RFLP Analysis of Chromosomal Segregation in Progeny From an Interspecific Hexaploid Somatic Hybrid Between *Solanum brevidens* and *Solanum tuberosum*

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ABSTRACT

Segregation of restriction fragment length polymorphism (RFLP) loci was monitored to determine the degree of homeologous pairing and recombination in a hexaploid somatic hybrid, A206, the result of protoplast fusion between *Solanum tuberosum* (PI 203900, a tetraploid cultivated potato) and *Solanum brevidens* (PI 218228), a diploid, sexually incompatible, distant relative harboring several traits for disease resistance. Somatic hybrid A206 was crossed to Katahdin, a tetraploid potato cultivar, to generate a segregating population of pentaploid progeny. Although the clones of the tetraploid *S. tuberosum* lines PI 203900 and Katahdin were highly polymorphic, the diploid *S. brevidens* clone was homozygous at all but two of the tested RFLP loci. Thus, homeologous recombination could be detected only when *S. tuberosum* and *S. brevidens* chromosomes paired and the *S. brevidens* homologs then segregated into separate gametes. A bias toward homologous pairing was observed for all 12 chromosomes. At least four and perhaps six chromosomes participated in homologous pairing only; each of 24 progeny contained all *S. brevidens*-derived RFLP markers for chromosomes *4*, *8*, *9* and *10*. The remaining six chromosomes paired with their homolog(s) about twice as often as expected if hexaploid pairings were completely random. Where detectable with RFLPs, homeologous recombinations (both single and double) occurred at a frequency of 1.31 per chromosome. Cytological observations of meiosis I in the somatic hybrid indicated that homeologous pairing had occurred. Enhanced recombinational activity was observed for chromosome *2*. A specific small deletion from chromosome *4* was detected in A206 and 11 other somatic hybrids out of 14 screened. These hybrids represent 13 independent fusion events between the same clones of *S. brevidens* and *S. tuberosum*. In one instance, this deletion occurred in one of two plants resulting from the same callus, indicating that the loss occurred in culture after fusion had taken place. It is possible that this deletion contributes to somaclonal variation.

CHROMOSOMAL instabilities are common in *Solanum* species. Although it may not be surprising that chromosome loss occurs in interspecific somatic hybrids (EHLENFELDT and HELGESON 1987), lagging chromosomes have also been reported for interspecific sexual hybrids, even those from narrow crosses that are achieved with relative ease (see summary by DVORAK 1983). Moreover, many tetraploid potato cultivars exhibit multivalents and univalents at diakinesis, a likely cause of aneuploidy and reduced fertility (SWAMINATHAN 1954). Unlike polyploid wheat, which employs genetic mechanisms to enforce diploid-like pairing between homologous chromosome sets (OKAMOTO 1957), polyploid potatoes seem to be constructed from diverse homeologous genomes that pair with each other (DVORAK 1983).

In an ongoing project aimed at introgressing genes for disease resistance from wild *Solanum* species into potato cultivars, interspecific somatic hybrids were generated from the fusion of *S. tuberosum* (potato) with the distantly related species *S. brevidens* (AUSTIN, EHLENFELDT and HELGESON 1986). The hybrids retained the disease resistances of both species (HELGESON *et al.* 1986). One concern about the newly transferred resistances was that incomplete pairing between homeologous genomes might result in chromosome instabilities and poor transmission of these traits through subsequent sexual crosses. Therefore, studies were initiated to characterize recombination and segregation in the hexaploid somatic hybrid ($4X + 2X$) and its pentaploid progeny that resulted from a sexual cross with the tetraploid cultivar, Katahdin. Restriction fragment length polymorphism (RFLP) markers were used to distinguish the chromosomes of *S. brevidens* and *S. tuberosum*. The somatic hybrid contained markers that would account for all 24 chromosome arms of both fusion parents (WIL-

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LIAMS, HUNT and HELGESON 1990). In addition, analysis of the pentaploid progeny indicated that no chromosomes appeared to be lost preferentially.

The present study characterizes the stability of *S. brevidens*-dervived DNA that has been introgressed into the potato genome. Segregation of RFLP markers was used to determine pairing bias, homeologous recombination (recombination between *S. brevidens*-derived and *S. tuberosum*-derived chromosomes) and locus stability in the pentaploid progeny of the somatic hybrid. The presence of homeologous recombinations provided evidence that introgression of wild species traits was possible from somatic hybrids of these sexually incompatible species.

