Cytological and molecular characterization of repetitive DNA sequences of *Solanum brevidens* and *Solanum tuberosum*

V.-M. Rokka, M.S. Clark, D.L. Knudson, E. Pehu, and N.L.V. Lapitan

Abstract: The chromosomal distribution, copy numbers, and nucleotide sequences were determined for four repetitive DNA clones, pSB1 and pSB7 of *Solanum brevidens* and pST3 and pST10 of *Solanum tuberosum*. Using fluorescence in situ hybridization (FISH), pSB1 and pSB7 were localized near the telomeres and in some centromeric and interstitial sites of *S. brevidens* chromosomes, but not in *S. tuberosum* chromosomes, after high stringency washes. The clone pST3 showed signals in the telomeric areas of a few chromosomes in *S. tuberosum*, but signals were not detected in *S. brevidens*. All three repeated sequences (pSB1, pSB7, and pST3) were detected in chromosomal areas that are typically known to contain tandemly repeated sequences. The *S. tuberosum* clone pST10 did not show signals in either species even at low stringency conditions. The estimated copy numbers of the four clones were 1500, 6750, 300, and 400 for pSB1, pSB7, pST3, and pST10, respectively, in the corresponding haploid genomes (*S. brevidens* and *S. tuberosum*). The inserts of the four clones pSB1, pSB7, pST3, and pST10 were 322, 167, 845, and 121 bp, respectively. After sequencing, no significant sequence homologies were found among the four clones. A homology search in sequence data bases showed that pSB7 has variable homology (78–100%) with another repetitive sequence of *S. brevidens* Sb4/2 depending on its subrepeat. It also showed some homology with one repeat of tomato (pLEG15) and one repeat of *Solanum circaeifolium* (pSC15).

Key words: chromosome, copy number, fluorescence in situ hybridization, FISH, nucleotide sequence, potato.

Résumé : La distribution chromosomique, le nombre de copies et la séquence nucléotidique de quatre clones d'ADN répétitif, pSB1 et pSB7 du *Solanum brevidens* ainsi que pST3 et pST10 du *Solanum tuberosum*, ont été déterminés. À l'aide de l'hybridation in situ à fluorescence (FISH), il a été déterminé que pSB1 et pSB7 étaient localisés à proximité de tous les télomères de même qu'au niveau de certains centromères ou en des sites intermédiaires chez les chromosomes du *S. brevidens* alors qu'ils étaient absents des chromosomes du *S. tuberosum* suite à des lavages sélectifs. Pour le clone pST3, des signaux ont été observés proche des télomères de quelques chromosomes du *S. tuberosum* mais aucun signal n'a été détecté chez le *S. brevidens*. Ces trois séquences répétitives (pSB1, pSB7 et pST3) ont été détectées dans des régions chromosomiques qui contiennent typiquement des séquences répétées en tandem. Le clone pST10 du *S. tuberosum* n'a pas produit un signal chez l'une ou l'autre espèce et ce même en conditions permissives d'hybridation. Le nombre de copies de chacune des ces séquences répétitives a été estimé à 1500, 6750, 300 et 400 par génome haploïde pour pSB1, pSB7, pST3 et pST10 respectivement. Les inserts des clones pSB1, pSB7, pST3 et pST10 mesuraient respectivement 322 pb, 167 pb, 845 pb et 121 pb. Suite au séquençage de ces quatre clones, aucune homologie significative entre eux n'a été notée. Des recherches d'homologie effectuées dans les banques de séquences ont révélé que pSB7 présentait une homologie variable (78-100 %) avec une autre séquence répétitive du *S. brevidens* (Sb4/2) selon la sous-unité. Une certaine homologie a aussi été décelée avec un ADN répétitif de la tomate (pLEG15) et un autre du *Solanum circaeifolium* (pSC15).

Mots clés : chromosome, nombre de copies, hybridation in situ à fluorescence, FISH, séquence nucléotidique, pomme de terre.

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Introduction

The cultivated potato (*Solanum tuberosum*) is a tetraploid ($2n = 4x = 48$) species and the fourth most important crop in total production in the world (Hawkes 1994). *Solanum brevidens* ($2n = 2x = 24$) is a wild potato species that is extremely resistant to economically important viral diseases, including potato leaf roll virus (PLRV) and potato virus Y (PVY), and is moderately resistant to potato virus X (PVX) (Valkonen et al. 1992). Sexual hybridization between these two species has been limited (Watanabe et al. 1995), but the production of somatic hybrids using protoplast fusion has been reported by several authors (e.g., Austin et al. 1985; Fish et al. 1987; Rokka et al. 1994).

