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Species-specific sequences in the genus *Solanum*: identification, characterization, and application to study somatic hybrids of *S. brevidens* and *S. tuberosum*

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Summary. To aid in the identification and analysis of somatic hybrids between potato (*Solanum tuberosum*, dihaploid line PDH 40) and the non tuber-bearing wild species *S. brevidens*, a series of species-specific repetitive DNA sequences have been isolated. This was accomplished by making libraries of HaeIII-digested total DNA of *S. tuberosum* and *S. brevidens*, by cloning fragments into the SmaI site of plasmid pUC18 and transforming them into *E. coli* (JM83). The *S. brevidens* library consisted of 1,000 recombinant clones, and that of *S. tuberosum*, 700. Nitrocellulose filters with recombinant clones were hybridised to nick-translated total DNA of *S. brevidens* and also *S. tuberosum*, and, following autoradiography, clones that hybridised strongly to the DNA of only one of the species were chosen. Two highly repeated *S. brevidens* clones (pSB1, 400 bp and pSB7, 210 bp), one high-copy-number *s. tuberosum* clone (pST10, 200 bp) and one low-copy-number sequence of *S. tuberosum* (pST3, 1.5 kbp) were selected for further analysis by Southern hybridisation to digested total DNA. Clone pSB7 gave a ladder pattern on hybridisation to EcoR1-digested total DNA of *S. brevidens*, with signals at multiples of 200 bp DNA. Using these probes it was possible to verify the hybridity of putative hybrids of dihaploid *S. tuberosum* and *S.brevidens*, and to confirm by Southern analysis and by slot blots the parental genome dosage of hexaploid hybrids (two *s. brevidens:* one *S. tuberosum*, and vice-versa). The *S. tuberosum*-specific probe, pST10, hybridised with DNA of three other tuber-bearing wild species (*S. hjertingii, S. capsicibaccatum* and *S. berthaultii*). A squash-blot procedure was developed using the probes that would allow early identification of somatic hybrid callus. There are a number of useful applications of such species-specific probes in the identification and analysis of somatic hybrids.

Key words: Potato – *Solanum brevidens* – Somatic hybrids – Species-specific probes – Squash blots

Introduction

The transfer of complete or partial genomes by somatic hybridisation provides opportunities for plant breeders to exploit germplasm of sexually incompatible species in breeding programmes (Austin et al. 1985; Pental et al. 1986; Fish et al. 1987; O'Connell and Hanson 1987; Vardi et al. 1987; Jones 1988; Smith et al. 1989). The most common methods used to identify the hybrid nature of putative hybrids include: intermediate morphology (Austin et al. 1986; Handley et al. 1986; Fish et al. 1987, 1988), isozyme markers (Schnabelrauch et al. 1985; Gleba et al. 1984; Gleddie et al. 1986; Chen et al. 1989), analysis of fraction I proteins (Morikawa et al. 1987) or complementary restriction fragment length polymorphism (RFLP) (Fish et al. 1988; Pehu et al. 1989; Pental et al. 1988). Most of these methods provide information on only a few genetic loci. In the case of RFLP analysis, the potential exists to examine more of the genome, because nontranscribed sequences can also be detected. However, the sequences most frequently used so far have been rDNA (Pental et al. 1988; Rosen et al. 1988) or relatively low-copy-number sequences (Fish et al. 1988; Pehu et al. 1989), which are both localised on one or only a few chromosome pairs. Use of repeated, interspersed

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sequences in RFLP analysis is limited because of the difficulty in interpreting the banding pattern.

In contrast to the above approaches, species-specific, highly repetitive DNA sequences are potentially useful as probes to identify nuclear hybridity at an early stage after hybridisation, such as in the identification of putative hybrid calli. These sequences may also be used to study the degree of transfer of donor DNA in experiments on partial genome transfer. In addition, it has been established that the frequency of stable transformation after direct gene transfer into protoplasts can be increased significantly when genomic DNA segments of repetitive sequences of the transformed species are introduced together with a selectable gene (Meyer et al. 1988; Marchesi et al. 1989). Recent developments in the in situ hybridisation of DNA probes to tomato and potato chromosomes have also made it possible to use repeated DNA sequences localised in discrete regions of certain chromosomes as chromosome-specific markers (Ganal et al. 1988; Visser et al. 1988).

