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Fertile somatic hybrids of *Solanum* species: RFLP analysis of a hybrid and its sexual progeny from crosses with potato

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Summary. Restriction fragment length polymorphism (RFLP) markers were used to distinguish the chromosomes of *Solanum brevidens* from those of potato (*S. tuberosum*) in a fertile somatic hybrid. The hybrid had markers that account for all 24 chromosome arms from each parent, indicating that the hybrid contained at least one copy of each chromosome from each parent. The markers were then used to follow segregation of chromosomes in sexual progeny that resulted from a cross of the somatic hybrid with the potato cultivar 'Katahdin'. Approximately 10% of the sexual progeny lacked one or more of the markers specific to *S. brevidens*. No one chromosome or marker appeared to be lost preferentially. This infrequent absence of a chromosome marker derived from the wild parent could be explained by intergenomic pairing and recombination. The loss of a marker band for chromosome 8, coupled with the retention of two flanking markers, suggested that a small region of DNA was deleted during regeneration of the somatic hybrid. These results show the value of RFLP analysis when applied to somatic hybrids and their progeny. Clearly, RFLPs will be useful for following the DNA from wild species during its introgression into potato cultivars.

Key words: Somatic fusion – *Solanum brevidens* – RFLP – *Solanum tuberosum* – DNA introgression

Introduction

The sexual incompatibilities between potato (*Solanum tuberosum*) and several wild species of *Solanum* were bypassed through the production of somatic hybrids by the technique of protoplast fusion (Austin et al. 1985; Austin and Helgeson 1987; Helgeson 1989). Many of the resulting hybrids were fertile despite their unusual ploidy and endosperm balance number (Ehlenfeldt and Helgeson 1987). The hybrids were intermediate for several parental traits such as leaf shape, flower color, and stem morphology (Austin et al. 1985, 1986). Moreover, some agronomic traits that were unique to one of the fusion parents have been incorporated into the hybrid. Some of the more valuable of these parental contributions include the ability to form tubers, and resistance to pathogens such as *Phytophthora infestans* and potato leaf roll virus (Helgeson et al. 1986), as well as tuber soft rot caused by *Erwinia carotovora* and related *Erwinia* species (Austin et al. 1988).

The ploidy of our somatic hybrids equaled the sum of the parental ploidy levels (2 × wild species + 4 × potato), yet some hexaploid hybrids could be crossed to tetraploid potato cultivars (Ehlenfeldt and Helgeson 1987). Many of the progeny from such crosses were pentaploids and were themselves fertile when crossed again to potato. The pentaploid population was phenotypically variable, appearing to segregate for many traits. However, the two parental species are highly divergent and pairing between chromosomes derived from the different species may be inhibited. Thus, the apparent phenotypic segregation may be due to loss of chromosomes during meiosis rather than segregation of characters.

This report addresses the question of whether a specific hexaploid somatic hybrid between *S. brevidens* and *S. tuberosum* contains all chromosomes of both spe-

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cies and whether this DNA segregates in a manner that is consistent with Mendelian genetics. Restriction fragment length polymorphisms (RFLPs) were used as molecular markers to characterize the chromosome complement of somatic hybrids as well as to determine whether true segregation of chromosomes could be detected among the pentaploid progeny.