Repetitive DNA elements in eukaryotic species are either arranged in tandem or are dispersed (Flavell 1980). Many tandemly repeated sequences in plants are present in numerous copies in the species from which they were isolated but are present in fewer copies in related species, such sequences are referred to as species-specific repeats (Lapitan 1992). Repetitive DNA sequences have been isolated from many species of the Solanaceae, including *Lycopersicon esculentum* (Ganal et al. 1988; Schweizer et al. 1988), *S. tuberosum* (Visser et al. 1988; Pehu et al. 1990; Schweizer et al. 1993), *Solanum phureja* (Visser et al. 1988), *Solanum acaule* (Schweizer et al. 1988), *Nicotiana tomentosiformis* and *Nicotiana tabacum* (Koukalová et al. 1989), *S. brevidens* (Pehu et al. 1990; Preiszner et al. 1994), *Solanum demissum* (Schweizer et al. 1993), *Solanum circaeifolium* and *Solanum bulbocastanum* (Stadler et al. 1995), and *Solanum spegazzinii* (Gebhardt et al. 1995). Some of these sequences have been shown to be useful tools for analyzing the nuclear genomes of hybrids produced by sexual (Watanabe et al. 1995) or somatic hybridization (Saul and Potrykus 1984; Schweizer et al. 1988; Pehu et al. 1990; Xu et al. 1993).

In situ hybridization (ISH) is useful for characterizing the chromosomal organization of repetitive sequences in plants. Tandemly repeated sequences are often found close to the telomeres and centromeres of chromosomes (Appels et al. 1978; Ganal et al. 1988), while dispersed repeated sequences can be distributed throughout the genome or in a few chromosomal regions (Ganal et al. 1988; Lapitan 1992). Although Visser et al. (1988) were the first to report ISH using radioactively labeled repetitive DNA sequences for mitotic potato chromosomes, ISH in potato is not very widely used. Genomic in situ hybridization (GISH) has been used in potato to analyse somatic hybrids and their derivatives (Wolters et al. 1994; Jacobsen et al. 1995; Ramulu et al. 1996). Pachytene chromosomes have also been used for tomato (Xu and Earle 1996), but their handling and analysis are technically more demanding (Wagenvoort et al. 1994). Tandemly repeated sequences as probes in ISH have been useful for constructing karyotypes of plant species (Jones and Flavell 1982; Lapitan et al. 1989). Species-specific sequence repeat probes can also be used to detect the parental origin of chromosomes in somatic hybrids (Piastuch and Bates 1990; Itoh et al. 1991; Rokka et al. 1998) and to study structural alterations, such as amplification and interchanges in chromosomes (Lapitan et al. 1984, 1988).

This study was conducted to characterize the four repetitive clones pSB1 and pSB7 of *S. brevidens* and pST3 and pST10 of *S. tuberosum* (Pehu et al. 1990), in terms of their chromosomal localization, copy number, and DNA sequence, and to determine their suitability as probes for genome analysis of somatic hybrids and somatohaploids (Rokka et al. 1997) between *S. brevidens* and *S. tuberosum*.

Materials and methods

Plant material

Solanum brevidens CPC 2451 and a dihaploid line of *S. tuberosum* (PDH40) derived from cv. Pentland Crown were maintained in culture on MS medium (Murashige and Skoog 1962) with 2% (w/v) sucrose under 16 h light : 8 h dark. The seeds of *S. brevidens* were obtained from Dr. Jari P.T. Valkonen, University of Helsinki, Finland; the seeds of tetraploid *S. tuberosum*, from a cross of cultivars Agria × Satu, were provided by Leena Pietilä, Boreal Plant Breeding, Jokioinen, Finland.

Species-specific clones

The clones pSB1 and pSB7 were isolated from *S. brevidens* and were shown to be highly repeated sequences based on genomic Southern hybridization. The clones pST3 and pST10 were isolated from *S. tuberosum* (Pehu et al. 1990). The clone pST10 was a high copy number sequence, but pST3 had fewer copies in the potato genome, according to Southern hybridization (Pehu et al. 1990).

DNA preparation

Plants from in vitro shoots were grown in the greenhouse with maximum day and night temperatures of 18 and 13°C, respectively. Total cellular DNA was extracted from the leaves of *S. brevidens* and PDH40 using the method of Draper et al. (1988).

Copy-number estimation

Copy-number estimation was carried out by slot blot analysis according to Cullis et al. (1984). Copy numbers (based on insert size) used for reconstruction were between 100 and 20 000 copies per haploid genome for the probes pSB1, pSB7, and pST10. The copy-number reconstructions used for the probe pST3 were between 10 and 500. The hybridization signal produced by 5 μg of total genomic DNA (equivalent to 2×10^6 genomes) was compared with the plasmid standards. Autoradiographs were scanned with a LKB Ultrascan XL laser densitometer connected to an Olivetti M24 personal computer, which was equipped with the LKB 2400 GelScan XL software. The relative intensity of the slot blot was determined according to the absorption values (peak areas) of the slot blot on the autoradiographs. Genome size was taken to be 1.05 pg for a haploid genome (Arumuganathan and Earle 1991). The stringency of the washes used for the copy-number reconstructions was equivalent to 5% mismatch according to calculations in Sambrook et al. (1989).