In this study the identification and characterisation of DNA sequences specific either to the *S. brevidens* or to *S. tuberosum* genomes are described, and their use in the study of euploid and aneuploid somatic hybrids is assessed.

Materials and methods

Plant material

The plants used in this study were the diploid wild *Solanum* species *S. brevidens*, a dihaploid line of *S. tuberosum* (PDH40) derived from cv Pentland Crown, and their somatic hybrids, regenerated after electrofusion and chemical fusion of protoplasts (Fish et al. 1987, 1988). A group of wild *Solanum* species (*S. hjertingii* Hawkes, *S. capsicibaccatum* Card. and *S. berthaultii* Hawkes) was also incuded. The plants were established in a glasshouse from in vitro shoots maintained on Murashige and Skoog's (1962) medium with 2% sucrose, at 16-h day length. The maximum day and night temperatures were 18 °C and 13 °C, respectively.

DNA preparation and cloning

Total DNA was extracted from 2 g fresh weight of frozen leaf tissue from glasshouse-grown plants by the method of Dellaporta et al. (1983). Total DNA from the somatic hybrids *S. brevidens* and *S. tuberosum* was digested with HaeIII and cloned into the SmaI site of plasmid pUC 18. The ligation products were used to transform *E. coli* strain JM83 (Viera and Messing 1982). Recombinant clones were selected on plates containing ampicillin (100 µg/ml) and 2% X-Gal, and were transferred to two identical nitrocellulose filters (Schleicher and Schuell, Dassel, FRG).

Selection of species-specific clones and Southern hybridization

The nitrocellulose filters with the recombinant clones were hybridised to total DNA of either *S. brevidens* or *S. tuberosum*, previously nick-translated to a specific activity of 5×10^6 cpm/µg DNA (Amersham). Prehybridisation and hybridisation were carried out in 50% formamide (5 × Denhardt's, 5 × SSPE, 0.1% SDS, 100 µg/ml denatured salmon sperm DNA) at 42 °C for 4 h and at 37 °C overnight, respectively. The filters were washed in 1 × SSC, 0.1% SDS at 62 °C for 1 h. Putative species-specific clones, which showed strong hybridisation to only one of the species, were selected. To verify the species specificity, 3–4 µg of the selected plasmid DNA was bound onto a DNA binding filter (Pall Biodyne) using a slot-blot apparatus (Bio-Rad Laboratories Ltd., UK). The slot-blot filter was probed with nick-translated total DNA of each fusion parent. Two species-specific clones of each fusion parent were selected for Southern analysis. Total DNA (4 µg) of the parental species was restricted by EcoRI and HindIII, and the fragments were separated by electrophoresis in 0.8% agarose gel. The DNA was transferred onto a nylon filter (Pall Biodyne) by capillary transfer (Southern 1975). The inserted sequences were excised from the plasmid using BamHI and HindIII, then electroeluted from the gel (McDonell et al. 1977) or separated in low gelling temperature agarose (Sea Plaque) and labelled in the presence of the agarose. The inserts were labelled by oligolabelling (Feinberg and Vogelstein 1983) and used to probe the filters containing the restricted total DNAs. All Southern hybridisations were carried out as described by Church and Gilbert (1984). The filters were washed at three different stringencies (1 × SSC, 0.1% SDS, 50 °C; 0.1 × SSC, 0.1% SDS, 50 °C; 0.1 × SSC, 0.1% SDS, 65 °C, for 60 min in all treatments).

Squash blots

To test the feasibility of identifying hybrids at the callus level, 5, 10 and 25 mg of *S. brevidens* and *S. tuberosum* callus was squashed onto Pall-Biodyne DNA binding filters, either using a pair of pliers or by hitting an aluminium rod (placed on the callus) with a hammer in the presence of denaturation solution. The filter was then neutralised in 3 *M* sodium acetate and baked for 45 min at 80 °C. After baking, any remaining callus tissue was removed and the filter was probed with the labelled sequences specific to the fusion parents, as described above.