MATERIALS AND METHODS

Plant material: All plants were vegetatively propagated from single individuals that are maintained *in vitro* (HABERLACH *et al.*, 1985). Somatic hybrid A206 resulted from protoplast fusion (method of AUSTIN, BAER and HELGESON 1985) between *S. brevidens* Phil. (PI 218228, clone 6A) and *S. tuberosum* L. (PI 203900, clone R4). *S. brevidens* (*Sb*) is a wild diploid ($2n = 2x = 24$) that does not form tubers, whereas *S. tuberosum* (*St*) PI 203900 is a tetraploid potato ($2n = 4x = 48$). Hybrid A206 is an interspecific hexaploid as determined by chromosome count (M. K. EHLENFELDT, H. KIM-LEE and J. P. HELGESON, unpublished results) and RFLP analysis (WILLIAMS, HUNT and HELGESON 1990). Sexual progeny were derived from a cross (EHLENFELDT and HELGESON 1987) between A206 and *S. tuberosum cv.* "Katahdin" (KAT), another tetraploid potato line. The 24 sexual progeny examined were at or near the pentaploid level as determined by chromosome count. The progenitor accessions for *Sb*, *St* and KAT were obtained from the *Solanum* collection at the Inter-regional Potato Introduction Project (IR-1), Sturgeon Bay, Wisconsin.

Plant DNA manipulations: Total cellular DNA was isolated from frozen apical leaves taken from young plants that had been clonally propagated and then grown in the greenhouse for 4–10 weeks. DNA isolation was as described previously (WILLIAMS, HUNT and HELGESON 1990). Approximately 7 μg of each DNA sample were digested with *Eco*RI, *Eco*RV or *Hin*dIII and subjected to electrophoresis in 0.7% agarose gels. DNA was transferred to Nytran membrane (Schleicher & Schuell) according to the manufacturer's instructions. Each blot included DNA from *St*, *Sb*, A206, KAT and 24 sexual progeny from the cross A206 × KAT.

Detection of polymorphisms: The probes used in this study were derived from tomato and have been mapped to single loci in both tomato (BERNATZKY and TANKSLEY 1986; TANKSLEY, MUTSCHLER and RICK 1987) and potato (BONIERBALE, PLAISTED and TANKSLEY 1988). The probe/enzyme combinations were chosen to detect polymorphisms that differentiated the *Sb* and *St* parental DNA in the progeny without being obscured by comigrating KAT bands. Each of the selected probes detected at least one unique band in each fusion parent. Each chromosome was marked by three to seven probes (Figure 1). Probe and blot manipulations were as described by WILLIAMS, HUNT and HELGESON. (1990).

RESULTS AND DISCUSSION

Sb and *St* exhibited distinct restriction fragment length polymorphisms, and the RFLP patterns of their somatic hybrids typically included the bands of both parents (WILLIAMS, HUNT and HELGESON 1990). Initially, 93 probes were screened with parental DNA cut with *Eco*RI, *Eco*RV and *Hin*dIII. Of these probes, 71 gave polymorphisms between *Sb* and *St* with at least one enzyme. A total of 55 of these probes also distinguished bands from *Sb*, *St* and A206 from those shown by potato cultivars such as KAT. These probes were used to score RFLPs in the pentaploid progeny resulting from a cross of KAT with the hexaploid somatic hybrid, A206.

Degree of heterozygosity: Heterozygosity of parental plants was determined by the detection of multiple bands that segregated with respect to each other in the pentaploid progeny. Because *St* and KAT are tetraploid, often more than two alleles were detected at a locus (Figure 2). In considering the 48 most easily scored loci, those with which polymorphisms between *Sb*, *St* and KAT were clearly visible, we determined that *Sb* was heterozygous at only two loci. An illustration of this is shown for TG122 on chromosome *10* (Figure 3). The other heterozygous locus was TG330 on chromosome *8*. *Sb* was homozygous at the 46 remaining loci. In contrast, *St* and KAT were heterozygous at 25 and 26 loci, and homozygous at 14 and 12 loci, respectively. Nine loci in *St* and 10 loci in KAT were ambiguous due to comigrating bands from each other or from *Sb*. Thus, *St* and KAT were heterozygous for at least 52% of the loci, whereas *Sb* was heterozygous at only 4% of the loci examined. Due to possible bias from probe selection, these numbers should be taken as only relative measures of heterozygosity. However, these data do support previous work indicating that polyploid potatoes contain diverse genomes (DVORAK 1983) and maintain a high level of polymorphism that is not evident in many diploid species (S. PELOQUIN, personal communication).