Sequencing

Sequencing was carried out using the Sequenase T7 DNA polymerase (Version 2) kit (United States Biochemical). The sequences were subjected to sequence homology searches in the GenBank, EMBL, DDBJ, and PDB data bases.

Chromosome preparation

Seeds of *S. brevidens* and *S. tuberosum* were germinated in the dark at 23°C in a petri dish on moistened paper after an overnight treatment with 1.5 $mg \cdot mL^{-1}$ GA_3 (gibberellic acid). When roots were approximately 2–3 mm in length in *S. brevidens* and 3–4 mm in *S. tuberosum*, the seedlings were treated with hydroxyurea (HU) (2.5 mM for *S. brevidens* and 1.25 mM for *S. tuberosum*) overnight in the dark at 23°C, as described by Pan et al. (1993). Seedlings were then washed and transferred to a clean petri dish with a fresh filter paper. After a 5-h treatment in the dark at 23°C, the root tips of *S. tuberosum* were collected and fixed in ice-cold 3:1 methanol – glacial acetic acid. The

Table 1. Stringency washes used in in situ hybridization.

Wash		pSB1		pSB7	
No.	Description	T_m (°C)	% mismatched nucleotides allowed	T_m (°C)	% mismatched nucleotides allowed
1	3× SSC at 37°C, 2× SSC at 37°C, *2× SSC at RT*; each for 10 min	84	59	89	64
2	2× SSC at 37°C, 2× SSC at 37°C, *1× SSC at RT*; each for 10 min	79	54	84	59
3	2× SSC at 37°C, 1× SSC at 37°C, *0.5× SSC at RT*; each for 10 min	74	49	79	54
4	2× SSC at 37°C, 1× SSC at 37°C, *0.1× SSC at RT*; each for 10 min	62	37	70	45
5	*25% (v/v) formamide in 2× SSC at 42°C*, 1× SSC at 37°C, 0.1× SSC at RT; each for 10 min	68	27	73	31
6	*40% (v/v) formamide in 2× SSC at 42°C*, 1× SSC at 37°C, 0.1× SSC at RT; each for 10 min	59	17	64	22

Note: T_m and the percentage of mismatched nucleotides allowed, were calculated according to the most stringent washing step (italic) of each wash. Values were calculated using the formulas described by Leitch et al. (1994).

root tips of *S. brevidens* were collected after a 6-h treatment in the dark, transferred to a vial containing ice-cold distilled water for 16–18 h, then fixed in ice cold 3:1 methanol – glacial acetic acid. Root tips of tissue cultured plantlets of *S. tuberosum* line PDH40 were used for some chromosome preparations. Roots were collected from tissue cultured plantlets and treated with HU, as described for *S. tuberosum* seed material. Metaphase chromosomes from root tips were prepared according to Lapitan (1997).

Probe preparation

The inserts of the clones pSB1, pSB7, pST3, and pST10 were amplified by PCR (polymerase chain reaction) using M-13 forward and reverse primers (Vieira and Messing 1982). The PCR reaction contained 50–100 ng of DNA, 1 μM of each primer, 0.2 mM of each dNTP, 1.5 mM $MgCl_2$, and 2.5 U of Taq polymerase (Promega) in 1× reaction buffer (50 mM KCl, 10 mM Tris, 0.1% Triton X-100) supplied by the manufacturer, in a 100-μL volume. The temperature cycle conditions were 4 min at 94°C, 1 min at 55°C, and 2 min at 72°C, then 35 cycles for 1 min at 94°C, 1 min at 55°C, and 2 min at 72°C, followed by 1 min at 94°C, 1 min at 55°C, 10 min at 72°C, and 20 min at 37°C, then maintained at 4°C.

Fluorescence in situ hybridization (FISH)

Amplified DNA inserts of pSB7, pST3, and pST10 were labeled by random priming with biotin-14-dATP (BRL) (Feinberg and Vogelstein 1983) and the insert of pSB1 was labeled with digoxigenin-11-dUTP (Boehringer Mannheim) as described by Leitch et al. (1994). Hybridization-mixture preparation and ISH followed the procedure of Rayburn and Gill (1985). The slides were incubated in a humidity chamber at 37°C for 15–16 h during the hybridization process.

