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Recombination of *Solanum brevidens* chromosomes in the second backcross generation from a somatic hybrid with *S. tuberosum*

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Abstract *Solanum brevidens* synteny groups were examined with 47 widely-distributed RFLP markers in 17 BC_2 progeny from six fertile BC_1 plants. The BC_1 plants were derived from a single *S. brevidens* + *S. tuberosum* somatic hybrid backcrossed with *S. tuberosum* (potato). Probes which were linked in potato and tomato were also found to be syntenic along each of the 12 *S. brevidens* chromosomes. More than half of the *S. brevidens* synteny groups had lost one or more *S. brevidens*-specific RFLPs in the BC_2, suggesting that recombination had occurred. For 8 of the 12 *S. brevidens* RFLP synteny groups, the frequency of recombinant chromosomes exceeded that of intact parental chromosomes. Using the RFLP data, 161 RAPD markers were tentatively located throughout the *S. brevidens* genome. Further analyses with 39 of these 161 RAPD markers generally showed that RAPD and RFLP results were comparable, but some inconsistencies were noted with 14 of the 39 RAPD markers. The extent of marker loss and the high frequency of synteny groups which were marked by a single *S. brevidens*-specific RFLP marker suggest that the *S. brevidens* chromosomes have some pairing affinity with potato chromosomes. This interaction should facilitate the transfer of novel disease-resistance traits into potato breeding lines. One plant was recovered with the chromosome number of *S. tuberosum* (2n=48) that carried a single *S. brevidens* RFLP marker, suggesting transfer of this *S. brevidens* marker into the genome of *S. tuberosum*.

Key words Potato · Introgression · Wide hybrids · RFLP RAPD

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Introduction

Somatic hybrids between *Solanum tuberosum* (potato) and a number of sexually-incompatible wild *Solanum* species have been obtained through protoplast fusion (Novy 1992; Austin et al. 1993; Helgeson et al. 1993). Many of the wild species genomes incorporated into somatic hybrids carry valuable genes conferring resistance to the major pathogens of potato (Hanneman and Bamberg 1986). To realize the breeding potential of these somatic hybrids, the transfer of desirable characters from the wild species into potato breeding lines needs to be accomplished. Fortunately, somatic hybrids are often fertile and viable seeds have been recovered from crosses of the somatic hybrids with potato (Ehlenfeldt and Helgeson 1987; Helgeson et al. 1993). Introgression of disease-resistance characters may thus be possible, though this potential has not yet been demonstrated.

As a model to follow introgression of wild characters into potato, we have chosen to examine in detail the progeny derived from a single somatic hybrid (designated A206) between *Solanum brevidens* (2n=2x=24) and *S. tuberosum* (2n=4x=48). This and related somatic hybrids are hexaploid (2n=6x=72) and contain most of the biochemical markers contributed by the parental species (Austin et al. 1985; Williams et al. 1990 a). Limited genetic polymorphism has been found in *S. brevidens* (Williams et al. 1990 a), as might be expected for an inbreeding species. In addition, the somatic hybrid produces tubers, unlike *S. brevidens* which is taxonomically placed in the series *Etuberosa* (Matsubayashi 1991 and references therein). *S. brevidens* also appears to carry resistance to potato tuber

soft rot (caused by *Erwinia* spp.), potato-leaf-roll virus as well as Early Blight (*Alternaria solani*) (Austin et al. 1985, 1988; W. Stevenson, unpublished, respectively).

To assess whether *S. brevidens* chromosomes would be transmitted through meiosis in the somatic hybrid, a first backcross generation was derived from crossing a hexaploid somatic hybrid with the tetraploid potato cultivar 'Katahdin'. These BC_1 progeny were generally pentaploid (2n=5x=60) but a range of chromosome numbers from 2n=58 to 2n=62 was seen in these plants (Williams et al. 1993). Analyses of restriction fragment length polymorphism (RFLP) markers showed that individual *S. brevidens* linkage (synteny) groups were absent in some BC_1 plants (Williams et al. 1993), which may account in part for the range of observed chromosome numbers. In other BC_1 plants, only partial synteny groups were detected such that some *S. brevidens*-specific RFLP markers for different chromosomes were missing (Williams et al. 1993). From a breeding perspective these results are significant for two reasons. First, they demonstrate that *S. brevidens* chromosomes are transmissible through meiosis. Secondly, they suggest that *S. brevidens* chromosomes have the potential to interact and recombine with those of potato, thus allowing introgression of *S. brevidens* characters into potato.

To better assess the potential for inter-genomic recombination we have analyzed 17 BC_2 progeny derived from six BC_1 plants. Ramanna and Hermsen (1981) described a lack of pairing affinity between *S. brevidens* chromosomes and those of *S. pinnatisectum* (*S. pinnatisectum* is also crossable with potato). Thus, it was surprising that the majority of the *S. brevidens* RFLP synteny groups present in our study had lost one or more *S. brevidens*-specific RFLP markers. The extent and pattern of marker loss is described in this report.