Results

The *S. brevidens* library consisted of 1,000 recombinant clones, and the *S. tuberosum* library of 700. A total of 34 putative *S. brevidens*-specific clones and 10 *S. tuberosum*-specific clones was selected following colony hybridisation, and the specificity was tested by slot-blot hybridisation. As shown in Fig. 1, the selected clones varied in their degree of specificity as well as in their copy number. Two highly repeated *S. brevidens*-specific sequences (sizes 400 bp and 210 bp, designated pSB1 and pSB7) were selected for further characterisation. One high-copy-number (size 200 bp, pST10) and one low-copy-number sequence (size 1.5 kb, pST3) of *S. tuberosum* were also isolated.

Southern hybridisation experiments using pSB1 (*S. brevidens*-specific clone) as the probe showed that the sequence was highly repetitive, and the hybridisation pattern obtained indicated that it was unlikely to be an interspersed sequence (Fig. 2a). The second clone, pSB7, gave a ladder pattern after hybridisation to EcoRI-di-

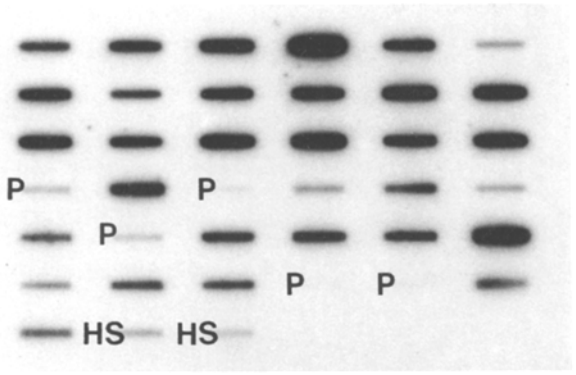

Fig. 1. Slot-blot hybridisation of plasmid DNA containing the putative species-specific inserts probed with total DNA of *S. brevidens*. *P*=putative *S. tuberosum*-specific clones, *HS*= herring sperm DNA; the unlabelled slots are putative *S. brevidens*-specific clones

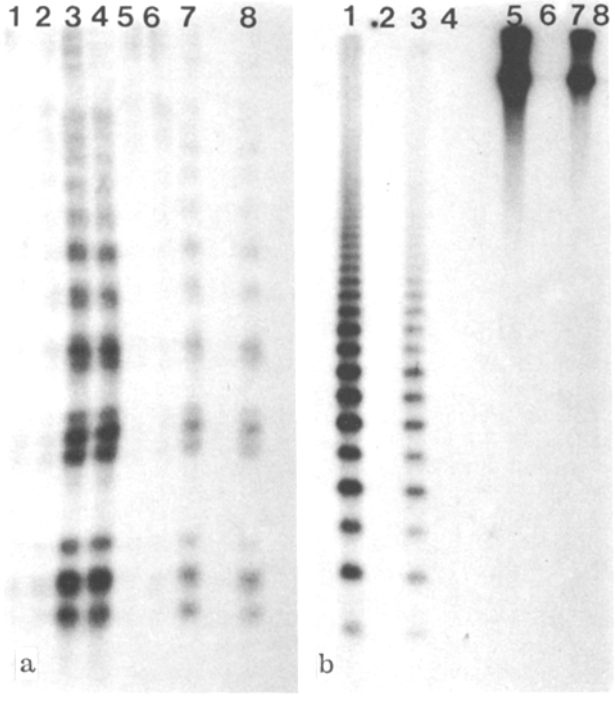

Fig. 2a, b. Verification of the species specificity of the selected *S. brevidens*-specific sequences. **a** Southern blot of HindIII-restricted total parental DNA probed with *S. brevidens*-specific sequence, pSB1. *Lanes 1, 2: S. tuberosum* DNA (4 µg); *lanes 3, 4: S. brevidens* DNA (4 µg); *lanes 5, 6: S. tuberosum* DNA (2 µg); *lanes 7, 8: S. brevidens* DNA (2 µg). **b** Southern blot of EcoRI- and HindIII-digested total parental DNA probed with *S. brevidens*-specific sequence pSB7. *Lanes 1, 3: S. brevidens* DNA (EcoR1 digest); *lanes 2, 4: S. tuberosum* DNA (EcoR1 digest); *lanes 5, 7: S. brevidens* DNA (HindIII digest); *lanes 6, 8: S. tuberosum* DNA (HindIII digest)