Each probe was hybridized to chromosome spreads of both *S. brevidens* and *S. tuberosum*. After hybridization, the slides were washed at different stringencies (Table 1) to establish the conditions under which the probes would hybridize only to chromosomes of the source species. The tested stringency conditions, the corresponding melting temperature (T_m), and the percentage of allowed mismatched nucleotides are shown in Table 1. In calculations, the following formulas (Leitch et al. 1994) were used:

[1] $$T_m = 0.41\,(\%\,GC) + 16.6 \log M_{Na^+} - (500/n) - 0.61\,(\%\,\text{formamide}) + 81.5$$

where

T_m = melting temperature

% GC = percentage of guanine and cytosine in the probe sequence

M_{Na^+} = the concentration of monovalent cations (Na^+) in the washing solution ($mol \cdot L^{-1}$)

n = probe length in base pairs

% formamide = concentration of formamide

[2] $$\text{Stringency}\ (\%) = 100 - M_f\,(T_m - T_a)$$

where

M_f = the concentration of monovalent cations (formamide) in the washing solution ($mol \cdot L^{-1}$)

T_m = melting temperature

T_a = temperature at which washing was done

The hybridized biotin-labeled probes were detected with 5 μg/mL of fluorescein avidin DN (Vector Laboratories) in PN buffer (0.1 M Na_2HPO_4, 0.1 M NaH_2PO_4, pH 8.0, containing 0.5% (v/v) Nonidet P-40 (Sigma)), and the digoxigenin-labeled probe (pSB1) was detected with 2.5 μg/mL of anti-digoxigenin–rhodamine (Boehringer Mannheim) in PN buffer. After washing three times in PN buffer, the chromosomes were counterstained with 50 μg/mL of 4',6-diamidino-2-phenylindole (DAPI) for 15 min in the dark, then slides were rinsed with distilled water and mounted in antifade solution (Johnson and Nogueira Araujo 1981). DAPI-stained chromosomes and hybridized signals were captured with a ×63 (*S. tuberosum*) or a ×100 (*S. brevidens*) Zeiss objective using a cooled array CCD (charge-coupled device) collector and digital imaging (Brown et al. 1995). Three to five metaphase chromosome spreads were examined in each treatment.

Results

Copy numbers and sequences of pSB1, pSB7, pST3, and pST10

The copy numbers per haploid genome of potato corresponding to the four probes pSB1, pSB7, pST3, and pST10 were approximately 1500, 6700, 400, and 300, respectively. Based on the estimates of the copy number, the length of the

cloned DNA insert, and the haploid genome size of potato, the repetitive sequences in pSB1, pSB7, pST3, and pST10 were estimated to comprise 0.04, 0.01, 0.028, and 0.003%, respectively, of each particular potato genome.

After sequencing, the accurate sizes of the four clones were determined to be 322 bp for pSB1, 167 bp for pSB7, 845 bp for pST3, and 121 bp for pST10 (Fig. 1). The nucleotide sequences of the clones can be found in the EMBL data base under the accession numbers, X63736 (pSB1), X63737 (pSB7), X63738 (pST3), and X63739 (pST10). There was a 22 bp internal repeat in pSB1 (Fig. 1).

No significant sequence homologies were found in the data base searches with pSB1, pST3, and pST10 and among the four clones. A search with pSB7 showed variable homology with the subrepeats of the fragment Sb4/2 isolated from *S. brevidens* (Preiszner et al. 1994). Depending on the subrepeat of Sb4/2, the homology with pSB7 varied from 78 to 100%. pSB7 also showed 87% homology with a *Lycopersicon esculentum* derived satellite DNA repeat (pLEG15) (Schweizer et al. 1988) and 62% homology with a DNA repeat (pSCH15) isolated from *S. circaeifolium* (Stadler et al. 1995).

FISH patterns of the repeated DNA sequences of *S. brevidens* and *S. tuberosum*

Four different chromosome pretreatment methods were tested to produce morphologically suitable potato chromosomes for ISH. The pretreatments used one of the following synchronization reagents: 8-hydroxyquinoline (Pijnacker and Ferwerda 1984), ice water (Pijnacker and Ferwerda 1984), aphidicolin (Lapitan et al. 1989), or HU (Pan et al. 1993). The quality of the metaphase chromosomes differed after each treatment. Treatment with HU produced more elongated chromosomes than did treatment with 8-hydroxyquinoline or ice water. The morphology of the chromosomes was also clearer after HU treatment than after aphidicolin treatment. The higher concentration of HU (2.5 mM) gave more metaphase-stage spreads (approximately 10 good spreads per slide) for *S. brevidens*; however, using the same concentration resulted in a low mitotic index in *S. tuberosum* (under 5 spreads). HU treatment followed by a 16- to 18-h ice-water treatment enhanced the spreading of *S. brevidens* chromosomes during the squashing.