Materials and methods

Plant materials

The plants used for this study were derived from a fertile somatic hybrid (designated A206; generated by the method of Austin et al. 1985) between *S. brevidens* (PI 218228, 2n=2x=24) and *S. tuberosum* (PI 203900, 2n=4x=48, R4). Somatic hybrid A206 (2n=6x=72) was crossed as the female parent with *S. tuberosum* cv Katahdin (KAT) to give BC_1 progeny at or near the pentaploid chromosome number (2n=5x=58 to 62). Six lines (C0, C31, C41, C46, C75 and C76) were crossed as seed parents with 'Katahdin'. A total of 17 BC_2 plants (two to four BC_2 progeny per parent) were chosen for analysis with RFLP markers. The somatic hybrid and its BC_1 progeny were previously characterized by RFLP analyses (Williams et al. 1990a, 1993). Helgeson et al. (1993 and unpublished) have characterized disease resistance, as well as other agronomic traits, in these and similar populations. All materials were clonally and aseptically maintained in-vitro (Haberlach et al. 1985). Thus, exact genetic copies of each parental line, the somatic hybrid, and two backcross generations, were available. Chromosome counts were made on root tips as described by Williams et al. (1993).

RFLP analyses

DNA was isolated from greenhouse-grown plants as described by Williams et al. (1990 a). A total of 47 tomato RFLP probes were used which had previously been characterized in the BC_1 generation (Williams et al. 1993). Blots were scored for the presence or absence of *S. brevidens*-specific fragments as described by Williams et al. (1990 a, 1993).

RAPD analyses

DNA was isolated by a micro-extraction procedure taken from Deragon and Landry (1992) and Cheung et al. (1993). Briefly, leaves from in-vitro grown plants were excised and leaf disks (6.5 mm) were obtained with a paper punch. One leaf disk was placed in a microcentrifuge tube (Kontes disposable tube with pestle, cat.# 749520) and ground in 160 µl of cold extraction buffer (50 mM TRIS-HCl, pH 8.0, 100 mM Na_2EDTA, 20 mM $Na_2S_2O_5$). After grinding, 40 µl of lytic mixture (50 mM TRIS-HCl, 100 mM Na_2EDTA, pH 8.0) was added and the mixture was incubated for 2 h at 55 °C. After incubation, the mixture was centrifuged at 14000 rpm for 15 min and DNA was precipitated by adding 90 µl of 10 M ammonium acetate and 200 µl of isopropanol and then pelleted for 15 min as above. Pellets were washed in 70% ethanol, dried, and resuspended in 60 µl of 'low EDTA' TE buffer (10 mM TRIS-HCl, 0.1 mM EDTA, pH 8.0). Aliquots of 3.0 to 5.0 µl contained sufficient DNA for amplification via the polymerase chain reaction (PCR).