Materials and methods

Plant material

Solanum brevidens Phil. (PI 218228), *S. tuberosum* L. cv 'Kathadin' and *S. tuberosum* L. (PI 203900) were obtained from the *Solanum* collection at the Inter-regional Potato Introduction Project (IR-1), Sturgeon Bay, Wisconsin and are maintained as clonal copies (Haberlach et al. 1985). Somatic hybrid A206 was produced by the fusion of protoplasts from *S. brevidens* with protoplasts from *S. tuberosum* by the method of Austin et al. (1985). The somatic hybrid was then crossed with 'Katahdin' as described by Ehlenfeldt and Helgeson (1987). The *S. brevidens* parent is diploid (2n = 2x = 24), self-fertile, and non-tuber bearing; *S. tuberosum* (PI 203900), the potato parent, is tetraploid and bears round yellow-fleshed tubers. Somatic hybrid A206 is hexaploid (chromosome counts 71, 72, 74; M. Ehlenfeldt, unpublished results) and bears yellow-fleshed elongated tubers. It is resistant to tuber soft rot caused by *Erwinia* spp. (Austin et al. 1988). Resistance to *Erwinia* spp. is probably derived from the *S. brevidens* parent (Lojkowska and Kelman 1989). Resistance of A206 to potato leaf roll virus (PLRV) is also derived from the *S. brevidens* parent, and resistance to *Phytophthora infestans* (late blight) is conferred by the *S. tuberosum* parent (Helgeson et al. 1986). The sexual progeny from a cross of A206 with 'Katahdin' are mostly pentaploid and show segregation for many traits such as resistance to tuber soft rot, late blight, PLRV, tuber morphology, yield, specific gravity and earliness (Austin and Helgeson, in preparation).

Plant DNA manipulations

Total cellular DNA was isolated from frozen young leaves according to the method of Saghai-Maroof et al. (1984). Approximately 7 µg of each DNA sample was digested with *Eco*RI, *Eco*RV or *Hin*dIII (BRL) and subjected to electrophoresis in 0.7% agarose gels. DNA was transferred to Nytran membranes (Schleicher and Schuell) according to the manufacturer's directions.

Plasmid labeling and hybridization

The clones that were chosen as hybridization probes are a subset of those used to map the tomato and potato genomes by restriction fragment length polymorphisms (Bernatzky and Tanksley 1986; Tanksley et al. 1987; Bonierbale et al. 1988). The selected tomato clones identify RFLPs that map to single loci in potato, marking each of the 24 chromosome arms (Bonierbale et al. 1988) and yield DNA polymorphisms between potato and *S. brevidens*. Another DNA probe, the 45s major ribosomal repeat from pea (pHA2 plasmid; Jorgensen et al. 1982), was used as a marker for the short arm of potato chromosome 2. Random hexamer labeling was used to incorporate ^{32}P-dCTP into linearized plasmids (Feinberg and Vogelstein 1983). Hybridization proceeded as described by the manufacturer of Nytran, and the membranes were washed to medium stringency (0.5 × SSC, 60 °C).

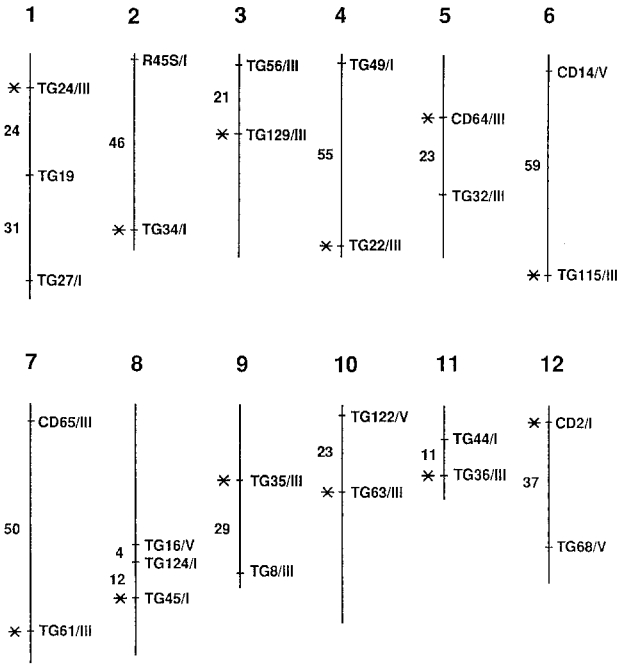

Fig. 1. Schematic representation of *Solanum* chromosomes (after Bonnierbale et al. 1988) indicating locations of markers that were used in this study to track the inheritance of parental chromosomes through the somatic hybrid and its sexual progeny. Each probe/enzyme combination detected polymorphisms between the *S. brevidens* and the *S. tuberosum* fusion parents; III refers to *Hin*dIII, I to *Eco*RI, and V to *Eco*RV. The estimated linkage distances between markers are based on the summation of two point distances between the intervening markers that were presented by Bonierbale et al. (1988). An attempt was made to choose markers that (A) detected at least one unique band from each parent, (b) mapped to a single chromosome, and (c) were distant enough to mark each of the 24 chromosome arms, based on centromere location in tomato and map distance. *Asterisks* indicate probe/enzyme combinations that appear in Fig. 2.