The FISH experiments were done to determine the chromosomal distribution of the repeated sequences in the potato genomes and to find the stringency conditions in which the DNA sequences would give species-specific signals. The two repeated sequences of *S. brevidens*, pSB1 and pSB7, hybridized mostly in the telomeric areas and at some centromeric sites of the *S. brevidens* chromosomes (Figs. 2A, 2B, and 2C), when 17% mismatched nucleotides for pSB1 (Table 1) and 22% mismatched nucleotides for pSB7 were allowed. pSB7 hybridized to all 24 chromosomes of *S. brevidens*, but one pair of chromosomes showed only slight signals. There was a variation in intensity of FISH signals in chromosomes (Fig. 2C). pSB1 hybridized to 17–18 chromosomes (Fig. 2B) under the same stringency conditions that were used for pSB7. The probes pSB1 and pSB7 partly co-localized in most chromosomes, though pSB7 clustered more than pSB1 at the other telomeric end or at both telomeric regions. For both probes, there were also a few signals that indicated hybridization to interstitial sites of some chromosomes.

Fig. 1. Nucleotide sequences (5´-3´) of the probes pSB1 and pSB7 of *Solanum brevidens* and pST3 and pST10 of *Solanum tuberosum*. The underlining marks the location of a 22 bp direct repeat in pSB1. The accession numbers for the probes are X63736 (pSB1), X63737 (pSB7), X63738 (pST3), and X63739 (pST10).

pSB1

ATTTTAGGAC AGTTACGAAA AGAAGAAAAG TTTTGTTCTT TTATTCATCA
CGATATTCTA AGGTCATTGC TTCAAGTCAA AAAAAATTTG AGGCGTTTTT
TGTTTTGTTT ATGTATTTTT CTTGATTTTC GGCCATTTTG GGGAATTTAC
GTAATATTCA GCCATTTTCG GACAGTTACG AAAAGAAGTA AGTTTTTTTC
TTTTATTCAT CAAGATATTT TAAGGTCATT GGTTCAAGTC AAAAAAAAAT
TGAGGCGTTT TTTGTTTTGT TTATGTATTT TTCTTGATTT TTCGGCCATT
TTGGGGTATT TACGTAATAT TC

pSB7

ATTTTCAAGG TCAAACGAAC CCTAGAGTAG GCAACCCCCC ATTTTGCCGA
TTTTCGTGTG CTATATAGTA CATGGATTTT TAGTGATCCG GCATTTCGAC
GTCATTTTTA CCAAAATTTT TCTTAGACGT CCGTTAAGAC CTTAGCTATA
GAGCCGGTTG GTCCGAC

pST3

ACCTCCTTCA ATTGGTATGA AGCCAATTCT GCTTTTTCTC TAGAAGATAC
TCCCATAGCA TCCACAAATT TGTAAATCTC ATCCAAGAAT CCTTGGGGAT
CTTCTCCAAT TCTAGAGCCC AAAAAGATAG GAGGATTCAT CCTCACGAAG
TCTCTGAACC TTGAGGCAGC CGTACTCTCA TTAACATTTA CCCTAGGCAC
CACATCGAGA ATTGCTTGAG CCGTCATAGC TTGAGCTAGA GTTAGAAAAG
CCGCCCTAAT CTCTTCATTA GTCATGACCG AGGGAACCAC CGGAACCTCA
TTCTCTTGAT TGCCTATGGG GACTTGGTCA CCTTGAAGAC CTTGGGGAGG
AACTCCCCCA TCCACATTCT CCTCTTCCGC TATCCTAGGA TTAGCCCTCG
ATAAACACAA GAGACGGATT AGGAAAGATA CTTATAGACC AAACTCTAAG
GCACGACGTC AAGATTAATG AAATAAGTGA AACTTCTACC ATCATATAGC
CTCTCGCTCA TAGATGTGTC GCGACTCACA CCGATGAACA AGACTCTACT
AGACGTGGCA TATGAGACTT TGAATCCTAA GGGACAATTT TCAAAACCTT
ATGCTCTGAT ACCAAGTTTG TAACGACCTG AGATTACCCC ATAGCCGTTA
CATGCGTATT CGACCTCCCG GAGGTCTCAT ACAAGCCTTA TCATTCATTC
ATCGCATTCG ATAATAAAAC CGTAAGGAAT TTAAACACTA TTTAAAACAA
GTTCATAATA CTCATAAAAT CTTTCAAATG TAACGAAGCA GAATTTCTAA
GTCTCATGTA CCATCTTAGA CACGAATAGA ACATCTTAGG GGCTC

pST10

ACCTTCCTAA CCACATCCAC TCGGTGCTAG CCTTGTTTCC CATAGAGTAT
TCAATCACAT ATGTACTTAG AGAAGGTTCC TTTTCTAGTT CAACCAACTC
ATAGTACCTC TTCCCAAGAA A

The FISH signals of pST3 were clustered in some telomeric chromosomal regions of tetraploid *S. tuberosum* (Figs. 3A and 3B), where a low stringency wash that allowed 44% mismatched nucleotides was used. Major signals were located at the ends of arms of four chromosomes. Many chromosomes also had some minor signals, mostly in their telomeric areas and at few interstitial sites. No FISH signals were detected when pST10 was used as a probe, even under very low stringency conditions, using the chromosomes of tetraploid potato or dihaploid PDH40 as the target.

