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Organization of a *Solanum brevidens* repetitive sequence related to the TGRI subtelomeric repeats of *Lycopersicon esculentum*

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Abstract A species-specific repetitive DNA fragment has been isolated from a genomic library of *Solanum brevidens*. Sequence analysis revealed a regular organization of three non-homologous subrepeats forming tandemly-arranged composite repetitive units. Interpretation of Southern hybridization patterns based on the known sequence data suggests that the isolated sequence element represents an abundant organization type, although the presence of simple tandem arrays of the subrepeats is also indicated. Seventy-four percent sequence similarity was found between one of the *S. brevidens* subrepeats (Sb4AX) and a satellite DNA (TGRI) localized as a subtelomeric repeat on almost all *Lycopersicon esculentum* chromosomes. In-situ hybridization indicated that, similarly to TGRI, the *S. brevidens*-specific repeats are located at the ends of the arms of several chromosomes. On the basis of the data obtained, a common ancestral sequence can be proposed for the tomato (TGRI) and the *S. brevidens* (Sb4AX) repeat however, the molecular organization of this element in these two species evolved in a basically different manner.

Key words Intergeneric homology · *Lycopersicon Solanum* · Repeat organization · Repetitive sequence Subtelomeric chromosome region

Introduction

Repetitive sequences constitute a considerable part of the nuclear genome in higher plants. Apart from functional multi-copy genes, a high number of non-transcribed small elements (with unit sizes up to 400 bp) have been described for several species (for a review see Lapitan 1992). These are organized as tandem arrays, interspersed with single-copy sequences, or else form complex repeats with each other. A group of repetitive elements called 'satellite DNA' has a copy number of 10^4–10^5, tandemly arranged and are located in the constitutive heterochromatin regions of chromosomes (Appels et al. 1978; Peacock et al. 1981; Grellet et al. 1986; Hutchinson and Lonsdale 1982). Considering the similar size and localization of satellite repeats, it has been proposed that they play a role in the organization of heterochromatic DNA (McIntyre et al. 1988; Peacock et al. 1981). Alternatively, according to other hypotheses, they can accumulate simply because they are neutral to selection (Doolittle and Sapienza 1980; Orgel and Crick 1980). The virtual lack of a strictly sequence-dependent function of these elements can result in inter- and intraspecific variation in nucleotide sequences and copy numbers. Translocations, rearrangements and the accumulation of mutations may lead to extensive divergences which can be responsible for the development of genus- or species-specific variants of an ancestral element (for reviews see Flavell 1980, 1986).

Highly-repeated sequences evolve more rapidly than either coding regions or random sequences with low copy numbers (Zamir and Tanksley 1988). Some of the tandemly-organized fast-evolving repetitive sequences undergo rapid and concerted changes within isolated species (Arnheim 1983). Recently, several studies have shown that closely-related species present variation in the organization of repeat families (Bedbrook et al. 1980; Dvorak et al. 1988; Gupta et al. 1989; McIntrye et al. 1988; Vershinin et al. 1990). On the basis of these observations, the detailed characterization of repetitive arrays in closely-related species may serve as a highly-efficient tool in biosystematic and phylogenetic research.

In the present paper we report on a DNA sequence which was isolated from *S. brevidens* and could not be detected in *S. tuberosum* genomic DNA. Detailed characterisation revealed sequence homology between the analyzed *S. brevidens* repeat and an *L. esculentum* satellite repeat. In spite

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of a similar chromosomal distribution, the organization of the related repeats is essentially different in the two species. A possible contribution of the described data to the understanding of the evolutionary development of genus *Solanum* will be discussed.

Materials and methods

Screening and cloning of *S. brevidens*-specific sequences

Total DNA was isolated from *S. brevidens* (PI 218228) suspension culture cells by cesium chloride density-gradient equilibrium centrifugation (Sambrook et al. 1989).

Genomic DNA of *S. brevidens* was partially digested with *Mbo*I in order to give the maximum number of fragments between 10 and 20 kb. Digested DNA was ligated to λpGY97 (Vincze and Kiss 1990) phage arms according to Sambrook et al. (1989).