Homologous pairing: The distribution of bands for the 55 probes which were used for recombination studies is shown in Figure 4. With these data it was determined that homologous pairing occurred between the two *Sb*-derived chromosome sets and among the four *St* sets. Evidence for homologous pairing is the high percentage of pentaploid progeny that exhibited all of the *Sb*-derived markers for any given chromosome. This situation would likely arise if *Sb*-derived chromosomes paired only with each other. The other situation that would give intact *Sb* chromosomes requires the formation of univalents (no recombination) instead of homologous pairing. However, this situation would typically result in poor transmission of the univalents, leading to many progeny that were missing entire chromosomes. None of the pentaploid progeny lacked the complete set of *Sb*-derived chromosomes. One individual lacked all of the *Sb*-derived markers

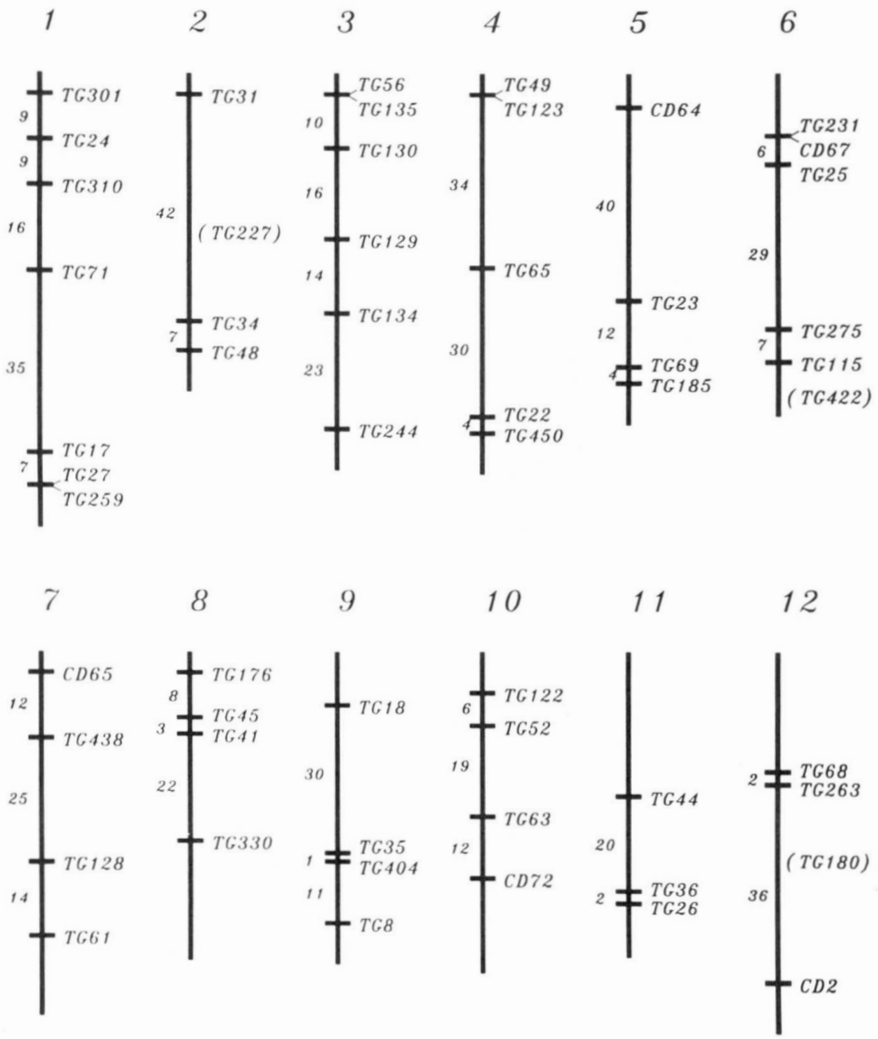

FIGURE 1.—Map locations of RFLP markers used in this study. Markers were chosen to cover as much as possible of each chromosome. Map distances are approximate and based on the map of BONIERBALE *et al.* (1988) and personal communication from S. TANKSLEY. Probes listed in parentheses have been reported only on the tomato maps.