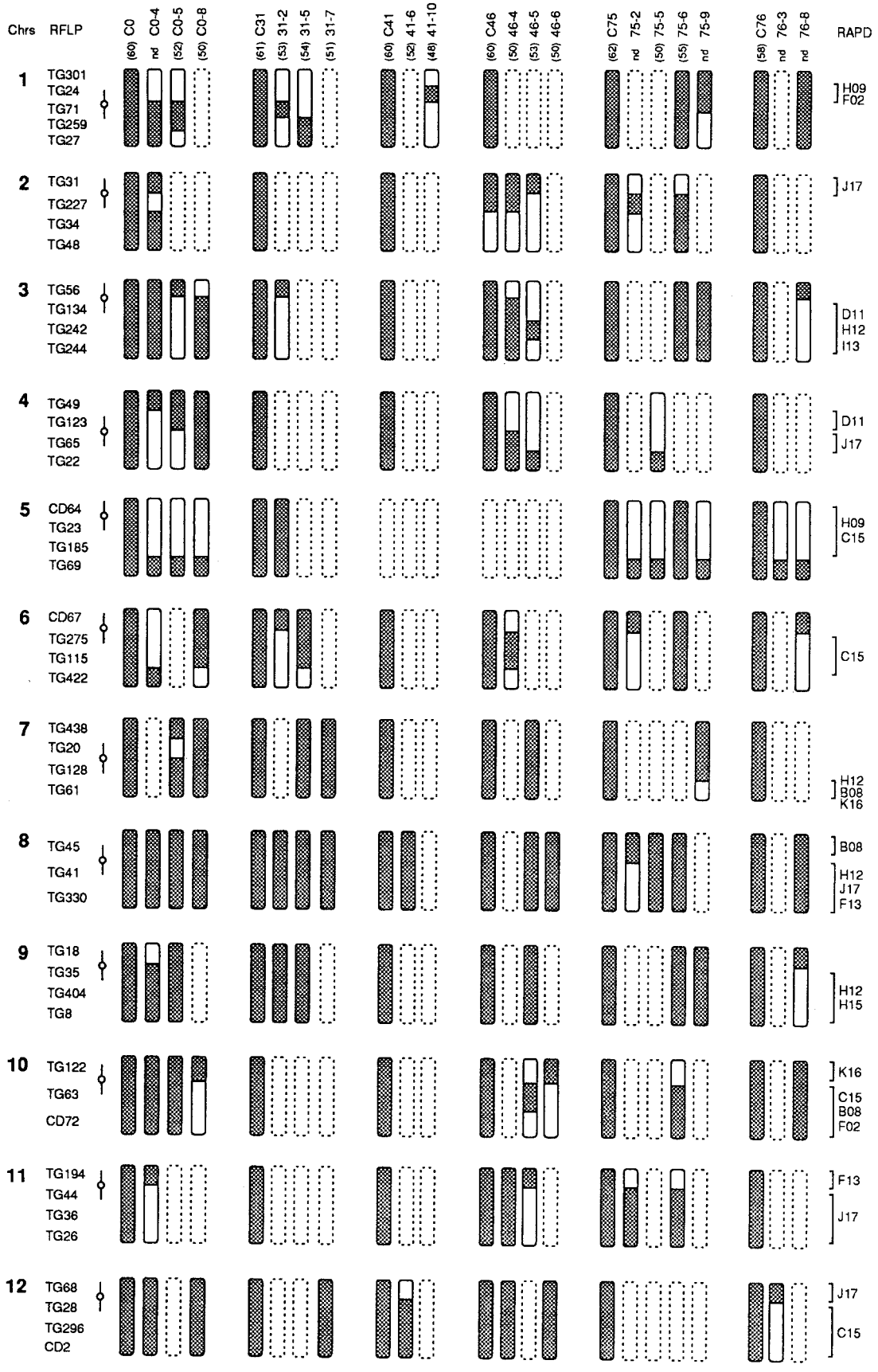
PCR amplification was performed as per Williams et al. (1990 b) in a Perkin-Elmer model 480 thermocycler with one cycle of denaturation at 94 °C for 2 min, followed by 40 cycles of 94 °C (denaturation) for 1 min, 35 °C for 1 min (annealing), a 2 min ramp to 72 °C followed by 2 min at 72 °C (extension); with a final extension at 72 °C for 7 min following the amplification cycles. Each 25-µl amplification reaction contained approximately 25 ng of template DNA in 10 mM TRIS-HCl pH 8.3, 50 mM KCl, 3.4 mM $MgCl_2$, 0.01% gelatin, 100 M each of dATP, dCTP, dGTP and dTTP (Pharmacia), 250 µM decameric oligonucleotide primer (Operon Technologies), and 1 Unit of AmpliTaq DNA polymerase (Cetus). Amplification products were size-separated in a 1% Synergel (Diversified Biotech, Newton Centre, Mass.), 0.6% agarose (BRL) matrix in 0.5 × or 1.0 × TBE buffer (Sambrook et al. 1989) and the bands were visualized and photographed after staining with ethidium bromide. RAPD markers were named by the primer used and, in subscripts, by the size of the amplified fragment, e.g., a 500 bp fragment amplified by primer H09 was represented as $H09_{500}$.

Results

RFLP analyses of second generation plants

Our results only considered changes that have been detectable in the *S. brevidens* genome. This is because the potato genome is tetraploid and highly heterozygous, and loci often show tetrasomic inheritance. Six first backcross (BC_1) generation plants (C0, C31, C41, C46, C75 and C76) served as female parents in a cross with the potato cultivar 'Katahdin'. Two, three or four individuals from each cross,

Fig. 1 Ideogram of *S. brevidens* chromosomes in BC_1 parents and BC_2 progeny lines determined with RFLP markers (note that *hyphenated numbers* denote BC_2 progeny, e.g., C0-4 was derived from the BC_1 parent C0). Each chromosome was marked with 3–5 RFLP markers, generally placed in the map order of potato (see text). *Shading* indicates that the marker was present in a given line. *No shading* or a *dotted outline* indicates that the RFLP marker(s) or RFLP synteny group was absent, respectively. For illustration purposes only, putative centromere positions are indicated with a *circle and line* (deduced from Tanksley et al. 1992). RAPD markers assigned to a specific chromosome region are indicated but their sizes are not. Chromosome counts for each plant are indicated in parentheses (nd=not determined)