Screening for species-specific clones was accomplished by hybridization of replica filters containing 1 200 recombinant clones with random primed *S. brevidens* and *S. tuberosum* total genomic DNA, respectively. Clones which gave a strong hybridization signal with the *S. brevidens* probe and a weak, or no, signal with the *S. tuberosum* probe were chosen for phagemid isolation. The isolated phagemids were digested with *Eco*RI and their fragments were separated on 0.8% agarose gels and transferred onto nylon (Hybond N, Amersham) membranes according to standard procedures (Sambrook et al. 1989). Subsequently, differential hybridization was carried out with total genomic DNA probes. On the basis of its differential hybridization, one fragment (Sb4/1, see Fig. 2), and the clone (λSb4) harbouring this fragment, were chosen for detailed characterisation.

Fragment isolation

DNA fragments from plasmid or phagemid molecules were isolated using electroelution from agarose blocks into dialysis bags (Sambrook et al. 1989). A and B subrepeats of Sb4/1 were isolated after *Taq*I digestion and electrophoretic separation in a 2.5% agarose gel. The isolated fragments were subcloned into the *Acc*I site of pUC 19 using standard procedures (Sambrook et al. 1989). For the Southern analyses, the 160-bp-long *Xba*I/*Pst*I fragments (A) and the 240-bp-long *Xba*I/*Pst*I fragments (B) of the recombinant pUC plasmids were labelled by random priming (Feinberg and Vogelstein 1983).

Southern hybridization

For Southern hybridization, 8 µg of DNA isolated both from *S. tuberosum* and *S. brevidens* was digested with *Dra*I, *Eco*RI, *Hae*III and *Taq*I restriction enzymes, according to the manufacturer's (Biolabs) instructions. The enzymes were applied in great excess (8 U/µg DNA) to ensure complete digestion. Restricted DNA fragments were separated in 1% agarose gels. Following denaturation, DNA was transferred onto nylon membranes (Hybond N) and crosslinked with UV irradiation. Labelling was accomplished by the random priming method with a minimum final specific activity of 10^8 cpm/µg of fragment DNA.

Hybridizations were carried out at 42 °C in a buffer solution containing 6 × SSC, 0.1% SDS, 10 mM Tris/HCl pH 8.0, 1 mM EDTA, 0.25% dried skimmed milk and 50% formamide, for 6–12 h. Washing of filters was performed under stringent conditions (2 × 45 min 0.1 × SSC, 0.1% SDS, at 62 °C).

Filters were exposed to Forte Medifort RP diagnostic films, using an intensifying screen (DuPont Cronex Lightning Plus), for 1–4 days at –70 °C. Probes were removed from the filters after autoradiography by hot (80–90 °C) 0.1% SDS solution.

Sequencing

The nucleotide sequence was determined by the dideoxy chain-termination method (Sanger et al.1977) after the cloning of the fragments into M13mp19. A USB Sequenase Kit was used according to the manufacturer's instructions. Sequence analysis was accomplished using MICROGENIE (Beckmann) and MULTALIN (Corpet 1988) programmes.

The nucleotide sequence of the cloned Sb4/2 *S. brevidens* genomic DNA fragment is available from the EMBL/Genebank databank under the accession number X63414.

In-situ hybridization

In-situ hybridization and signal detection were accomplished according to Pinkel et al.(1986). Root tips of young, greenhouse-grown plants were used for cytological preparations as described previously (Preiszner et al. 1991). The *S. brevidens*-specific Sb4/1 fragment (Fig. 2) was labelled by nick translation with bio-11-dUTP (Sigma) according to the instructions of the supplier of the nick translation kit (Amersham). The labelling reaction resulted in the incorporation of at least 30% of the bio-11-dUTP. Labelled probe was denatured in hybridization solution (25 ng probe, 50% formamide, 10% dextran-sulphate, 0.5 mg/ml tRNS, total volume 40 µl/slide). Hybridization was carried out at 37°C in a humidity chamber for 12 h. After hybridization, slides were washed for 4 × 3 min in 50% formamide/1 ×SSC at 42 °C, 4 × 3 min 2 × SSC at 42 °C, and once in TSSC (4 × SSC, 0.5% Triton-X 100) at room temperature.