Cross-hybridization of the repeated sequences to the chromosomes of the other species was tested. Using low stringency washes that allowed 37–59% mismatched nucleotides for pSB1 (Table 1), both *S. brevidens* clones pSB1 and pSB7 showed FISH signals also on the chromosomes of *S. tuberosum*. When the stringency was increased so that only 17% mismatched nucleotides for pSB1 and 22% mismatches for pSB7 were allowed (Table 1), no FISH signals were observed on *S. tuberosum* chromosomes, while FISH signals on the chromosomes of *S. brevidens* remained (Figs. 2A, 2B, and 2C). Under very low stringency conditions (washes 1 and 2; Table 1), *S. tuberosum* clone pST3 hybridized to both *S. brevidens* and *S. tuberosum* chromosomes. Using higher stringency conditions with formamide (washes number 5 and 6;

Fig. 2. Localization of two repetitive DNA sequences in *S. brevidens* (pSB1 and pSB7) onto chromosomes using FISH. (A) A spread of the 24 chromosomes of *S. brevidens* counterstained with DAPI. (B) In situ hybridization signals from the digoxigenin-11-dUTP labeled probe (pSB1), distributed mostly in the telomeric areas and at some centromeric and interstitial sites, on the same chromosome spread shown in A. (C) In situ hybridization signals of the biotin-14-dATP labeled probe (pSB7) located mostly in the telomeric regions of the chromosomes.