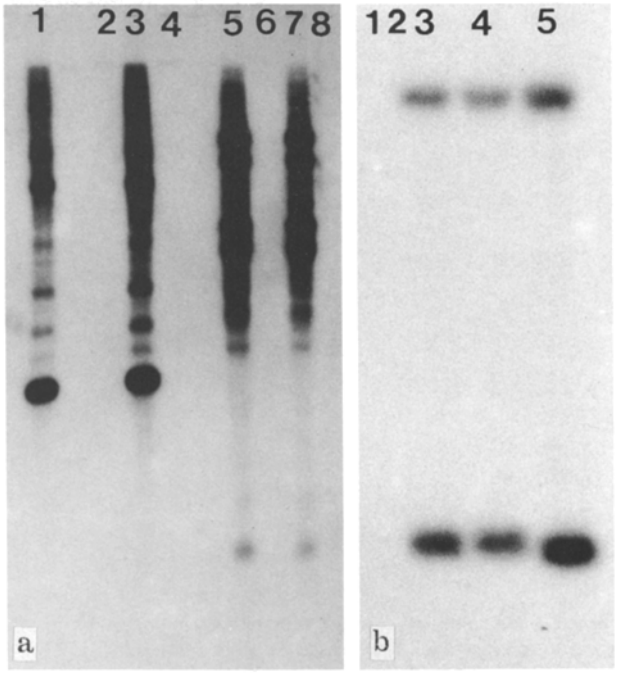

Fig. 3a, b. Verification of the species specificity of the selected *S. tuberosum*-specific sequences. **a** Southern blot of HindIII- and EcoRI-restricted total parental DNA probed with *S. tuberosum*-specific sequence, pST10. *Lanes 1, 3: S. tuberosum* DNA (EcoRI digest); *lanes 2, 4: S. brevidens* DNA (EcoRI digest); *lanes 5, 7: S. tuberosum* DNA (HindIII digest); *lanes 6, 8: S. brevidens* DNA (HindIII digest). **b** Southern blot of HindIII-restricted total parental DNA probed with *S. tuberosum*-specific sequence, pST3. *Lanes 1, 2: S. brevidens* DNA; *lanes 3–5: S. tuberosum* DNA

gested total DNA of *S. brevidens* (Fig. 2b, lanes 1–4). The hybridisation signal to a 200-bp DNA fragment was the strongest, and the signal gradually decreased with the higher molecular weight DNA fragments, which were multiples of the basic monomer unit. With HindIII digestion, the clone pSB7 hybridised very strongly to a high-molecular-weight fragment (Fig. 2b, lanes 5–8). Of the two *S. tuberosum* clones, pST10 hybridised strongly to EcoRI- and HindIII-restricted total *S. tuberosum* DNA (Fig. 3a, lanes 1, 3, 5 and 7). In contrast, the hybridisation pattern of the other *S. tuberosum*-specific clone, pST3, indicated that it is represented in moderately low copy number in the *S. tuberosum* genome (Fig. 3b). Neither of the *S. tuberosum*-specific sequences shared homology with *S. brevidens*, even after a very low stringency wash (1 × SSC, 0.1% SDS, 50 °C).