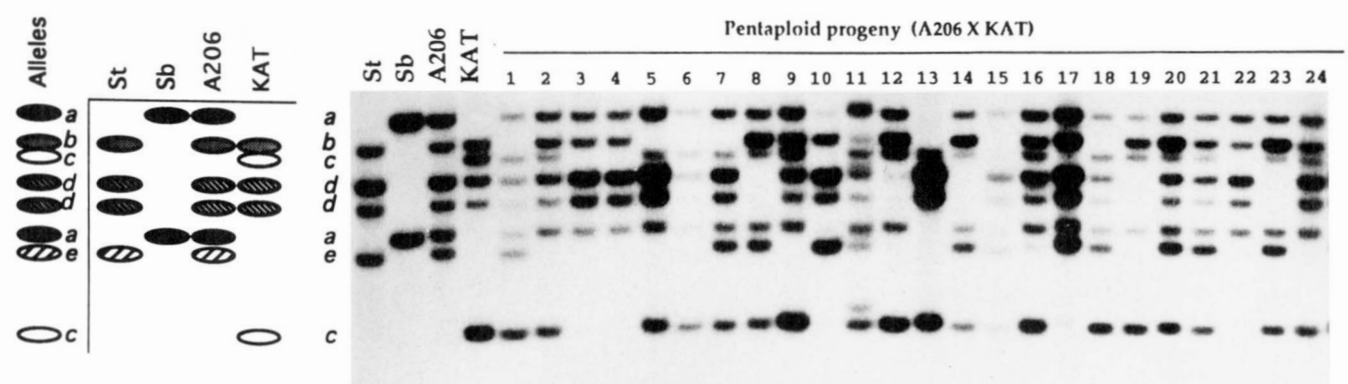

FIGURE 2.—Alleles and segregation for TG115, chromosome 6. Collectively the parents contained five alleles that segregated among the pentaploid progeny from a cross of A206 × KAT. A diagrammatic representation of the alleles is given at the left of the figure. Certain alleles (*a*, *c* and *d*) each constitute two bands due to internal restriction sites. Allele dosage: *St* = *bdde*, *Sb* = *aa*, A206 = *aabdde*, and KAT = *bccd*. The banding patterns for *St*, *Sb*, the somatic hybrid (A206), KAT, and 24 pentaploid sexual progeny from the cross of A206 × KAT are given at the right of the figure. Genomic DNA was digested with *Eco*RV.

for two chromosomes and six other individuals were lacking markers for a single Sb-derived chromosome (Figure 4).

For the set of 24 pentaploid progeny ($n = 12$) there were 288 cases ($24 \times 12 = 288$) where homologous pairing could be assessed by scoring for the presence of all of the *Sb*-derived markers on a given chromosome. In 264 out of those 288 cases (91.7%) all of the *Sb*-derived markers were present (Figure 4). This value indicates a strong bias toward homologous pairing. However, the value for homologous pairing may be slightly high because of the obscuring of homeo-

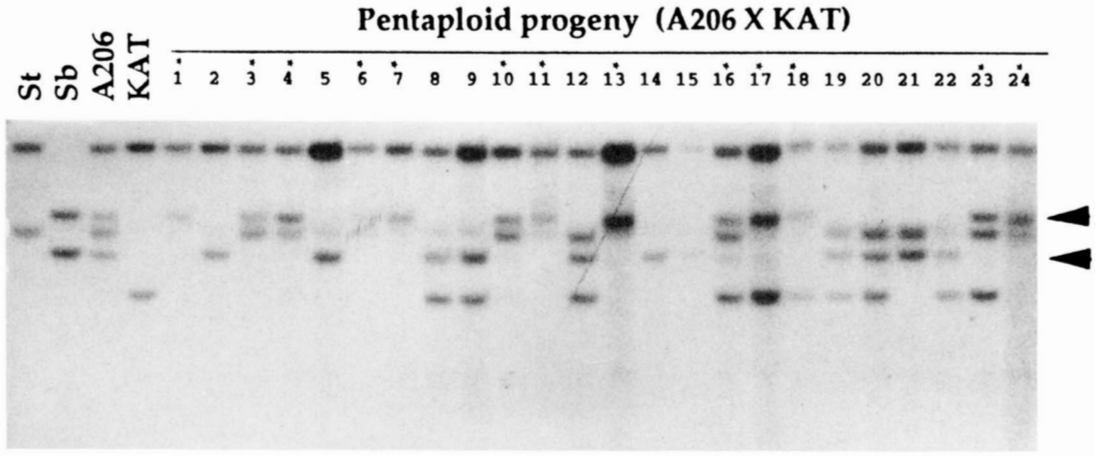

FIGURE 3.—Heterozygosity in *S. brevidens*. One-to-one segregation of *Sb*-derived bands. Lines with asterisks lack the lower molecular weight *Sb*-derived band whereas those without asterisks lack the higher molecular weight *Sb* band. Genomic DNA was digested with *Hin*dIII and probed with TG122, a probe specific to chromosome *10*. *Sb*-derived bands are indicated by arrowheads.

logous recombination by one of several situations. These include: (a) homeologous recombination distal to the terminal marker or double cross overs in unmarked regions, (b) cosegregation of two recombinant chromatids that completely masked each other's homeologous recombinations or (c) cosegregation of a recombinant chromatid with a nonrecombinant *Sb*-derived chromatid. In any event, a bias toward homologous pairing is probably responsible for a large proportion of the cases that exhibited all of the *Sb*-derived markers for a given chromosome.