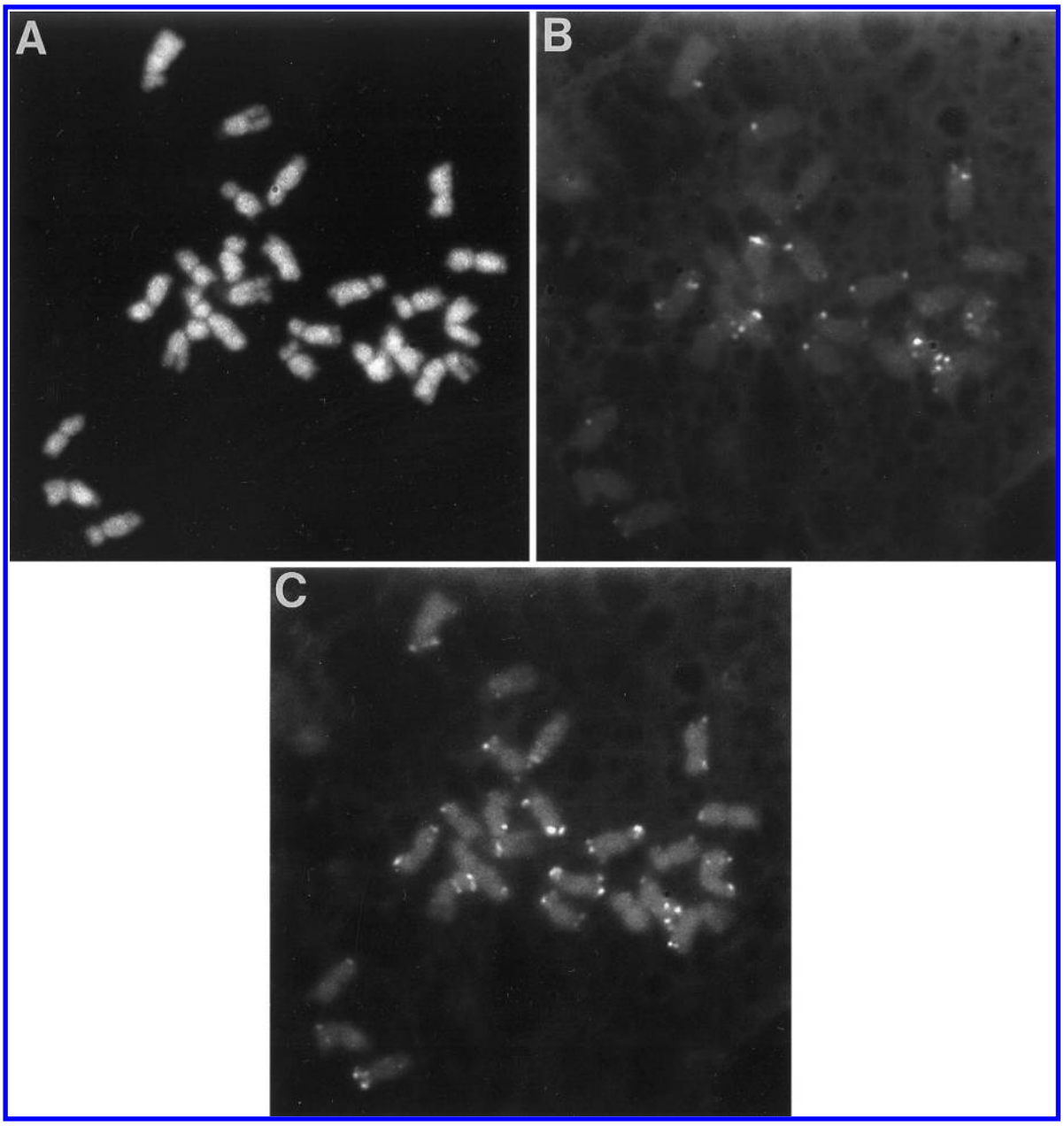


Table 1), no signals were detected either on *S. tuberosum* or on *S. brevidens* chromosomes. Allowing 44% mismatched nucleotides (wash 4; Table 1), hybridization signals were detected only on *S. tuberosum* chromosomes (Fig. 3B) and not on *S. brevidens* chromosomes.

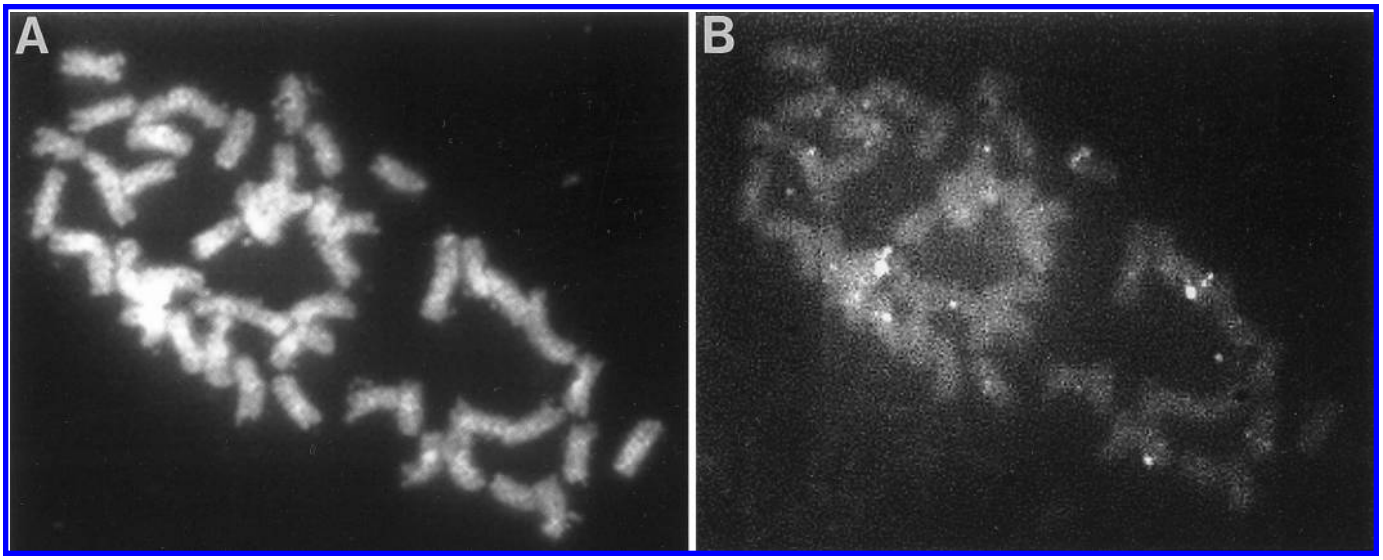
Discussion

In situ hybridization

In situ hybridization is a primary tool in chromosome identification and integration of genetic and physical maps. The highly condensed nature of plant chromosomes and their similar morphology and small size represent bottlenecks in ISH experiments for many plant species, including the potato. Using ISH, the genomic distribution of a number of different repetitive DNA sequences may provide an exact identification (idiogram) of all potato chromosomes, as has already been shown for tomato (Lapitan et al. 1989). Also, the identification of alien chromosomes using specific DNA sequences provides a useful tool for cytological characterization of interspecific hybrids (Piastuch and Bates 1990).

