 56 chromosomes, respectively. The expected chromosome number for a BC_1 individual is 60, 12 from *blb* and 48 from *tbr*.

Discussion

We expected the *blb* genome to be similar but not identical to the *tbr* genome. *S. bulbocastanum* and *S. tuberosum* are distantly related (Hawkes 1990; Spooner and van der Ber 1992). According to Matsubayashi (1991) the genome formula for *blb* is A^bA^b and for *tbr* is AAA^tA^t. These species have the genome "A", which is regarded as the most basic one; the superscript "b" and

"t" indicate that minor structural differences in the chromosomes have been found. We chose RAPD analysis to search for markers specific to the *blb* genome and found that on average 64% of the *blb* RAPD fragments scored were common between both species. As Klein-Lankhorst et al. (1991) found for tomato species, the RAPD technique provides a highly effective means to fingerprint potato species and interspecific hybrids (Xu et al. 1993). The gel pattern of RAPD products of the somatic hybrid should be the sum of the parental patterns. However, we found exceptions to this prediction. The appearance of products that are not present in the parents, or the disappearance of some parental products, could be explained by primer competition during annealing (Williams et al. 1990) or by somaclonal variation (Xu et al. 1993).

We attempted the conversion of eight *blb*-specific RAPDs into SCARs (or STSs) by cloning and sequencing the RAPD products, thereby allowing the lengthening of the primers from 10 to 18 nucleotides. Although these longer primers were expected to be specific for *blb*, at low stringency they amplified the homeologous *tbr* loci. In general, increasing the annealing temperature made it possible to eliminate the *tbr* PCR product. These results confirm the assumption of Williams et al. (1990) and of Paran and Michelmore (1993) that RAPD polymorphisms are caused by mismatches in 1 or a few nucelotides in the priming sites, rather than by inversion or deletions. The 18-mer primer pairs $LPC19_{1300}$, $LPA9_{900}$ and $LPA2_{900}$ yielded single products, while $LPC20_{400}$ amplified a predominant product of the same size as the original RAPD marker from which it was derived along with other products. We could not eliminate the *tbr* product for the primers $LPA7_{560}$, $LPA16_{500}$, $LPA8_{560}$ and $LPB17_{500}$. Presumambly, the longer primers tolerated the mismatch(es) at the priming site and the *tbr* product was amplified, thereby losing the polymorphism obtained with the 10-mer primers. Such a response may depend on the position of the mismatch. For example, a mismatch in the 5′ region of the primer may affect extension of a 10-mer but allow extension of an 18-mer primer. In conclusion, conversion from RAPD to SCAR was successful in four of eight cases.

The linkage map in potato has been constructed based on diploid crosses (Bonierbale et al. 1988; Gebhardt et al. 1989). The map distances calculated for diploids may differ in tetraploids. On the other hand, many characters present in tetraploids as quantitative traits may not be mapped in diploids (Yu and Pauls 1993). RFLP maps of polyploids are more difficult to construct than those of diploids due to the high number of genotypes expected in the segregant population. Wu et al. (1992) proposed a method for mapping polyploids based on the segregation of single-dose restriction fragments (SDRFs) and analysis of the segregation of each restriction fragment based on its presence or absence in the progeny. The SCARs are promising tools for mapping polyploid species because these markers segregated 1:1 in the progeny. Therefore, these SCARs could be used as SDRFs to map tetraploid potatoes by the method of Wu et al. (1992).

These SCARs, although defined by specific reaction conditions, allowed a more reproducible assay than the corresponding RAPDs. Therefore, it is possible to use RAPDs for a quick and efficient screen for species-specific DNA markers in *Solanum* and then to convert these markers into SCARs. It may be possible to develop these dominant SCARs for use as a quick plus/minus assay, such as an ELISA test, in the segregant progeny. In addition, the cost for PCR-based analysis can be reduced by scaling down the volume of the reaction and increasing the number of polymorphic bands by the use of primer combinations.

When we tested the inheritance of the SCARs in the backcross progeny, we found that the segregation of $LPC19_{1300}$ and $LPC20_{400}$ fit a 1:1 (present:absent) ratio. This is the expected ratio for dominant markers and random chromosome segregation in the progeny of a pentaploid in the simplex condition (Aaaaa). The other two SCARs did not amplify any product. There are several explanations for the loss of these markers. First, they could be plastid loci. However, because these markers represent single or low-copy-number sequences in the *blb* genome, they are unlikely to be plastid sequences. Second, since the SCARs are dominant markers, the locus corresponding to the lost markers could have been heterozygous, and the BC1 parent could have inherited the recessive allele (lacking the markers). According to Dr. C. Brown (personal communication) all RFLPs sampled over both arms of the twelve *blb* chromosomes were homozygous; therefore, this possibility is unlikely. Third, if homeologous pairing occurs, the *blb* markers could be lost by recombination and segregation in the CBP parent. Fourth, if homeologous pairing does not occur, some haploid *blb* chromosomes in the BC_1 parent could be lost during meiosis. Fifth, chromosome loss may occur in CBP because of disrupted meiotic pairing.