Hybridization of the species-specific probes to restricted total DNA of the putative somatic hybrids of *S. tuberosum* (PDH40) and *S. brevidens* verified the hybridity of all but one of them (Fig. 4a, b). The latter was shown, in fact, to contain *S. brevidens* DNA alone. Hexaploid hybrids (products of three protoplasts fusing together) have two complements of one parental genome

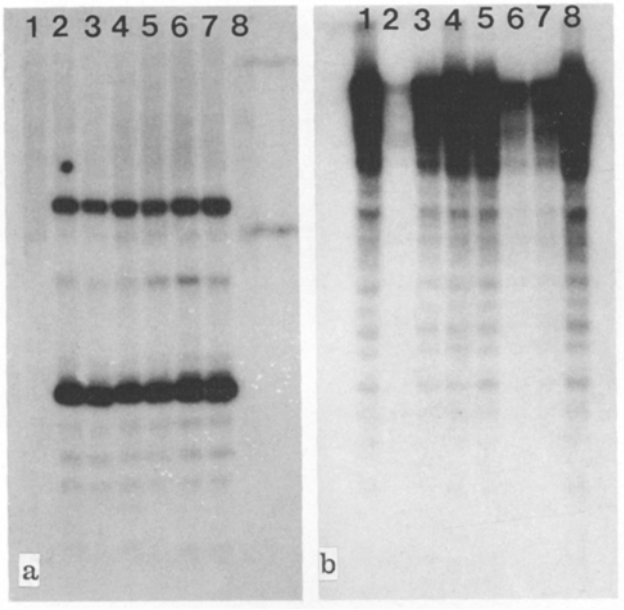

Fig. 4 a, b. Verification of nuclear hybridity of somatic hybrids using species specific sequences. **a** Southern blot of HindIII-restricted total DNA of fusion parents and selected putative hybrids probed with *S. tuberosum*-specific probe, pST3. *Lane 1: S. brevidens; lane 2: S. tuberosum; lanes 3–7:* somatic hybrids; *lane 8:* putative hybrid shown to have only *S. brevidens* DNA. **b** Southern blot of the same filter as in **a** probed with *S. brevidens*-specific sequence, pSB1. Lanes as above