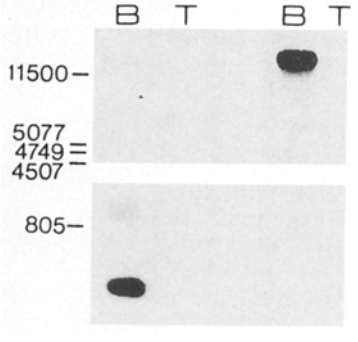
The detection of hybridization signal was performed with fluorescein-avidin DCS (Vector Laboratories). The signal was enhanced by three repetitions of biotinilated goat anti-avidin antibody and FITC-avidin treatment. Chromosomal DNA was stained with propidium-iodide. The cells were investigated by fluorescent microscopy (Leitz) at 450–490 nm excitation for fluorescein and 530–560 nm for propidium-iodide.

Results

Cloning and sequence analysis

As a result of the random cloning of large fragments of *S. brevidens* genomic DNA into phagemid vectors, approximately 5×10^3 recombinant bacterial clones have been obtained. Twenty-two of the one-thousand and two-hundred clones tested in colony hybridization exhibited considerable differences in the intensity of the hybridization signals after probing with the labelled genomic DNA of *S. brevidens* and *S. tuberosum*, respectively. After *Eco*RI di-

Fig. 1 a, b Species specificity of the isolated Sb4/1 fragment. Genomic DNA samples of *S. tuberosum* (*T*), and *S. brevidens* (*B*) were digested with the restriction enzymes *Dra*I (**a**) and *Hin*dIII (**b**), respectively. In the 1–4 kb size region only very weak hybridizing bands appeared and this part of the autoradiogram is not shown



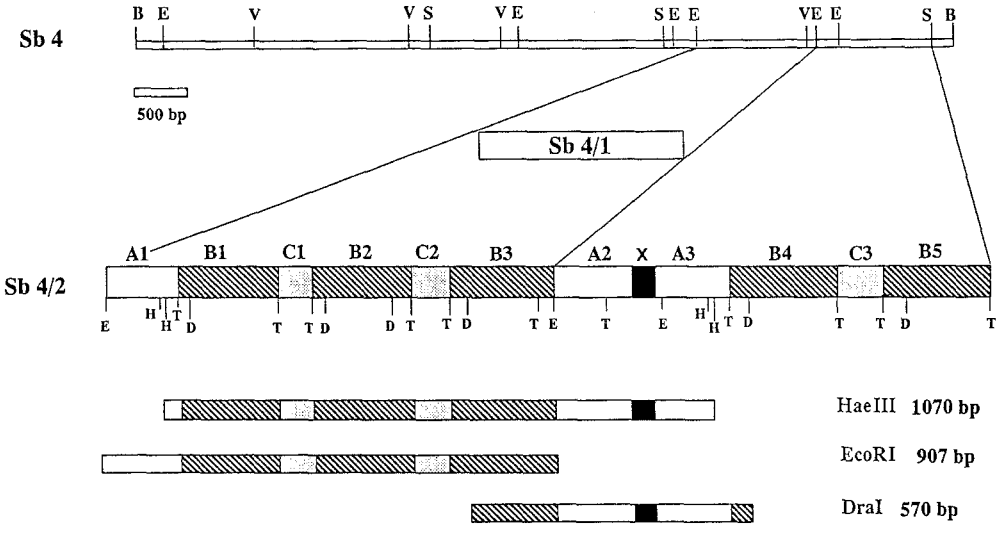


Fig. 2 Physical map of the cloned fragment (Sb4) from S. brevidens genomic DNA and the structure of the sequenced region (Sb4/2). Sb4/1: the *S. brevidens*-specific *Eco*RI fragment that was used for in-situ hybridization. The organization of the A,B and C subrepeats on the Sb4/2 fragment is described. Characteristic restriction fragments corresponding to bands of southern hybridization are shown in lower part of the figure. Restriction sites: *V*, *Eco*RV; *E*, *Eco*RI; *S*, *Sal*I; *B*, *Bam*HI; D, *Dra*I; *H*, *Hae*III; *T*, *Taq*I