Results

DNA content of the fusion parents and hybrid A206

The probe/enzyme combinations that were used to detect each chromosome arm and to determine its parental origin are given in Fig. 1. In an attempt to identify markers for each of the 24 chromosome arms of the fusion hybrid, the probes were chosen by the following three criteria: (1) each probe had mapped to a single chromosomal locus in potato (Bonierbale et al. 1988), (2) probes that marked the same chromosome were separated by relatively large recombination distances (Bonierbale et al. 1988), and (3) each probe detected at least one unique band from each parent in this study. The constraints of criteria 1 and 3 occasionally required the use of markers that were separated by less than maximal recombination distances.

A total of 26 probes were tested for the ability to detect polymorphisms between the *S. tuberosum* and the

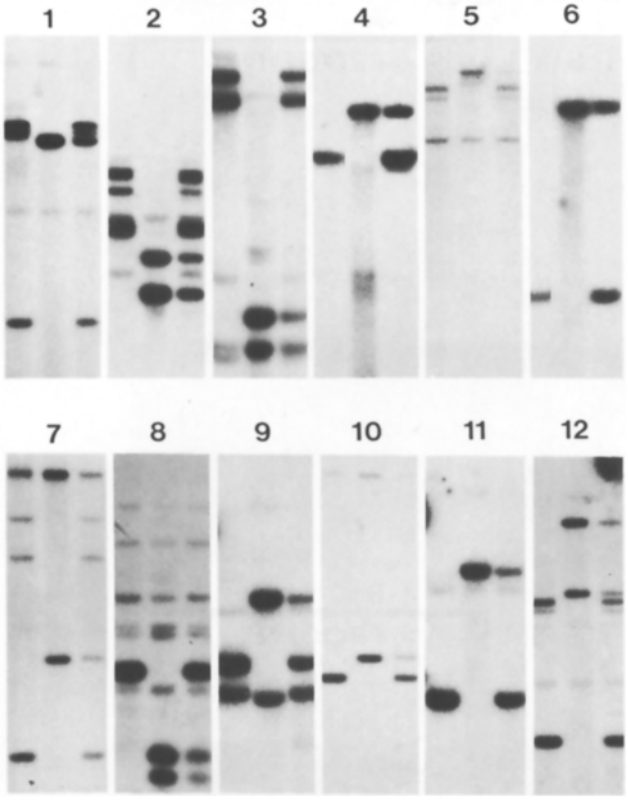

Fig. 2. Somatic hybrid A206 contained DNA from all 12 chromosomes of each fusion parent. *Numbers* in the figure denote the chromosome for which the tomato DNA probe is specific. The lanes in each panel correspond to DNA from the *S.* tuberosum fusion parent (*left*), the *S. brevidens* fusion parent (*center*), and the somatic hybrid A206 (*right*). Data are shown here for 12 of the probe/enzyme combinations from Fig. 1. Probe and enzyme combinations are: *chromosome 1* probe TG24/*Hin*dIII, *chromosome 2* probe TG34/*Eco*RI, *chromosome 3* probe TG129/*Hin*dIII, *chromosome 4* probe TG22/*Hin*dIII, *chromosome 5* probe CD64/*Hin*dIII, *chromosome 6* probe TG115/*Hin*dIII, *chromosome 7* probe TG61/*Hin*dIII, *chromosome 8* probe TG45/*Eco*RI, *chromosome 9* probe TG35/*Hin*dIII, *chromosome 10* probe TG63/*Hin*dIII, *chromosome 11* probe TG36/*Hin*dIII, *chromosome 12* probe CD2/*Eco*RI