Pairing bias: Although all chromosomes showed some degree of pairing bias, four chromosomes (*4*, *8*, *9* and *10*) appeared to participate exclusively in homologous pairing. This pairing bias was indicated by the fact that none of the 24 pentaploid progeny lacked any of the *Sb*-derived markers for these chromosomes (Figure 4). For example, the *Sb*-derived allele of TG 65 did not segregate, indicating that the two copies of *Sb*-derived chromosome *4* always paired with each other and that each of the progeny contained one copy of that chromosome (Figure 5). Additional evidence for exclusive homologous pairing was seen in the near 1:1 segregation ratios of loci on chromosomes *8* and *10* that were scored as heterozygous for *Sb*-derived markers. For example, Figure 3 shows that none of the pentaploid progeny contained both or lacked both of the *Sb*-derived alleles for TG122 on chromosome *10*, an indication that these differentially marked *Sb* chromosomes had always paired with each other. Two additional chromosomes (*11* and *12*) might have undergone only homologous pairing. Four progeny lacked all *Sb*-derived markers for one or the other of these chromosomes. This could be due to univalent formation and chromosome loss in these individuals. Alternatively, recombination might have occurred so that the *Sb*-marked segments were not included in the scored individuals.

Homeologous pairing: The markers were chosen to cover the genome so that homeologous recombinations could be detected readily (Figure 1). Because *Sb* was diploid and homozygous for all but two markers, its alleles were known to be in the *cis* configuration. Thus, it was possible to detect homeologous recombinations by scoring the recombinational loss of *Sb*-derived markers.

Locations of homeologous recombinations can be derived from Figure 4. Each oblong bar in the figure represents the phenotype of an individual with respect to the *Sb*-derived markers on a single chromosome. The absence of an *Sb*-derived marker is indicated by white space. Thus the majority of *Sb*-derived markers were present in all progeny, but homeologous recombinations occurred in regions where one marker was present (indicated by shading) but the adjacent marker was absent.

Six chromosomes participated to some extent in homeologous pairing and recombination. The number of progeny showing recombinations ranged from five progeny for chromosome *2* to one for chromosomes *1* and *7* (Figure 4). Enhanced recombinational activity was detected on chromosome *2*. Out of 288 chromosomes scored, a total of 21 recombinations were detected on 16 chromosomes. Thus, for those chromosomes that underwent recombinations, 1.31 recombinations per chromosome were detected.

The major consequence of low heterozygosity in the *Sb*-derived chromosomes was that some homeologous recombinations probably went undetected, resulting in a conservative prediction of introgression success. Pentaploid progeny were scored as having resulted from homeologous recombination if they lacked one or more *Sb*-derived markers, but not all *Sb*-derived markers for a given chromosome. However, homeologous recombination would not have been detected when one *Sb*-derived chromosome par-