Chrs
RFLP
C0
C0-4
C0-5
C0-8
C31
31-2
31-5
31-7
C41
41-6
41-10
C46
46-4
46-5
46-6
C75
75-2
75-5
75-6
75-9
C76
76-3
76-8
RAPD
1
TG301
TG24
TG71
TG259
TG27
H09
F02
2
TG31
TG227
TG34
TG48
J17
3
TG56
TG134
TG242
TG244
D11
H12
I13
4
TG49
TG123
TG65
TG22
D11
J17
5
CD64
TG23
TG185
TG69
H09
C15
6
CD67
TG275
TG115
TG422
C15
7
TG438
TG20
TG128
TG61
H12
B08
K16
8
TG45
TG41
TG330
B08
H12
J17
F13
9
TG18
TG35
TG404
TG8
H12
H15
10
TG122
TG63
CD72
K16
C15
B08
F02
11
TG194
TG44
TG36
TG26
F13
J17
12
TG68
TG28
TG296
CD2
J17
C15

giving a total of 17 BC_2 plants, were examined with 47 tomato RFLP probes that previously had been shown to hybridize with specific *S. brevidens* restriction fragments (Williams et al. 1993). Most, if not all, of these probes are syntenic (i.e., are located on homoeologous chromosomes) between potato and tomato (Tanksley et al. 1992). These RFLP markers appeared to be syntenic in *S. brevidens* as well, as supported by the following results. First, markers located to chromosome *5* in both potato and tomato were lost as a group in BC_1 plants C41 and C46, indicating that *S. brevidens* chromosome *5* was absent in these plants (Williams et al. 1993, and Fig. 1). Similarly, all *S. brevidens* RFLP markers located on potato and tomato chromosomes *1*, *2*, *6*, *11* and *12* were also lost as a block in some of the individual BC_1 plants (Williams et al. 1993). Markers assigned to these chromosomes in the BC_1 retained their synteny associations in BC_2 plants (Fig. 1). Second, due to random chromosome assortment from their pentaploid parents, each BC_2 plant had a different complement of *S. brevidens* chromosomes. Thus, *S. brevidens*-specific RFLP fragments could be assigned to 1 of the 12 different *S. brevidens* synteny groups, and these synteny groups corresponded to those of potato and tomato. It should be noted that the order of markers along syntenic chromosomes differs between tomato and potato (Tanksley et al. 1992), and therefore may differ for *S. brevidens* as well. However, with one exception (see below) markers were ordered according to their map position in potato. Assuming that the marker order between *S. brevidens* and potato chromosomes is equivalent, both arms of each *S. brevidens* chromosome and one or more interstitial regions were marked with RFLPs (Fig. 1).

For the purposes of our analyses, plants carrying all *S. brevidens*-specific RFLP markers for a particular chromosome were assumed to be non-recombinant for that chromosome and thus were assumed to carry intact chromosomes. Conversely, plants which carried one or more, but not all, *S. brevidens* chromosome-specific RFLPs were assumed to be recombinant for that particular chromosome. Since only 3% of *S. brevidens* RFLPs showed evidence of heterozygosity in a previous study (Williams et al. 1993), and only one (TG330 on chromosome *8*) was used in the present analysis, the failure to detect a marker was not due to allelic segregation but rather to the loss of that particular marker. For the sake of convenience, loss of a marker (e.g., a deletion) was considered as one of many possible modes of recombination. It is possible that some non-recombinant chromosomes had recombined in regions not detected by the RFLP probes because of the large genetic distances between RFLP markers. It is also possible that recombinant chromosomes would have been scored as non-recombinant chromosomes if both rearranged segments were present in the same plant (e.g., a reciprocal translocation involving two *S. brevidens* chromosomes). Each of these biases leads to an underestimate in the number of recombinant chromosomes.

Using these assumptions, the majority of *S. brevidens* chromosomes were recombinant since 51 recombinant chromosomes and 40 non-recombinant chromosomes were scored in the BC_2 plants (Table 1 and Fig. 1). Most plants which carried markers on chromosomes *7*, *8*, *9* and *12* carried non-recombinant chromosomes (Table 1). For each other chromosome the number of recombinant chromosomes exceeded non-recombinant types. At the extreme, all BC_2 plants carrying *S. brevidens* markers for chromosome *2* were recombinant (Table 1 and Fig. 1). Chromosome *2* also appeared to recombine frequently in the BC_1 (Williams et al. 1993).

Table 1 Transmission of non-recombinant and recombinant *S. brevidens* synteny groups in BC_2 plants. Non-recombinant chromosomes represent the number (and percent in parentheses) of BC_2 plants which carried all *S. brevidens* RFLP markers for a given chromosome (see text). Recombinant chromosomes represent the number (and percent) of BC_2 plants for which only a portion of the *S. brevidens* chromosome-specific RFLPs were detected. Chromosome *2* from plant 46-4 was not considered recombinant in the BC_2 because its parent chromosome from C46 was itself recombinant (see Fig. 1)

Chromosome	Non-recombinant	Recombinant
1	2 (12)	6 (35)
2	0 (0)	4 (25)
3	3 (18)	6 (35)
4	1 (6)	5 (29)
5	2 (12)	8 (47)
6	1 (6)	7 (41)
7	4 (24)	2 (12)
8	12 (71)	1 (6)
9	6 (35)	2 (12)
10	3 (18)	4 (24)
11	1 (6)	4 (24)
12	5 (29)	2 (12)
Total	40 (19.7)	51 (25.1)
Mean:	3.33	4.25
SD	3.26	2.22