The last three hypotheses can be tested by examining meiosis. Disrupted meiosis in CBP would suggest that the last hypothesis is correct. Due to the hexaploid hybrid having four sets of *tbr* and two sets of *blb* chromosomes, we expected to have homologous pairing. Hexaploid hybrids between *S. acaule* and *S. bulbocastanum* show nearly complete homologous pairing of chromosomes, normal separation of chromosomes at anaphase and high pollen fertility (Hermsen and Ramanna 1969). However, the pollen of CBP is sterile (Austin et al. 1993), suggesting that meiosis may be defective. We examined meiosis in CBP anthers and counted the chromosomes in root tip cells of the first backcross plants.

Meiotic observations of CBP showed the presence of lagging and disperse chromosomes that were not incorporated into the nucleus of telophase II cells(Fig. 3D), leading to the formation of micro-pollen (Fig. 3F) and, probably, to gametes with unbalanced chromosome

numbers. The somatic chromosome number of DG17 was 54 rather than the 60 chromosomes expected for a pentaploid individual. According to our results, $LPA9_{900}$ and $LPA2_{900}$ had segregated in the BC_1 progeny and were absent in the parent used in BC_2. Therefore, the loss of these markers is most likely due to the irregular meiosis in CBP leading to the elimination of chromosomes. It remains to be investigated whether homeologous pairing and recombination takes place in the hybrid.

Ehlenfeldt and Helgeson (1987) described meiotic abnormalities in somatic hybrids between diploid *S. brevidens* and *S. tuberosum*. However, Williams et al. (1993) reported that loss of *S. brevidens* chromosomes was uncommon, possible because of a bias toward homologous pairing for the *S. brevidens* chromosomes. McGrath et al. (1994) found evidence for homeologous recombination in the backcross progeny of *S. brevidens-S. tuberosum* hybrids. In our material widespread irregularities were observed early in the meiosis of CBP, in diakinesis or metaphse I early separation of univalents were observed (Fig. 3A). These irregularities suggest the existence of pairing problems during prophase I, presumably caused by the genomic differences between *tbr* and *blb*. The precise cause of this phenomenon is unknown. However, as suggested by Dvorak (1983) the genotypes of diploid species in *Solanum* could promote heterogenetic pairing in polyploid hybrids. If the *blb* chromosomes compete with the *tbr* chromosomes for pairing sites, "losing" chromosomes could be left as univalents. Benavente and Orellana (1991) suggest that in colchicine-doubled rye hybrids the pairing ability is an inherent property of each chromosome and does not depend on its potential pairing partners. Also, they found that in competitive situations the associations between more similar partners are not always favored. The presence in diploid species of enhancers of pairing is well-known in the graminae (Sears 1976): it is possible that such determinants may be carried by the *blb* genome and may affect the pairing of chromosomes in the hybrid.

The production of aneuploids by backcrossing the somatic hybrid to *tbr* suggests a powerful approach to the mapping of the *blb* genome and to the isolation of markers linked to genes of interest. Mc Grath et al. (1994) found that BC_1 plants from the *S. bervidens-S. tuberosum* somatic hybrid lacking the *S. brevidens* RFLP-synteny group were of greater utility in mapping RAPDs than the aneuploids in BC_2.

According to our results, the backcross progeny is either deficient for entire *blb* chromosomes or for fragments of chromosomes, depending on whether homeologous recombination took place. In either case, groups of linked markers are expected to be absent from the genome of most individuals. Linkage could thus be established by the comparative testing of a relatively small number of backcross progeny (20–100, depending on the rate of chromosome loss). If no homeologous recombination is taking place, linkage groups will correspond to *blb* chromosomes. If homeologous recombination takes place, linkage groups as defined by a single individual may only cover part of a chromosome. However, chromosomal groups could be defined by comparing individuals containing overlappinig sets of missing markers.

If the backcross 1 progeny is tested phenotypically for the presence of genes of interest, positive and negative plants could be bulked and subjected to bulk segregant analysis as described by Michelmore et al. (1991). For dominant genes, the markers absent in all the negative plants should be linked to the gene of interest.

Therefore, in order to facilitate the transfer of nematode resistance genes from *blb* to *tbr* we propose to search the first backcross progeny for *blb*-specific RAPD markers linked to the resistance gene by bulk segregant analysis and then convert these RAPD markers to SCARs for a more reproducible assay.

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