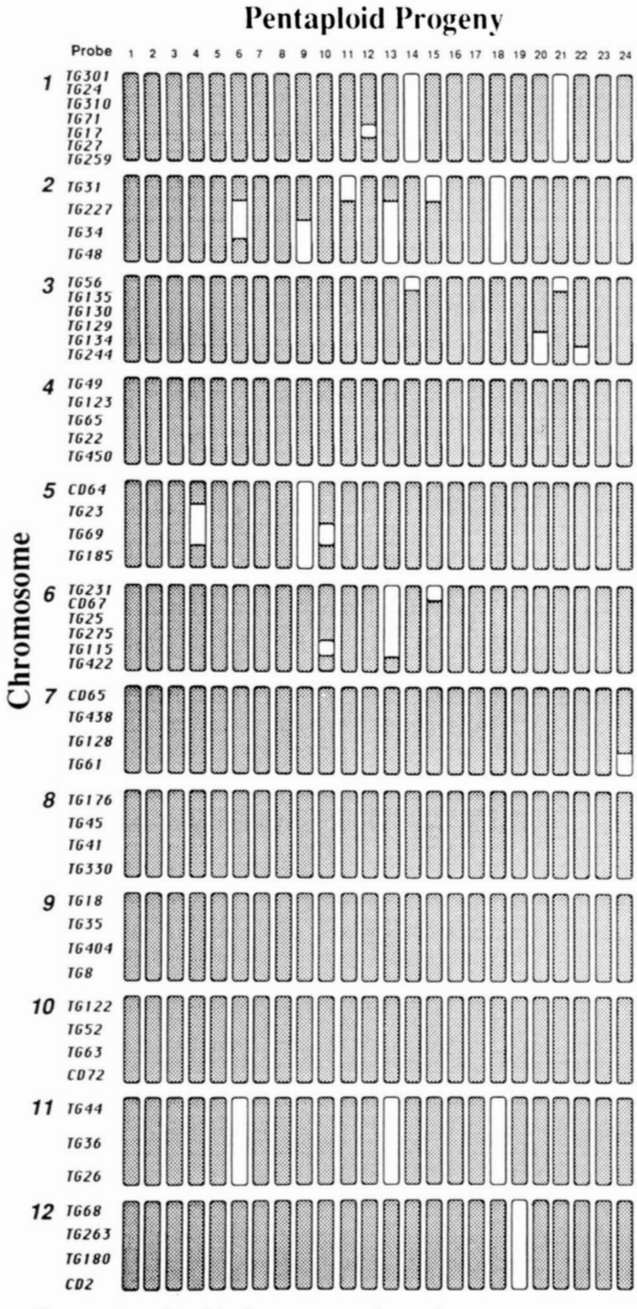

FIGURE 4.—Graphical representation of progeny genotypes. Twenty four pentaploid progeny from the cross (*St* + *Sb*) × KAT were scored for *Sb*-derived markers. Each column displays a genotype for the 12 chromosomes of an individual progeny. Shaded areas of the bars indicate that with the specified probe, all *Sb*-derived bands were detected for an individual. Blank regions indicate loci where *Sb*-derived bands (with a specific probe) were absent due to homeologous recombination.

tially or completely obscured the presence of a second *Sb*-derived chromosome that was recombinant. Although overlapping homeologous recombinations would result in fewer scored recombinations, it is likely that the presence of all *Sb*-derived markers in all 24 of the progeny would usually be due to homologous pairing rather than undetected homeologous recombinations.

Although the frequency of detectable homeologous recombination was not high, many regions of six different chromosomes were able to recombine to some degree (Figure 4). Therefore, it appears that exotic resistance genes could be crossed onto potato chromosomes and introgressed into cultivars.

Chromosome associations: Due to the tendency of polyploid potatoes to form multivalents, the outcome of segregation is not always predictable. Thus, in addition to RFLP studies the potential for homeologous pairing was also evaluated by cytological analyses. Both A206 and KAT showed multivalent formation. Average frequencies for 17 KAT cells counted were 1.9 univalents, 17.6 bivalents, 1.0 trivalents and 1.9 quadrivalents. For 11 A206 cells, average frequencies were 5.6 univalents, 21.8 bivalents, 5.3 trivalents and 1.7 quadrivalents. A photograph of meiosis I of A206 is shown in Figure 6. In this figure, it was possible to count 7 univalents, 21 bivalents, 5 trivalents, and 2 quadrivalents. The chromosome arrangement requiring the fewest homeologous pairings would be: 6 *St* + 1 *Sb* = 7 univalents, 1 *St/Sb* + 9 *St/St* + 11 *Sb/Sb* = 21 bivalents, 5 *St/St/St* trivalents and 2 *St/St/St/St* quadrivalents. Observations of 11 other A206 cells indicated a range of 0–4 homeologous pairings with an average of 1.1/observed cell, a figure that is in reasonable agreement with the RFLP data (21 homeologous recombinations in 24 progeny, Figure 4).

RFLP evidence for multivalent pairing that resulted in double reduction was seen with TG115 on chromosome *6* (Figure 2). Double reduction can be detected when a multi-allelic locus in a polyploid is on a chromosome that is undergoing multivalent formation at meiosis I. In order to detect double reduction, the point of recombination must be between chromatids carrying different alleles and proximal to the locus. During meiosis, the recombinant and the nonrecombinant chromatid (which are sister chromatids carrying identical alleles but are now attached to different centromeres) segregate to the same gamete. The result is a plant with the correct number of chromosomes but fewer than the expected number of alleles due to duplicated chromatids. Several of the progeny in Figure 2 had too few alleles, with the most obvious being #13. Bivalent segregation dictates that in this cross (A206 genotype = *aabdde* × KAT genotype = *bccd*, Figure 2), each pentaploid must receive three chromosomes carrying at least two different alleles from A206; #13 (phenotype = cd) received only allele *d* from A206. A comparison of band intensities for #13 with A206 and KAT suggests that the genotype of #13 is *dddcc* or *ddddc*. Double reduction would provide a mechanism by which progeny #13 could result from a gamete carrying *ddd* from A206 that joined with one carrying *cd* or *cc* from KAT. An alternative explanation could be that the plant is aneu-