Although recombinant *S. brevidens* chromosomes were detected with RFLPs for all chromosomes, the frequency and distribution of marker loss varied with the particular chromosome. For instance, four different combinations of RFLP marker loss occurred on chromosome *4*: plant C0-4 carried RFLP TG49, plant C0-5 carried both TG49 and TG123, plant 46-4 carried TG65 and TG22, and plant 46-5 carried only TG22 (Fig. 1). A similar complex pattern of recombination was seen for *S. brevidens* chromosomes *1*, *2*, *3*, *6* and *10*. For chromosomes *7*, *8*, *9*, *11* and *12*, only two types of recombinant chromosomes were seen, and all eight recombinants detected on chromosome *5* carried the single RFLP marker TG69 (Fig. 1).

Many recombinant chromosomes (60.8%) carried only one *S. brevidens*-specific RFLP marker (31 chromosomes represented by a single marker among 51 recombinant chromosomes). Of these, 26 carried a terminal marker and five carried a single RFLP which resided in the middle of a synteny group (Fig. 1). The remaining 20 recombinant chromosomes carried two or more *S. brevidens*-specific RFLP markers. Generally, these recombinant synteny groups carried RFLP markers that were located next to one another, and often appeared to reside on one chromosome arm or the other (e.g., chromosome *1* in plant C0-4, Fig. 1).

Seven plants had recombinant *S. brevidens* chromosomes which either carried or lost only interstitial markers (Fig. 1). Loss of internal RFLP markers, or loss of markers at both ends of a chromosome, suggested that more than one recombination event had occurred on these chromosomes. Two of these plants (C0-5 and 46-5) carried two doubly-disrupted chromosomes (chromosomes *1* and *7*, and *3* and *10*, respectively; Fig. 1). These putatively double-recombinant chromosomes may have been derived as the result of homoeologous chromosome pairing and recombination or, they may represent artifacts due to changes in the order of RFLP markers in *S. brevidens* relative to potato.

The observed proportion of multiply-recombinant chromosomes was dependent in part on assumptions about the order of RFLP markers. If the strict potato map order was used, 17 doubly-recombinant chromosomes were observed. However, eight of these were contributed by a single marker (TG69) on chromosome *5*. By inverting the order of the markers TG69 and TG185 in *S. brevidens* relative to potato, where they are tightly linked at 3.5 cM (Tanksley et al. 1992), there were only nine doubly-recombinant chromosomes. Since there should be no a priori assumption that gene order in *S. brevidens* exactly parallels that of potato, and the frequency of observed doubly-recombinant *S. brevidens* chromosomes was less than those scored as single recombinants, we chose to invert markers TG69 and TG185 on *S. brevidens* chromosome *5* relative to the potato RFLP map (Fig. 1).

On chromosome *1*, markers TG27 and TG259 mapped to the same locus on both the potato and tomato genetic maps (Tanksley et al. 1992). In *S. brevidens*, loss of marker TG27 in plant C0-5 suggested recombination had occurred between these two markers (Fig. 1). Thus, considering that double recombinants are less frequent in these plants than the loss of a single terminal marker, we have provisionally located TG27 as the terminal marker of *S. brevidens* chromosome *1* (Fig. 1).

Number of RFLP-detected synteny groups versus the number of counted chromosomes

Chromosome number was determined for 18 of the 23 RFLP-tested plants listed in Fig. 1 in order to ascertain whether the chromosome counts were correlated with the number of RFLP-detected *S. brevidens* synteny groups. In only two cases (plants C0 with 2n=60 chromosomes and 31–7 with 2n=51 chromosomes, Fig. 1) did the chromosome counts agree exactly with the expected number of *S. brevidens* RFLP synteny groups. For instance, assuming the entire somatic complement of 48 potato chromosomes was present in plant 31-7, the three RFLP-detected *S. brevidens* synteny groups (i.e., chromosomes *7*, *8* and *12*) would have given a total of 2n=51 chromosomes. For the other 16 plants, the chromosome counts did not agree with the number of RFLP-detected *S. brevidens* chromosomes (Fig. 1). For example, RFLPs for plant 41-6 showed that it carried two *S. brevidens* synteny groups, an entire synteny group for chromosome *8* and most of group *12* (Fig. 1). Root-tip cells in this plant contained 2n=52 chromosomes. One possible explanation for this disparity is that both copies of *S. brevidens* chromosomes *8* and *12* were present in this plant. Similarly, the number of counted chromosomes was greater than the number of *S. brevidens* RFLP synteny groups in plants C31, 31-5, C41, 41-6, C46, C75, and C75-5 (Fig. 1). Aneuploid gametes have been noted in both the somatic hybrid A206 as well as the recurrent potato parent 'Katahdin' (data not shown). Thus, multiple (disomic) copies of *S. brevidens* or potato chromosomes could explain these results.