Fig. 3 Differences in the nucleotide sequence of the homologous subrepeats of the Sb4/2 DNA fragment. *Dots* represent identical residues as compared to the consensus sequence

```
      1       10        20        30        40        50        60
cons  GACGTCGGAATTCCGGATCACCAAAAATCCTaG-ACTAcAGCACAcGAAAATcgGaAAAA
  A1  -------.........................-..........T.................
  A3  ..............................-..................T.......
  A2  ............................AT.G....T.............T.C....

             70        80        90       100       110       120
cons  TgGGgGGTtTGCttaCTCTGGGGcTCGTTtGACCTTGAAAAaGgGCCGGTTTGgCCGTGG
  A1  .A..........................................................
  A3  ..........................................................
  A2  ....A...C...CCG........A.....C...........T.A.........C......

            130       140       150     158
cons  GGACCAACcGGCTCtATAGCTAAGGTCTTAAcGGctcT
  A1  ........T.........................
  A3  .....................................
  A2  ..............C................A..ACG.


      1       10        20        30        40        50        60
cons  ATCGAACATTcACCGATAAAGTTGAATTTAAACTTAAAcGGAAggGGTAATAATGTAATt
  B1  ..........A.................................T................A
  B4  ..........A.................................TA...............A
  B2  ....................................T.....................
  B3  ....................................A.....................
  B5  ..........................................................

             70        80        90       100       110       120
cons  tCAcCAATAAATAAGAAGAGActGAAATTTGTACATAAatCGcAtt  c GAAtgCgTTT
  B1  ...T.....................................--------.G......
  B4  A..T.....................................--------.G......
  B2  ....................A.............A......TA.T....A.....
  B3  ....................A.............C...T...CG.C......A...
  B5  ....................A.............C...T...CG.C..........

            130       140       150       160       170       180
cons  TATGCCTTATTTTctcAgcTCTTTCTATTTAGGcAtctaagAAGGgtTTTt-CAaGTTTT
  B1  ..............AA................T.AG----.....G....T..T.....
  B4  ..............AA................T.A..T.......G....T........
  B2  ....................................C...........-........
  B3  ..............T...A.............................-........
  B5  ..............T...AT.............................-........

            190       200       210       220       230 234
cons  CAACGGCaTgAcCTTTTTcTAAAAcACAAaAaatcaaatt tg gtCGAgAAAA
  B1  .......G.........................A.G...G..C..........
  B4  .......G.................T.........AGG...G..C..........
  B2  .........A.T......T.............TA...T.GTGTGT-........
  B3  ..........................G.T......A.A..G..........
  B5  ..........................G........A.A..T.....C----


      1       10        20        30 32
cons  aAAAAATAGGGaAGGGGAGTAAAATTgTGGAGA
  C1  ........................A......
  C3  ............................
  C2  G.........G.................
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Table 1 Extent of similarity between related subrepeats of the Sb4/2 sequence

	A2	A3		B2	B3	B4	B5
A1	81%	93%	B1	85%	84.5%	96%	83%
A2	–	86.7%	B2	–	89.3%	85%	89.3%
			B3	–	–	83.8%	94.4%