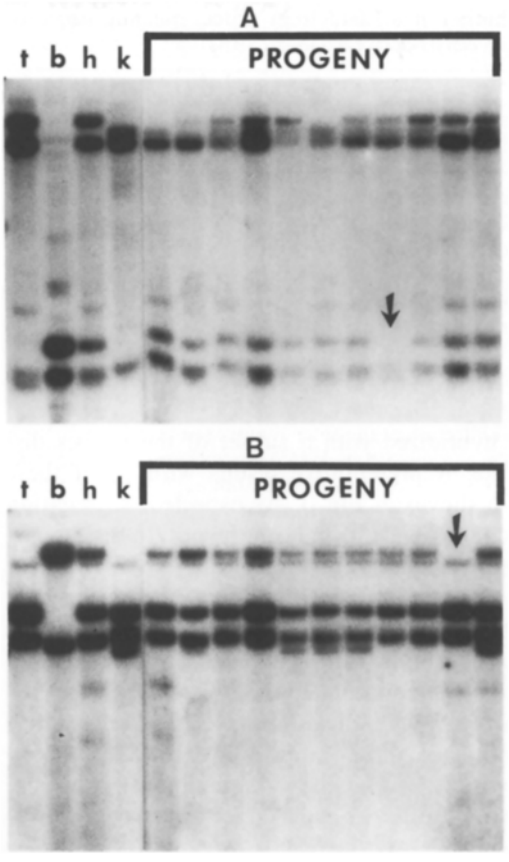

Fig. 3A and B. Sexual progeny exhibited segregation of parental bands. Gel blot hybridization was performed with DNA from *S. tuberosum* PI 203900 (*t*), *S. brevidens* (*b*), hybrid A206 (*h*), *S. tuberosum* cv 'Katahdin' (*k*), and 11 progeny from the cross A206 × 'Katahdin'. **A** The DNA samples were digested with *Hin*dIII, and the blot was hybridized with probe TG129 from chromosome 3. **B** After probe removal the blot was hybridized with probe TG35 from chromosome 9. *Arrows* indicate positions where *S. brevidens*-derived bands are absent

S. brevidens fusion parents. Because these parental species are only distantly related, a high degree of sequence divergence has resulted in detectable polymorphisms at 25 of the 26 loci. One of the probes detected polymorphisms when the DNA was cut with any of three different restriction enzymes (*Eco*RI, *Eco*RV, or *Hin*dIII). Nine of the probes detected polymorphisms with two of these enzymes, and 15 probes detected polymorphisms with only one enzyme. Figure 1 shows the probe/enzyme combinations utilized and the estimated map positions of marker loci.

Twelve of the RFLP patterns can be seen in Fig. 2. As can be seen from this figure, many probe/enzyme combinations resulted in hybridization to several bands, indicating that the parental plant was heterozygous or that the enzyme cleaved within the target sequence. Also, the parents often had one or more bands in common when multiple bands were detected. The presence of all parental bands from the 24 chromosome markers showed that at least one copy of each parental chromosome was present in the somatic hybrid A206. Although the dosage of each parental chromosome cannot be determined accurately with these analyses, A206 is known to be a hexaploid (M. Ehlenfeldt, unpublished data) and, with one exception, the banding pattern for A206 is the sum of the two parental patterns with no bands missing at any of the 25 marker loci. The one exception, TG124, is considered below.

Segregation of S. brevidens-derived marker bands in sexual progeny

In order to evaluate segregation of marker bands, blots containing DNA from sexual progeny (A206 × 'Katah-

Table 1. Comparison of data from probes yielding single or multiple *S. brevidens*-derived bands (SBBs)

	Single SBB probes	Multiple SBB probes
Probes detecting nulliplex progeny	4	11
Total number of probes	7	15
Percentage detecting nulliplex progeny	57	73
Number of nulliplex lanes detected	7	20
Total number of lanes scored	91	180
Percentage nulliplex lanes	8	11

din') were hybridized with a subset of the probes discussed above. Representative results with 11 of these progeny are shown in Fig. 3. As expected, segregation was detected for bands from both the potato fusion parent and the sexual potato parent. The *S. brevidens* parent appeared to be homozygous for all of the bands detected by the probes, with the exception of TG 124, whereas the *S. tuberosum* parents showed a high degree of band heterozygosity. In addition, 15 of the 22 probes detected at least one nulliplex individual (lacking all copies of a marker in polyploids) for one or more of the *S. brevidens*-derived bands (SBBs). When the 7 remaining probes were used, no loss of SBBs was detected for any of the individuals tested.