In nine plants (C0-5, C0-8, 31-2, 41-10, 46-4, 46-5, 46-6, 75-6 and C76), the number of counted chromosomes was less than the number of RFLP-detected *S. brevidens* synteny groups. For instance, plant 46-4 (2n=50 chromosomes) had two intact and four recombinant *S. brevidens* RFLP groups. If all 48 potato chromosomes were present and all six *S. brevidens* RFLP synteny groups could be observed through the microscope, the expected chromosome number would have been 2n=54. A reduction in the number of counted chromosomes relative to the number of RFLP-synteny groups could be explained by chromosome substitution. Since we have only focused on the *S. brevidens* genome with RFLPs, there is no information about the number of potato chromosomes present in these lines. Alternatively, chromosome translocation, fusion, or homoeologous pairing and exchange could also lead to a reduction in the number of counted chromosomes relative to those detected with *S. brevidens*-specific RFLPs.

Correlation of RAPD markers with RFLP markers on *S. brevidens* synteny groups

Initially, 224 decameric oligonucleotide primers (Operon Co. primers A01 through L04) were screened to identify *S. brevidens*-specific RAPD markers. For 90 primers (40%), at least one amplified fragment was present in both the somatic hybrid A206 and *S. brevidens*, but was absent in *S. tuberosum*. For example, using primer H01, an amplification product of 1 000 bp derived from *S. brevidens* was present in somatic hybrid A206 (Fig. 2). This fragment was considered an *S. brevidens*-specific RAPD marker. Other amplification products were not specific to *S. brevidens*. For instance, fragments of 450 bp and 1l900 bp in A206 were derived from the *S. tuberosum* parent and the fragment at 500 bp was common to both *S. brevidens* and *S. tuberosum*. Similar results were obtained with primers H02, H03 and H04 (Fig. 2). Taken together, these results confirmed that the somatic hybrid A206 contained genomes from both *S. brevidens* and *S. tuberosum*. These data are in agreement with previous RFLP results for this somatic hybrid (Williams et al. 1990 a, 1993).

Assignments of RAPD markers to specific *S. brevidens* chromosomes and chromosome regions were facilitated by comparisons with RFLP data. For instance, as illustrated in Fig. 3, chromosome *5* RFLP marker TG23 was present in four of six pentaploid lines but absent in two others (C41

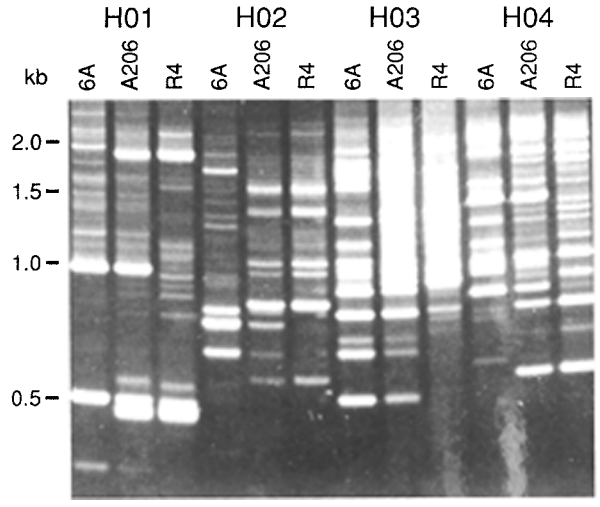


Fig. 2 Amplification products obtained with RAPD primers H01, H02, H03 and H04 showing polymorphisms between *S. brevidens* (*6A*) and *S. tuberosum* (*R4*), and the somatic hybrid *A206*. Note that most fragments present in the fusion parents 6A and R4 are also present in the somatic hybrid

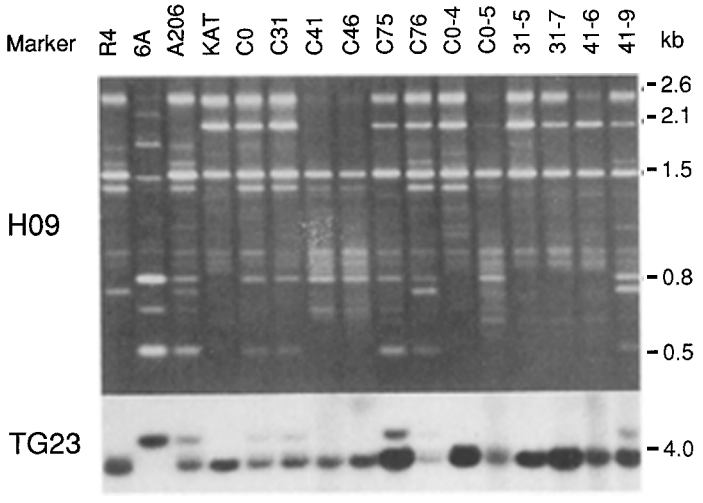


Fig. 3 Co-inheritance of *S. brevidens* chromosome *5*-specific RFLP TG23 (lower panel, at approximately 4 kb) and RAPD H09 (upper panel, at 500 bp) markers among six BC_1 and five BC_2 lines. The RAPD fragment amplified with H09 at 800 bp was co-inherited with chromosome-*1* RFLP markers