	C2	C3					
C1	90.6%	96.9%					
C2	–	93.8%					

gestion of these phagemid clones, species-specific fragments were identified and used as probes in Southern hybridization to *S. brevidens* and *S. tuberosum* genomic DNA samples. One of these fragments proved to be highly species-specific under stringent hybridization conditions (Fig. 1). A physical map of the whole cloned genomic fragment (Sb4) carrying the highly species-specific *Eco*RI fragment (Sb4/1) is shown in Fig. 2. Mapping and hybridization of the restriction fragments of the Sb4 clone with the Sb4/1 fragment revealed that next to SB4/1 there is a further region on the cloned DNA which is homologous to the probe.

Sequence analysis of the whole 1728 bp long species-specific *Eco*RI-*Sal*I region (Sb4/2, see Fig. 2) revealed three types of subrepeats (designated A, B and C), which are not related to each other (Fig. 3). The B repeat was found to be especially rich in AT residues (68.4%). The subrepeats are organised as follows: $A_1B_1C_1B_2C_2B_3A_2$ $XA_3B_4C_3B_5$ (Fig. 2). The highest degree of similarity was detectable between the A_1-A_3, B_1-B_4, B_3-B_5 and C_1-C_3 elements (Table 1). The sequence differences reflect point mutations and small deletions. The similarity between the related subrepeats ranged between 81–97% (Table 1). A non-homologous 23-bp-long interruption (X) was found between the A_2 and A_3 sequence elements (Fig. 2). The X sequence seems to be an integral part of the A repeat (see below); that is why we will refer to these repeats as Sb4AX.

Sequence homology between the Sb4AX and TGRI repeats

No homology with other known sequences was found in the EMBL/Genebank databank. On comparison of the nucleotide sequence of the *S. brevidens* DNA fragment to other published sequences, 74% similarity was found to a 162-bp-long satellite DNA repeat (TGRI, Fig. 4), which was previously detected in several *Lycopersicon* species and in *Solanum lycopersicoides*, but was undetectable in other members of the Solanaceae (Ganal et al. 1988; Schweizer et al. 1988). This homology was restricted only to the AX sequences of the analyzed Sb4/2 fragment (Fig. 4).

The TGRI repeat was isolated as a 162-bp *Hae*III fragment from the *Lycopersicon* genome (Ganal et al. 1988; Schweizer et al. 1988). On the basis of the sequence alignment of TGRI and the appropriate Sb4/2 regions, it is likely that the TGRI element contains not one but two *Hae*III recognition sites. Therefore 162- and 18-bp-long fragments are expected to be produced when TGRI repeats are digested by this enzyme (Fig. 4). Due to single base changes the corresponding *Hae*III sites do not exist in Sb4/2; however there are two other *Hae*III sites in A_1 and A_2 repeats close to these sites (Fig. 4). The X region which connects the A subrepeats in Sb4/2 is also present in the TGRI sequence. These observations can serve as a basis for the hypothesis that the natural repeat borders are not the *Hae*III sites of the TGRI repeats but that the repeat units are the AX sequences in both species.