When blots containing DNA from sexual progeny were probed, the occurrence of lanes that were nulliplex for SBBs suggested that inter-genomic pairing had occurred, i.e., both copies of an *S. brevidens* chromosome had paired with *S. tuberosum* chromosomes and then had segregated together. This co-segregation would leave some gametes from A206 nulliplex for the *S. brevidens* chromosome. With 22 probes hybridized to blots carrying DNA from the same 11–14 sexual progeny, 27 out of a possible 271 lanes (10%) were missing SBBs.

It appeared that no one chromosome from *S. brevidens* was excluded more frequently than any other chromosome. Only 1 of the 22 probes detected as many as four nulliplex individuals. The other 21 probes detected one–three nulliplex individuals (average of 1.1/probe). In general, the two markers for each *S. brevidens* chromosome segregated together. However, nine cases were found in which there was a lack of co-segregation. This could be due to recombination between *S. brevidens* and *S. tuberosum* chromosomes.

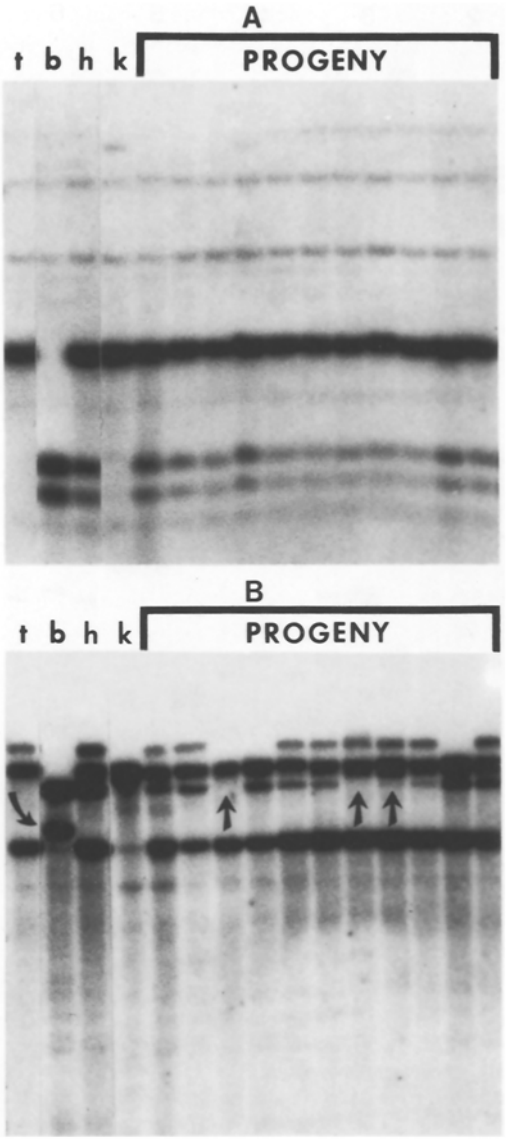

Fig. 4 A and B. Heterozygous deletion of the TG124 locus on chromosome 8 from *S. brevidens*. Gel blot hybridization was performed with DNA from *S. tuberosum* PI 203900 (*t*), *S. brevidens* (*b*), hybrid A206 (*h*), *S. tuberosum* cv 'Katahdin' (*k*), and 11 progeny from the cross A206 × 'Katahdin'. **A** The DNA samples were digested with *Eco*RI, and the blot was hybridized with probe TG45. All progeny lanes contained *S. brevidens*-derived bands. **B** After removal of TG45, the blot was hybridized with probe TG124. One band (indicated by the *curved arrow*) was missing in A206 and all of its progeny. *Straight arrows* indicate the positions where *S. brevidens*-derived bands were missing due to segregation among the progeny