and C46; also see Fig. 1). Comparison of TG23 with a 500-bp fragment amplified with primer H09 showed a one-to-one correspondence with the presence of a 4.1-kb *S. brevidens* fragment detected with RFLP probe TG23. Thus, this RAPD was keyed to chromosome *5*. In addition, H09 amplified an 800-bp fragment which co-segregated with chromosome *1* RFLPs (data not shown). Other fragments (e.g., approximately 2.1 and 2.6 kb) were segregating among these lines, and these can be traced to one or both of the *S. tuberosum* genomes (R4 or KAT) used to derive these materials (Fig. 3).

For the purpose of assigning RAPDs to chromosomes, a set of 15 BC_1 and BC_2 plants were chosen such that each RAPD could be initially assigned to a chromosome with minimum ambiguity. These plants included one BC_1 plant with all *S. brevidens* specific RFLPs (C75) and six BC_1 lines that were lacking all *S. brevidens*-specific RFLPs for one or two *S. brevidens* chromosomes. These BC_1 lines were C64 (lacking chromosome *1*), C113 (lacking *12*), C2 (lacking *11*), C12 (lacking both *6* and *11*), C41 (lacking *5*), and C76 (lacking both *2* and *11*) (Williams et al. 1993). Markers assigned to these chromosomes were scorable by their absence in the BC_1. Eight additional plants were drawn from the second backcross generation (C31-5, C31-7, C41-6, C46-4, C46-6, C75-5, C75-6 and C75-9; Fig. 1). These BC_2 plants carried only one or a few different *S. brevidens* RFLP synteny groups (Fig. 1). Thus, the presence or absence of a RAPD marker could be correlated with the presence or absence of an RFLP-marked chromosome in these eight BC_2 plants.

Within the set of 90 primers which showed putative *S. brevidens*-specific markers, 191 potentially-informative bands were scored. Of these 191 bands, 161 (amplified with 82 different primers) were tentatively assigned among the 12 *S. brevidens* chromosomes. Each chromosome was marked by 6–20 RAPDs (mean=13.4 markers per chromosome, SD=4.62). Thirty amplified fragments (16%, from the remaining eight primers) did not unambiguously associate with any synteny group.

To assess whether RFLP and RAPD data were in agreement, most BC_1 parents and their BC_2 progeny previously tested with RFLPs were retested with selected RAPD markers. Eleven primers (B08, C15, D11, F02, F13, H09, H12, H15, I13, J17 and K16) were chosen for this purpose because they amplified a total of 39 fragments with an expected distribution among all 12 *S. brevidens* chromosomes. Overall, RAPD data was concordant with RFLP data. For 25 of the 39 *S. brevidens* RAPDs, the initial chromosomal assignments were in agreement with the RFLP data. For example, identical results were obtained with RAPD marker H09 and RFLP TG23 (Fig. 3). However, 11 of the 39 RAPD markers initially scored were not reproducibly amplified or were otherwise difficult to score because they co-migrated with other amplification products. This observation underscores the necessity to confirm the assignments of all *S. brevidens* RAPD markers. In addition, three RAPDs required a change from their initial synteny group assignments. RAPD marker $C15_{1900}$ assorted with other RAPD markers from chromosome *5*, although it had initially been assigned to chromosome *10*. In this case, we noticed that certain DNA preparations would consistently under-amplify products >1 000 bp, and this was apparently the reason that $C15_{1900}$ was initially located incorrectly. Marker $D11_{900}$ assorted with chromosome *4* rather than retaining its initial association with chromosome *10*, apparently as the result of an ambiguity in the lines initially used to key RAPDs between *S. brevidens* chromosomes *4* and *10*. In general, BC_1 plants lacking an *S. brevidens* RFLP-synteny group were of greater utility in assigning RAPDs to chromosomes than were the aneuploid

lines in the BC_2 generation, and neither chromosomes *4* nor *10* were nullisomic in the BC_1. Finally, RAPD marker $H15_{850}$ was re-assigned from chromosome *5* to chromosome *9* because the key diagnostic line for chromosome *5* (plant C41) apparently lacked a portion of chromosome *9* that was not detected with RFLPs.