One characteristic of Sb4A repeats is the presence of an *Eco*RI site that is absent in TGRI (Fig. 4b). On the basis of Southern hybridization of Sb4A repeat probes to *Eco*RI-digested *S. brevidens* DNA (Fig. 5) it can be presumed that this site is absent, or else inactivated by methylation, in numerous repeats, which results in a ladder-type hybridization pattern. The *Eco*RI recognition sequence seems to be missing from all of the TGRI repeats (Schweizer et al. 1988; Ganal et al. 1991; Lapitan 1992) most likely as a consequence of a one base.pair difference (Fig. 4).

Genomic organization of the repetitive sequence

Hybridization to digested *S. brevidens* DNA with the Sb4A and Sb4B probes (see Fig. 2) resulted in different patterns depending on the restriction enzyme used (Fig. 5). However, with each enzyme/probe combination, we obtained a ladder-type hybridization pattern with different band intensities probably reflecting either variation in numbers of fragments or of subrepeats on the fragments.

This pattern can be the consequence of gaining and losing restriction sites via changes in the nucleotide sequence of the subrepeats, as well as due to a different methylation pattern. *Eco*RI, *Hae*III and *Taq*I, used for the digestion of genomic DNA, are sensitive to methylation in their recognition sequences. The simplest hybridization pattern was obtained by *Hae*III digestion. It can be explained by the two close *Hae*III sites in A repeats and the two methylation sites in the recognition sequence, both of which should be methylated to inhibit digestion. On the other hand, differences in the restriction sites of the subrepeats were detected even in the sequenced region, e.g., *Hae*III sites are not present in the A2 repeat (Fig. 2).

The fact that, in most cases, both A and B probes hybridize to the same fragments with all enzymes used (Fig. 5) indicates that AB composite repeats may predominate in the organization. However, the restriction sites obtained from the sequence analysis do not correspond perfectly to the sizes of the restriction fragments obtained through Southern hybridization. Only the 907-bp-long *Eco*RI, the 1070-bp-long *Hae*III, and 570-bp-long *Dra*I fragments, calculated from the sequence of the cloned region, can be related to the sizes of hybridizing bands on the Southern filter (see Figs. 2 and 5). The low-molecular-weight hybridizing fragments which cannot be correlated with the

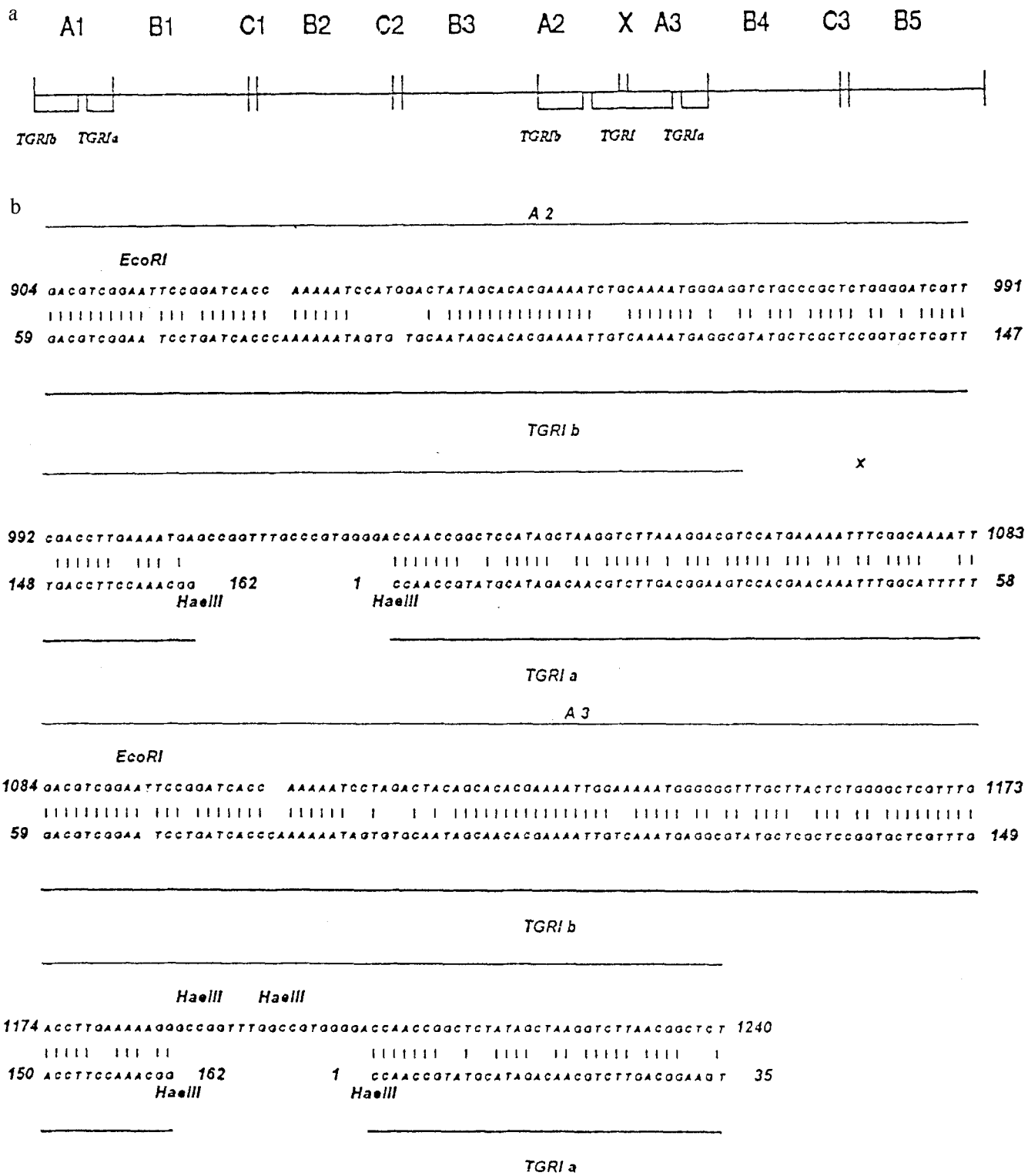

Fig. 4 a, b The TGRI genomic repeat of tomato exhibits 74% similarity to a region of the Sb4/2 sequence. **a** Position of TGRI-homologue sequences on the Sb4/2 fragment. *TGRIa,* 1–58 bp fragment of TGRI; *TGRIb,* 59–162 bp fragment of TGRI. **b** Alignment of the corresponding Sb4/2 and TGRI sequences. The characteristic restriction enzyme recognition sites are indicated.

known restriction sites may result from subrepeats with different restriction patterns (like A repeats with or without *Hae*III sites) or from a different higher-order organization. For example, the characteristic ladder pattern of the short (360 bp, 540 bp and 720 bp) fragments obtained in the *Eco*RI/Sb4A combination with a regular 180-bp (AX) repetition which do not hybridize to the B probe, or else do so very weakly, may suggest the existence of A tandem arrays (multiple repetitions of the 180-bp AX sequence).

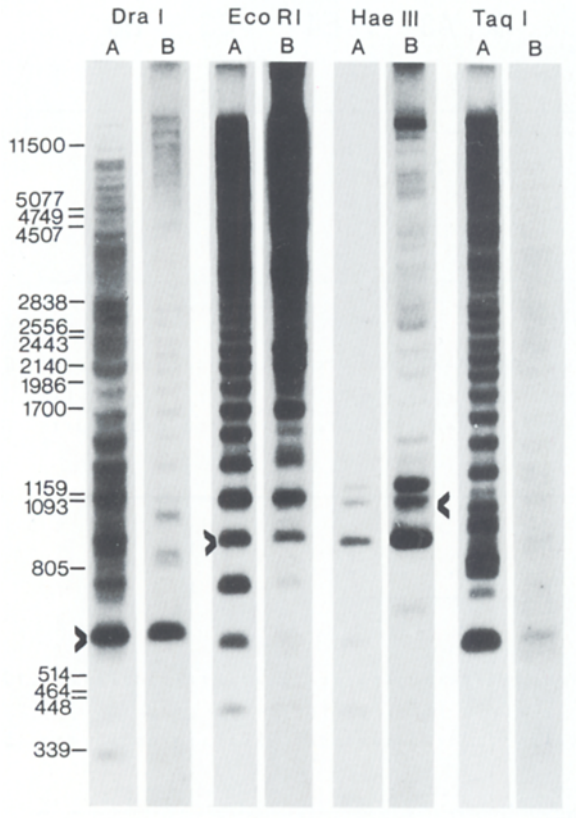