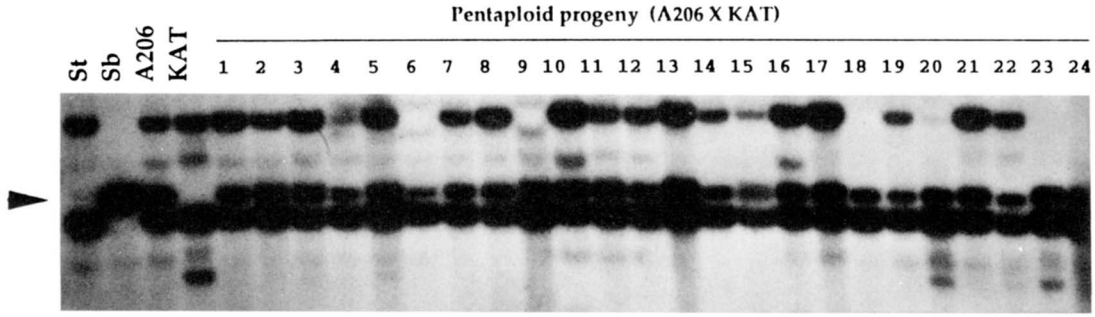

FIGURE 5.—Chromosome *4* appeared to participate exclusively in homologous pairing. All progeny contain the *Sb*-derived band (indicated by the arrowhead) for TG65 on chromosome *4*. Genomic DNA was digested with *Eco*RI.

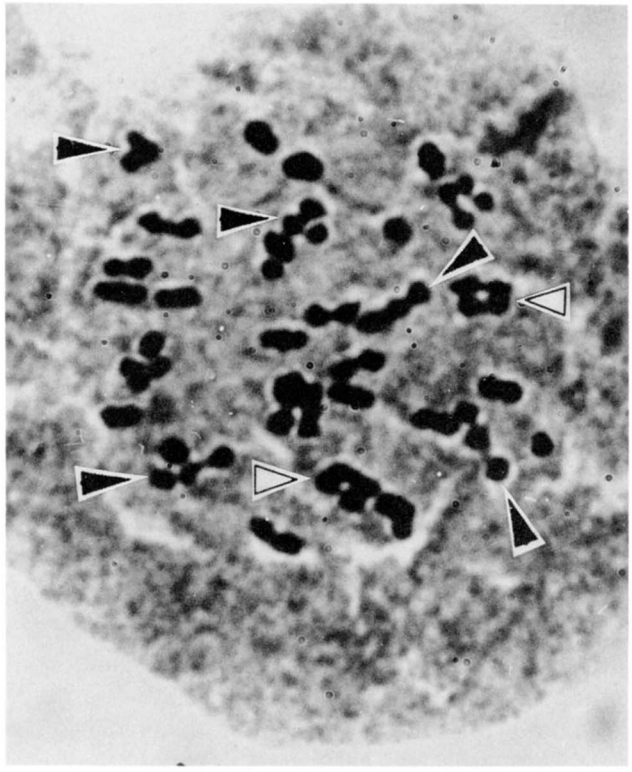

FIGURE 6.—Meiosis I in A206. Seven univalents, 21 bivalents, 5 trivalents (black arrowheads), and 2 quadrivalents (white arrowheads) were detected in this cell.

ploid, even though it has at least one marker from each *Sb* chromosome.

Other abnormalities with TG115 were seen among the progeny of A206. In addition to #13, double reduction may have caused the following progeny to contain too few alleles (the estimate of genotype was based on band intensity): progeny #12 (A206 gamete = *abb*, KAT gamete = *cc*), #19 (A206 gamete = *abb*, KAT gamete = *bc* or *cc*). Three individuals (5, 18 and 20) appeared aneuploid from chromosome count and showed abnormal RFLP patterns. Number 5 (62 chromosomes, possible genotype *acddde*) had an unusually intense band for allele *d* indicating that the plant might have an extra chromosome *6*. Progeny #20 (possible genotype *abbcde*) also had a chromosome count of 62. Finally, progeny #18 (possible genotype of *acde* by band analysis) had only 58 chromosomes. In contrast, other progeny appeared to be normal by RFLP pattern and by chromosome count. These plants (all with 60 chromosomes) include progeny 1, 7 and 9. Chromosome counts are not available for all of the 24 progeny. However, these results indicate that multivalent formation did occur in A206 and Katahdin, resulting in abnormalities detected in the progeny.