For the RAPDs that could be reliably and reproducibly scored, many were located within an interval defined by RFLPs. For instance, the two chromosome-*4* RAPDs $D11_{900}$ and $J17_{400}$ were bounded by RFLPs TG123 – TG65 and TG65 – TG22, respectively (Fig. 1). Similarly, RAPD markers on chromosomes *8*, *10*, *11* and *12* were located on opposite sides of the putative centromere region (Fig. 1), assuming that the position of each centromere in *S. brevidens* roughly correlates with their position(s) in potato and tomato (Tanksley et al. 1992).

Discussion

One concern for the introgression of desirable genes from *S. brevidens* into *S. tuberosum* was that their chromosomes would fail to interact. For instance, little or no inter-genomic recombination occurs in wide-hybrids involving sugar beet and its wild relatives (Jung et al. 1992 and references therein). We were particularly encouraged to find that one plant (41-10) had the normal potato complement of 48 chromosomes and still carried a single *S. brevidens*-specific RFLP marker. We suspect that this *S. brevidens* marker became associated (i.e., introgressed) with an *S. tuberosum* chromosome in this plant.

Extensive interactions between the potato genome and that of *S. brevidens* would not have been expected on the basis of cytological investigations on hybrids between non-tuberous *Solanum* species in the series *Etuberosa*, containing the E genome, and tuber-bearing species which are considered to carry a basic A genome (Matsubayashi 1991). Ramanna and Hermsen (1981) critically characterized chromosome pairing within and between three E-genome species (including *S. brevidens*), as well as with an A-genome species, *S. pinnatisectum*. Their results showed that interspecific hybrids involving the E genome showed a high proportion of multivalent chromosome associations. However, A × E genome interspecific hybrids showed a high proportion of univalents. They proposed two alternative explanations for these results. The first possibility was that 'genomes of non-tuberous and tuberous species are differentiated because of the loss of pairing affinity between homoeologous chromosomes'. The second possibility was that the A and E genomes are extensively modified by gross structural changes but still maintain a high affinity for pairing between homoeologous chromosome segments. Our results support the latter alternative since it is unlikely that recombination would occur if chromosomes did not interact, either through homoeologous pairing or in some as yet undefined manner.

Our observation that many *S. brevidens* recombinant synteny groups had lost terminal RFLP markers might be expected if 'gross structural changes' included paracentric inversions. Through homoeologous chromosome pairing and crossing-over, paracentric inversions can produce telosomic fragments that contain a centromere and others that are acentric and are subsequently lost through cell division. Five paracentric inversions involving whole chromosome arms differentiate the chromosomes of potato and tomato (Tanksley et al. 1992). Since *S. brevidens* shows a high degree of synteny conservation with potato and tomato, it is possible that some *S. brevidens* chromosomes differ from their potato homoeologs by paracentric inversions, as well. This may in part explain some of the marker loss we observed.

Many of the RFLP markers used here appear to be located in euchromatic regions of tomato (see Tanksley et al. 1992). However, cytological analyses of chromosome translocations in tomato showed that more than 80% of chromosome breakpoints occurred within centromeric and heterochromatic chromosome regions (Gill et al. 1980). If the tomato results can be extrapolated, much of the recombination we have observed along *S. brevidens* chromosomes may also have involved centromeres and heterochromatin. If so, then a loss of *S. brevidens*-specific RFLP markers would suggest that (1) the majority of 'deletion' breakpoints have occurred within the heterochromatin and (2) entire euchromatic chromosome arms have been lost. Loss of chromosome arms (i.e., recovery of telo-trisomic chromosomes) has been observed among progeny of tomato trisomics (reviewed in Quiros 1991).

We have considered the loss of *S. brevidens* RFLP markers from their characteristic synteny groups as evidence that *S. brevidens* chromosomes are recombinogenically active. This is justified if paracentric inversions are involved (see above), but additional and undefined mechanisms may also have contributed to marker loss in these populations. From the pattern of *S. brevidens* markers that are present (as well as our cytological analyses), it is apparent that some *S. brevidens* markers are no longer associated with their characteristic synteny group, but are probably now present elsewhere in the genome. This suggests that chromosome segments were translocated to other chromosomes, or that markers were exchanged between *S. brevidens* and *S. tuberosum*. The proof of recombination, either non-homologous or homoeologous, will depend on determining the new locations of recombined DNA segments.

The relative frequencies of different types of recombination, such as inversion, translocation, 'deletion', or homoeologous chromosome pairing and crossing-over, are not yet known. Our data suggest that all *S. brevidens* chromosomes are capable of recombining. Ultimately, it will be important to: (1) obtain better estimates for the frequency of recombination by looking at larger and more populations, (2) determine whether recombination frequencies are similar for different chromosomes and for a particular chromosome over successive generations, and (3) locate and recover recombined chromosome segments for clues as to the mechanism(s) of recombination. The demonstration of that all 12 *S. brevidens* chromosomes have the potential to recombine while residing in a potato

nucleus is an important step towards the introgression of disease-resistance characters through the use of somatic hybrids with potato.

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