Fig. 5 Hybridization pattern of the Sb4A and Sb4B sequences to digested genomic DNA of *S. brevidens*. The same filter was subsequently hybridized with Sb4/1 A and B sequences labelled to the same level of specific activity by random priming. The hybridizing bands, the sizes of which can be correlated with the restriction map of the sequenced region, are labelled by *arrowheads* (see also Fig. 2)

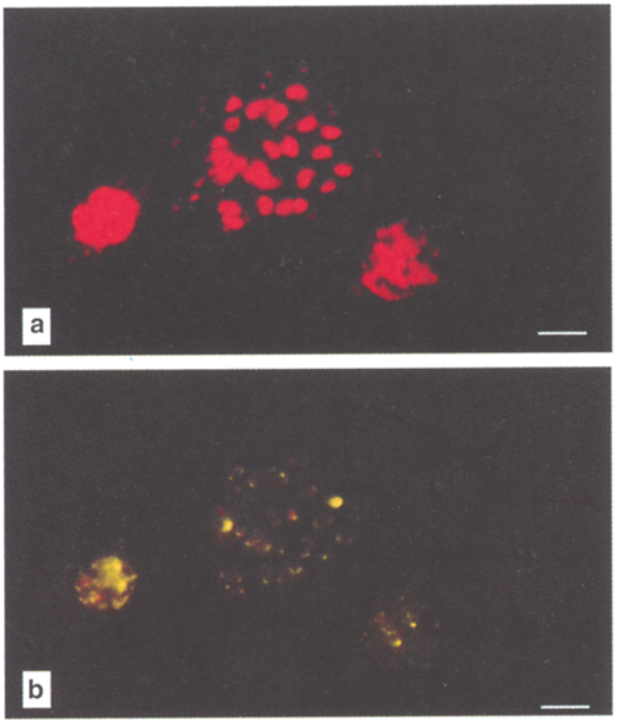

Fig. 6 a, b In-situ hybridization of the isolated species-specific sequence to *S. brevidens* chromosomes. **a** Nuclei and chromosomes stained with propidium-iodide. **b** Green fluorescent signals indicate the hybridization sites of the Sb4/1 fragment. The *bar* represents 6 m

A ladder pattern typical of tandem repeats could also be observed due to hybridization of the A repeats to *Hae*III-restricted DNA with fragment sizes of 180 bp, 360 bp and 540 bp, although the signals were relatively weak. A high-molecular-weight band hybridizing with the B sequence after *Hae*III digestion may be an indication of tandem B repeats not having this site.

Copy number and chromosomal location

Dot-blot hybridization of the Sb4A and Sb4B fragments to the serial dilutions of the *S. brevidens* genomic DNA revealed approximately 10000 copies/haploid *S. brevidens* genome with both probes (data not shown). Both repeats form around 1% of the haploid *S. brevidens* genome assuming that the genome size is about the same as that estimated for *L. esculentum* (Ramanna 1988).

Fluorescent in-situ hybridization with the Sb4/1 fragment was carried out in order to detect the distribution of the repeats on *S. brevidens* chromosomes. Signals indicate that the repeats are clustered and located on at least 20 of the 24 *S. brevidens* chromosomes (Fig. 6). In metaphase plates, as in interphase nuclei, in addition to the several relatively-faint fluorescent signals, two very strong ones could be recognized on two chromosomes (Fig. 6). Although the exact chromosomal localisation of the repetitive sequences needs further investigation, it is evident that most of the hybridization signals are located close to the ends of the chromosome arms (Fig. 6), which is consistent with the chromosomal distribution of TGRI repeats in *L. esculentum* (Ganal et al. 1988; Lapitan 1992).

Discussion

This paper describes a *S. brevidens* genomic DNA sequence element (Sb4/2) that is not present in *S. tuberosum;* consequently, in this respect, it can be regarded as species specific. A similar *S. brevidens* repetitive sequence (designated pSB7) with the same species specificity has been recently isolated by Pehu et al. (1990). At the moment, there are no molecular data available about the relationship between the pSB7 and the Sb4 sequences.

The Sb4/2 repeat consists of non-related repetitive nucleotide sequence regions with copy numbers of approximately 10^4 per haploid *S. brevidens* genome. The organization of the A, B and C subrepeats follows a non-random

distribution and they form complex repetitive arrays. The results of Southern hybridization of the isolated A and B subrepeat sequences to *S. brevidens* DNA digested by different restriction enzymes revealed that these composite repeats occur in the genome of this species in a high copy number.