Locus instability: Somaclonal variation is a common occurrence in plants that are regenerated from tissue culture (SCOWCROFT, LARKIN and BRETTELL 1983). Chromosome deletions and rearrangements may be the genetic basis of this phenomenon. One such deletion was detected on the Sb-derived chromosome *4*. In general, the hexaploid somatic hybrids had all bands from both fusion parents. An example of this is TG22 (Figure 7a). However, in the process of reviewing screening blots, a deletion of one band was seen with TG123, another marker on chromosome *4* (Figure 7b). Two bands were detected for TG 123 in *Sb*, whereas only the top band was present in hybrid A206 and its progeny. In order to confirm these findings, TG123 was used to probe DNA from 14 somatic hybrids, representing 13 independent fusion events between clonal protoplasts of *Sb* and *St*. Twelve of these hybrids lacked the band, whereas two retained it. Representative results with 11 of the hybrids are shown in Figure 7b. Hybrids A903 and A1674 were regenerated from the same callus but one plant retained the band whereas the other lost it. Thus, the deletion probably occurred after fusion, once cell division had begun. TG123 is located between TG49 and TG65 on chromosome *4* (see Figure 1). These flanking markers and the remaining TG123 band were present in all progeny of A206 as well as all of the independent somatic hybrids. Thus, the independent deletions included only part of the TG123 locus and occurred at a very high frequency. The TG123 probe appeared to span the junction of a highly mutable locus and an adjacent stable locus. No new bands were detected with the TG123 probe, thus

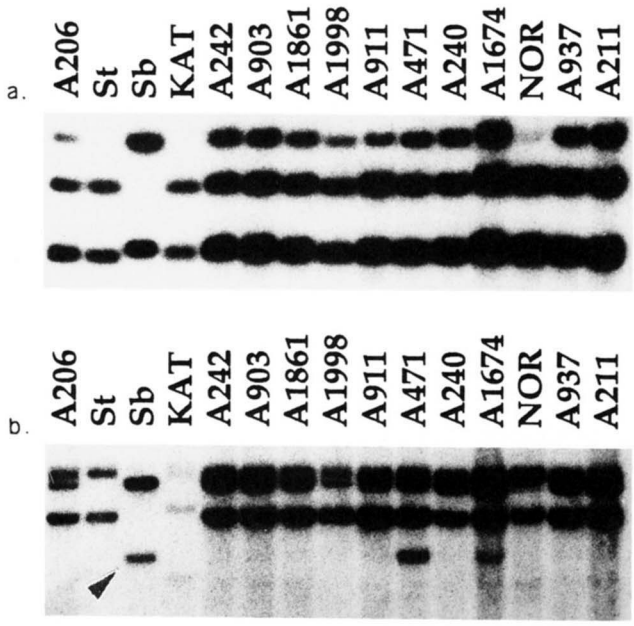

FIGURE 7.—Deletions detected in somatic hybrids at TG123 but not TG22, a second locus on chromosome *4*. RFLP patterns of genomic DNA cut with *Hin*dIII shown for A206, *St*, *Sb*, KAT, NOR (Norland, another potato cultivar), and 10 other somatic hybrids from fusions of *Sb* and *St*. The same blot was used for successive probings with TG22 and TG123. (a) All bands for TG22, were present in somatic hybrids. (b) Deletion of the lower *Sb*-derived band (indicated by the arrow) for TG123. Somatic hybrids A903 and A1674 were regenerated from the same callus, yet differed for the lower *Sb*-derived band at the TG123 locus.

the region of the mutable locus that is covered by TG123 was not found to reinsert.

CONCLUSIONS

The data presented above clearly indicate that introgression of DNA from wild, sexually incompatible *Solanum* species into potato breeding lines is possible. RFLP analyses of progeny from crosses of hybrid A206 with a potato line revealed that homeologous recombination occurred on 6 of the 12 chromosomes. Also, loss of *Sb*-derived chromosomes was uncommon. These findings may have practical significance. Somatic hybrids between *S. brevidens* and *S. tuberosum* express disease resistances to pathogens such as potato leaf roll virus, (HELGESON *et al.* 1986) or *Erwinia* spp. (AUSTIN *et al.* 1988). Introgression of the genes for these resistances could provide new, highly effective resistances against these important disease-causing agents. Efforts are currently underway to determine if *Sb*-derived disease resistances are introgressed.

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