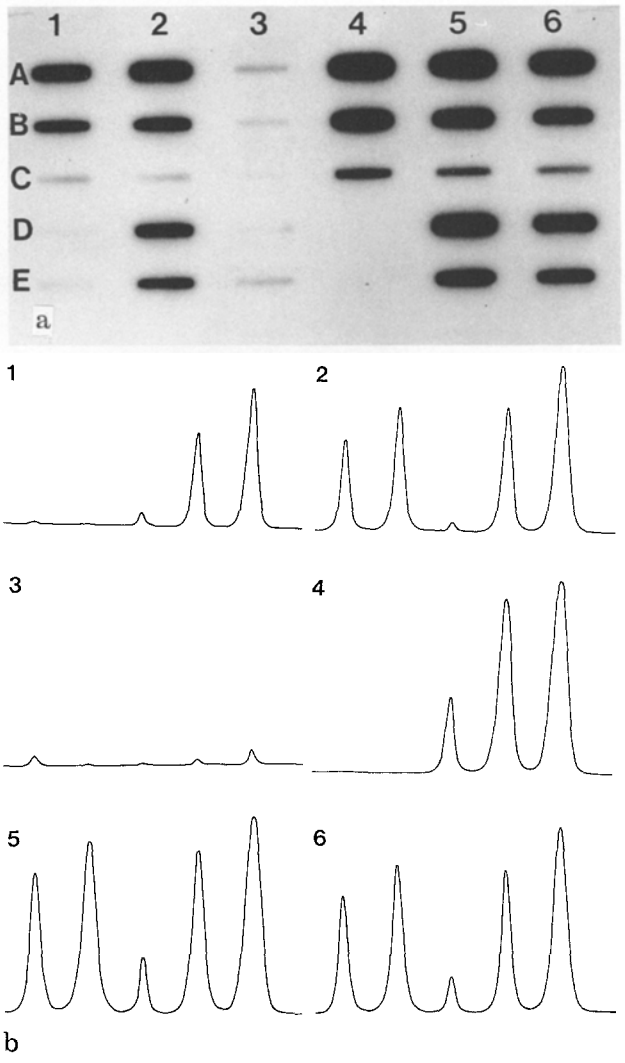

Fig. 5 a, b. Application of *S. brevidens*-specific probe to show parental nuclear genome dosages of two hybrids, and their quantification by densitometry. **a** Slot blot: *Lane 1 A–C:* tetraploid hybrid (65013); *lane 2 A–C:* tetraploid hybrid (84104); *lane 3 A–C: S. tuberosum; lane 4 A–C: S. brevidens; lane 5 A–C:* hexaploid hybrid (81136; two *S. brevidens:* one *S. tuberosum*); *lane 6 A–C:* hexaploid hybrid (81158; two *S. tuberosum:* one *S. brevidens*). DNA loadings *row A:* 1 μg; *row B:* 0.5 μg; *row C:* 0.1 μg. *Slot 1D:* 1 μg; *slot 1E:* 0.5 μg; *slots 1D, E:* herring sperm DNA. *Slot 2D:* 0.5 μg *S. brevidens* + 0.5 μg *S. tuberosum; slot 2E:* 0.25 μg *S. brevidens* + 0.25 μg *S. tuberosum. Slots 3D, E* and *4D, E* – blank. *Slot 5D:* 0.67 μg *S. brevidens* + 0.3 μg *S. tuberosum; slot 5E:* 0.33 μg *S. brevidens* + 0.15 μg *S. tuberosum. Slot 6D:* 0.3 μg *S. brevidens* + 0.67 μg *S. tuberosum; slot 6E:* 0.15 μg *S. brevidens* + 0.33 μg *S. tuberosum.* **b** Densitometry traces of the slot blot for quantification of DNA binding by the probe. (Note: *rows A, B* are probably overloaded for accurate quantification)

and one of the other (Pehu et al. 1989). The latter results were confirmed by slot-blot hybridisations, in which the parental genome dosage combinations were reconstructed from parental DNAs. The results were compared to those of DNA extracted from the hybrid plants of the different combinations, using the species-specific probes (Fig. 5). Densitometer readings of the autoradiogram verified the dosage results of the RFLP analysis.

Hybridisation of the *S. tuberosum*-specific probe, pST10, to restricted total DNA of wild *Solanum* species (*S. hjertingii* Hawkes, *S. capsicibaccatum* Card., *S. berthaultii* Hawkes) in shown in Fig. 6. All these species gave a hybridisation signal with all of the probes, indicating that the probe sequence is present in their genomes.

In the squash-blot hybridisation experiments, there was a strong specific signal of pSB1 to filters from the *S. brevidens* callus tissue squashes, whereas there was no signal on the equivalent callus squashes of *S. tuberosum* (Fig. 7).

Discussion

In this paper we report the identification of species-specific sequences of *S. brevidens* and *S. tuberosum*. Of the libraries containing 1,000 recombinant clones of *S. brevidens* and 700 of *S. tuberosum*, 3.4 and 1.4%, respectively, exhibited a strong hybridisation signal that was species-specific. The frequency of detection of species-specific clones in this work is approximately one-third of that reported by Saul and Potrykus (1984) and Metzlaff

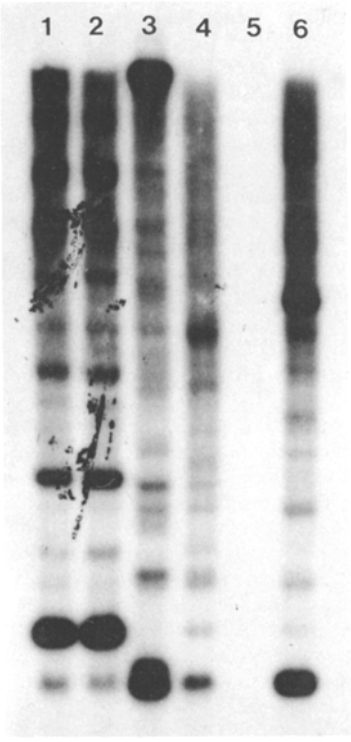

Fig. 6. *S. tuberosum*-specific sequence, pST10, used as a probe against restricted total DNA of wild *Solanum* species. *Lanes 1, 2: S. hjertingii; lane 3: S. capsicibaccatum; lane 4: S. berthaultii; lane 5: S. brevidens; lane 6: S. tuberosum*