Segregation of chromosome 8

In addition to possible recombination between chromosomes of the two parent species, it appears likely that a deletion in one chromosome occurred. A blot containing DNA from sexual progeny of A206 crossed with 'Katahdin' was probed consecutively with TG16, TG124 and TG45 (Fig. 4.). None of the progeny were lacking those SBBs that were detected by TG16 or TG45, markers that flanked TG124 (see Fig. 1). Thus, all progeny appeared to have at least one copy of chromosome 8 from *S. brevidens*. Probe TG124 gave two bands with the *S. brevidens* parental DNA. The high molecular weight

band marked by TG124 was detected in A206 and segregated among the progeny so as to detect three nulliplex individuals, a situation not unlike that with the other chromosomes of A206 and its progeny. However, all progeny, and even A206, lacked the low molecular weight TG124 band from *S. brevidens*. We have concluded, therefore, that the *S. brevidens* fusion parent was heterozygous at the TG124 locus. The low molecular weight band marked one homologue and the high molecular weight band marked the other. During the process of somatic fusion and regeneration of hybrid A206, DNA was deleted at the TG124 locus from the homologue marked by the low molecular weight band. Because both flanking markers (TG16 and TG45) were present, the progeny individuals that were nulliplex for the high molecular weight marker of TG124 actually possessed the modified chromosome. Individuals with the high molecular weight TG124 band possessed the unmodified chromosome and may or may not have had the modified chromosome as well. TG124 is the only marker for which the *S. brevidens* fusion parent was proven to be heterozygous for a band polymorphism.

Discussion

Chromosome loss is a common event in interspecific sexual crosses (Davies 1974; Lang 1971), including those between *Solanum* species (Ramanna and Hermsen 1971). In plant tissue culture, chromosome elimination may occur at random (Bayless 1980; D'Amato 1985). In somatic hybrids, elimination may be preferential for certain chromosomes (Pijnacker et al. 1987) or certain parental sets (Pental et al. 1986). In addition, Ehlenfeldt and Helgeson (1987) reported abnormal chromosomal behavior in meiosis for several *S. brevidens* + *S. tuberosum* somatic hybrids. It is apparent from chromosome counts (71, 72 and 74, M. Ehlenfeldt, unpublished data) that hybrid A206 probably contains the expected hexaploid chromosome number of 72, but the resolution of cytological examinations is too low to determine whether certain chromosome arms have been lost or whether two complete sets of *S. brevidens* and four complete sets of *S. tuberosum* chromosomes are present. Pijnacker et al. (1987) reported chromosome rearrangement resulting from somatic hybridization of *Solanum* species. In addition, some hybrid plants were missing two or three nucleolar chromosomes yet retained a higher chromosome number than expected after the loss.

Implications for the ploidy of hybrid A206

Segregation data for the present study with RFLPs suggests that hybrid A206 contains two sets of *S. brevidens* and four sets of *S. tuberosum* chromosomes. The presence of all 24 polymorphic marker loci indicates that at least one copy of each chromosome arm from each parental set is present. Several bands are known to represent different alleles because they appear to segregate in the sexual progeny, yet all parental bands are accounted for in A206, with the notable exception at the TG124 locus. If the hybrid were missing one entire copy of an *S. brevidens* chromosome, the sexual progeny should be nulliplex for that pair of chromosome markers much more often than for other markers. Because this has not been the case, it appears that A206 contains two complete sets of *S. brevidens* chromosomes.

Evidence for chromosome stability and preferential pairing

None of the sexual progeny lacked all or most of the SBBs. This indicates that the loss of entire sets of *S. brevidens* chromosomes did not occur, as has been seen with somatic hybrids between *Nicotiana* and *Petunia* (Pental et al. 1986). Also, no one chromosome was absent more frequently than the others. Thus, the production of nulliplex progeny is probably due to segregation rather than chromosome loss resulting from meiotic abnormalities. Indeed, the segregation of SBBs for chromosome 8 demonstrated that meiosis proceeded normally even after a deletion had occurred.