In the present study, two *S. brevidens* clones, pSB1 and pSB7, showed FISH signals that localized to chromosomal areas known to contain tandemly repeated DNA sequences

Fig. 3. Chromosomal distribution of a repetitive DNA sequence of *S. tuberosum* (pST3) using FISH. (A) The chromosome spread of a tetraploid *S. tuberosum* counterstained with DAPI. (B) The same chromosomes hybridized in situ with the probe pST3. The major signals are in the telomeric regions of four *S. tuberosum* chromosomes. Also there are some minor signals distributed at the telomeric and some interstitial sites of a few chromosomes.



(satellite DNAs). In plants, tandemly repeated sequences are usually found at the telomeres, around centromeres, and (a few) at interstitial sites. Satellite DNAs consist of thousands of copies of a short (150–500 bp) repeating unit (Lapitan 1992). They are assumed to be important for chromosome structure, because they are closely associated with constitutive heterochromatin (Appels et al. 1978; John and Miklos 1979). Satellite DNA sequences are nontranscribable and therefore are neutral to selection (Doolittle and Sapienza 1980; Orgel and Crick 1980), which contributes to their species-specificity. Preiszner et al. (1994) localized a highly repetitive sequence (Sb4/1) to the ends of several chromosome arms of *S. brevidens*. In an earlier study, Pehu et al. (1990) showed that the clone pSB7 had characteristics typical of a satellite DNA sequence. The Southern hybridization pattern of pSB1 was more dispersed than that of pSB7 (Pehu et al. 1990). In the present study, FISH showed that the two repeats hybridized mostly in the telomeric areas of *S. brevidens* chromosomes. Part of the pSB1 clone may belong to an interspersed family, as indicated by Southern hybridization patterns (Pehu et al. 1990), but according to ISH, pSB1 appears to be a tandemly repeated sequence. The size of hybridization signals from the pSB7 and pSB1 clones varied among the different chromosomes. The different sizes of the FISH signals may be related to the differences in copy number of the target sequence.

The *S. tuberosum* clone pST3 produced only a few FISH signals, which were also located in chromosomal areas that typically contain tandemly repeated DNA. Pehu et al. (1990) concluded that pST3 was not as redundant in the *S. tuberosum* genome as pSB1 and pSB7 were in the *S. brevidens* genome. Thus, FISH results support the Southern blotting experiments of Pehu et al. (1990), indicating that pST3 is mostly clustered in only a few areas of the potato genome. However, pST10 produced no signals on the *S. tuberosum* chromosomes even under very low stringency conditions. This may be due to its low copy number (300) in the potato genome, which is contrary to the earlier study of Pehu et al. (1990), who reported that pST10 was a high copy number repeated sequence. This may also be due to the short nucleotide sequence length (121 bp) of pST10 combined with its dispersed genomic organization, as described by Pehu et al. (1990).

Species-specificity and characteristics of pSB1, pSB7, pST3, and pST10

The *S. brevidens* DNA sequences pSB1 and pSB7 have recently been shown to be specific to the whole Etuberosa group of *Solanum* species (Malkamäki et al. 1996). The sequences gave strong Southern hybridization signals not only for *S. brevidens*, but also for other Etuberosa-group species (*Solanum etuberosum* and *Solanum fernandezianum*) (Malkamäki et al. 1996). pSB7 is also significantly identical to a tomato-specific repeat, as previously described by Stadler et al. (1995). In dot blot analysis, under high stringency conditions (15% mismatch with pSB1 and 5% with pSB7), no hybridization to *S. tuberosum* or *L. esculentum* was detectable (Malkamäki et al. 1996). Only under low stringency conditions (30% mismatch) did pSB7 show a weak signal in tomato (Malkamäki et al. 1996). In the present study, using FISH, both hybridized probes were *S. brevidens* specific under slightly less stringent conditions (17% mismatch allowed with pSB1 and 22% mismatch with pSB7). There were no detectable signals on *S. tuberosum* chromosomes, which provides evidence of their specificity to the Etuberosa group rather than to the Potatoe group (Hawkes 1994) (e.g., *S. tuberosum*).

This paper described the organization of repeated sequences in *S. tuberosum* and *S. brevidens*. The species-specific repeated DNA sequences reported here provide a potential tool for distinguishing FISH labeled alien *S. brevidens* chromosomes from the chromosomes of *S. tuberosum* in their hybrids using flow sorting (Gray et al. 1986). FISH-labeled flow-sorted chromosomes known to carry resistance genes could be used for microinjection, bombardment, or electroporation into

potato tissues to make chromosome-specific transfectans (Heslop-Harrison and Centre 1995). The probes pSB1 and pSB7 can also be used to distinguish the chromosomes of *S. brevidens* from those of *S. tuberosum* in cytological characterization of hybrids between these two species.

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