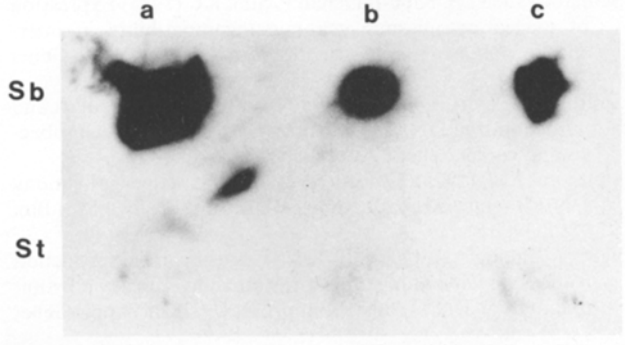

Fig. 7. Squash blot of *S. brevidens* and *S. tuberosum* callus tissue probed with *S. brevidens*-specific sequence, pSB1. *Sb* = *S. brevidens; St* = *S. tuberosum; a* = 20 mg; *b* = 10 mg; *c* = 5 mg callus tissue

et al. (1986). However, the species combinations used in their experiments were taxonomically more distant than those used here. In the case of *Secale* and *Triticum* (Metzlaff et al. 1986), the likelihood of identifying species-specific sequences was increased by using 'relic' DNA to form the library.

One of the *S. brevidens*-specific clones, pSB7, is likely to be a satellite ('relic') DNA sequence, because it produced a ladder pattern having a repeating unit of about 200 bp after digestion with EcoRI, indicating that there is a tandem repeat in the sequence. After HindIII restriction, the probe hybridised strongly only to a high-molecular-weight fragment, and it is likely that HindIII restriction sites are underrepresented in the satellite DNA of *Solanum* species, as has been reported for cereals (Junghans and Metzlaff 1988; Metzlaff et al. 1986). A similar, highly repetitive sequence, specific for *S. tuberosum,* has been identified by Visser et al. (1988). As was shown in their study, this type of probe would be very useful for in situ hybridisation, because it is likely to be localised in discrete blocks in the telomeric and centromeric regions of the chromosomes.

In a Southern hybridisation to genomic DNA, the other *S. brevidens*-specific clone, pSB1, showed a more dispersed pattern compared with pSB7. In addition, it hybridised strongly to the high-molecular-weight fragment produced in the HindIII restriction. This sequence also hybridised to other *Solanum* species, indicating that it is only specific with respect to *S. tuberosum*. It is therefore likely that part of this sequence belongs to the fast repetitive DNA family, which is interspersed in the euchromatin present throughout the genome (D. Schweizer, personal communication). This DNA family has been shown to evolve more slowly than the satellite DNA, which would explain the hybridisation to the other wild *Solanum* species (D. Schweizer, personal communication).

The repetitive *S. tuberosum* sequence, pST10, gave a similar hybridisation pattern to pSB1, whereas the other *S. tuberosum*-specific sequence, pST3, bound strongly to only two fragments on Southern hybridisation to genomic DNA. This indicates a lower degree of redundancy than for the other identified sequences. An interesting feature of these sequences is their high degree of *S. tuberosum* specificity, because even the lowest stringency wash removed the signal from the *S. brevidens* lanes.

The identified sequences were successfully used to verify the nuclear hybridity of somatic hybrids of *S. brevidens* and *S. tuberosum*. As a result of the high copy number and species specificity of these sequences, results from hybridisation experiments will be easier to interpret than when using complementary RFLP patterns (e.g. Fish et al. 1988; Pehu et al. 1989). Species-specific sequences are more likely to be distributed throughout the genome than are individual RFLP probes. The hybrid nature of putative aneuploid hybrids will also be easier to demonstrate than when using low-copy-number probes. As shown by the squash blot experiment, these sequences can be applied to screen and identify putative hybrids at the callus level. We are currently using them to study the presence of donor DNA in asymmetric hybrids of *S. brevidens* and *S. tuberosum*.

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