The observed frequency of nulliplex progeny (ca. 10%) suggests that there is a small bias against completely random pairing in meiosis. Completely random bivalent pairing between the genomes would produce 20% nulliplex gametes. On the other hand, if pairing were limited to homologous chromosomes, the production of nulliplex gametes would approach 0%. Alternatively, if inter-genomic pairing resulted in univalents and then chromosome loss, which has been reported for some interspecific crosses in *Solanum* (Ramanna and Hermsen 1981, 1982), the nulliplex frequency would be greater than 20%. In fact, when 22 probes were hybridized to blots containing DNA from the same 11–14 sexual progeny, 27 of 271 lanes (10%) were nulliplex for SBBs. These results indicate that although there is a small bias toward intra-genomic pairing, some inter-genomic pairing also occurred. The small bias toward intra-genomic pairing appears to be equal for the eleven chromosomes tested because their incidence of nulliplex production was similar. Thus, inter-genomic pairing should be common during meiosis and would provide ample opportunity for inter-genomic recombination.

Comparison of data from probes yielding single SBBs versus those yielding multiple SBBs indicated that the *S. brevidens* parent was largely homozygous for the probed loci. However, in cases where a probe detected multiple SBBs, it is possible that lanes may have been incorrectly scored as nulliplex due to the masking of still-present SBBs by co-migrating *S. tuberosum*-derived bands. These

inaccuracies would occur if the *S. brevidens* parent had been heterozygous, with one allele producing a unique band and the other allele producing bands that co-migrated with the *S. tuberosum* bands. However, because of the following observations, we feel that the lanes were scored correctly. In a multiple SBB pattern, the bands that were unique to *S. brevidens* always segregated as a unit, suggesting homozygosity. In addition, the results obtained with probes yielding single SBBs were similar to the results with probes yielding multiple SBBs; in both cases over 50% of the probes detected nulliplex lanes, and the frequency of nulliplex lanes was near 10% (Table 1). Thus we conclude that the *S. brevidens* parent was homozygous and that the bias against inter-genomic pairing was small.

Cytological evidence suggests that several species in series Etuberosa, including *S. brevidens,* contain individuals that are heterozygous for one or more reciprocal translocations (Hermsen and Taylor 1979; Ramanna and Hermsen 1981, 1982). These rearrangements are detected in the formation of chains that involve several chromosomes and result in a high percentage of aneuploid gametes, aborted seed, and progeny sterility. Although aneuploidy is better tolerated in polyploids than in diploids, A206 is very fertile and gives little evidence of meiotic abnormalities that could be detected with molecular markers. Thus it appears that the *S. brevidens* individual that was used as a fusion parent was not a translocation heterozygote.

Partial deletion of S. brevidens-derived chromosome 8

Somaclonal variation is a common occurrence during plant regeneration from tissue culture, often resulting in detectable phenotypic changes. Chromosomal deletions and rearrangements may be the genetic basis of this phenomenon (Scowcroft et al. 1983). Although phenotypic change due to somaclonal variation would be difficult to document in interspecific somatic hybrids, the present study has used RFLPs to reveal the occurrence of a deletion during the process of cell fusion and regeneration. Because the markers TG16 and TG45 flank the deletion, it can be no larger than the region that is defined by these markers. Bonierbale et al. (1988) estimated this region to be 16 map units in a interspecific cross involving diploid *Solanum* species.

Practical applications for RFLPs in conjunction with somatic hybridization

Somatic hybrids between *S. brevidens* PI 218228 and *S. tuberosum* PI203900 are intermediate for many parental traits and fully resistant to several pathogens (see Helgeson 1989; Helgeson et al. 1986; Austin et al. 1986, 1988). Sexual progeny from the cross of A206 with 'Katahdin' segregate for these resistance traits as well as for the ability to form tubers, tuber shape, earliness, and other agronomic traits (Austin et al. in preparation). These results indicate that, just as RFLP analysis has aided in the detection and mapping of multigenic traits in tomato (Osborn et al. 1987; Neinhuis et al. 1987), it may be used to identify and mark genes of interest in the sexual progeny of A206. Also, because these individuals are fertile, marker-enhanced selection may aid in generating useful breeding lines. Finally, we conclude that pairing between *S. brevidens* and potato chromosomes occurs to a degree that allows recombination. Thus introgression of desirable *S. brevidens* traits into potato cultivars should be possible.

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