A similar complex organization of non-related subrepeats that formed new repetitive units has been described for the telomeric heterochromatin of *Secale* species (Bedbrook et al. 1980) as well as other eucaryotes like *Chironomus* (Saiga and Edstrom 1985). Considering the results of in-situ hybridization experiments, it may be proposed that the presently-isolated repetitive sequence element is also related to the DNA of subtelomeric chromosomal regions. Moreover, analysis revealed that the sequence of one of the subrepeats (Sb4AX) is 74% similar to the TGRI repeat, which is tightly associated to the telomeric regions of 20 out of the 24 chromosome arms of *L. esculentum* (Ganal et al. 1988, 1991; Schweizer et al. 1988). However, in *L. esculentum* the TGRI clusters contain a single continuous array of the TGRI repeats, and there are no other sequences present in these clusters (Lapitan 1992).

In spite of the inter-specific differences of certain repeat families, the higher-order arrangement was not subject to drastic changes in the genus *Avena* (Solano et al. 1992), *Beta* (Schmidt et al. 1991) or in the family *Cruciferae* (Grellet et al. 1986; Sibson et al. 1991); however, similar species-specific differences have been observed in cereal species (for a review see Flavell 1980).

For example, in *Secale* species, the so-called 630-bp subtelometric repeat family was found to exist in *S. cereale* in three forms (Bedbrook et al. 1980): one simple form of tandem 120-bp repeats, and two complex forms with unrelated 116- or 300-bp sequences, respectively, interspersed with the above mentioned 120-bp repeats. In *S. silvestre* only the simple 120-bp tandem repeat could be detected. Bedbrook et al. (1980) assumed that prior to the amplification of *S. cereale*-specific repeats the complex repeat units were formed from preexisting simple repeats by the insertion of a non-related DNA element provoking subsequent amplification. In our study, the similarities between the sequenced members of the A, B and C subrepeat families may lead to a similar presumption for the development of the repeated organization. Physically-close units do not exhibit the highest degree of similarity, but such similarity is present between the A_1-A_3, B_1-B_4, B_3-B_5 and C_1-C_3 elements which belong to adjacent composite repeat units $A_1B_1C_1B_2C_2B_3A_2$ and $A_3B_4C_3B_5(\ldots)$. It can be presumed that, parallel to the independent amplification of different subrepeats, the larger arrays comprising a regular organization of the ABC sequences have been amplified as new units. However, the presence of tandem arrays of A and B repeats, respectively, in the genome of *S. brevidens* is also indicated by Southern analysis. On the basis of studies of repeated sequences in higher plants by reassociation kinetics (Flavell 1980, Hutchinson and Lonsdale 1982; Evans et al. 1983; Dhar et al. 1988) and with cloned repetitive units (Evans et al. 1983; Ganal et al. 1988; Martinez-Zapater et al. 1986; Wu et al. 1991) the amplification and translocation of sequences, which result in different variations of an ancestral array, proved to be fundamental processes, as has been shown for other higher organisms (Singer 1982).

In *S. brevidens* there are two, most probably homologous, chromosomes which show very strong signals in in-situ hybridization experiments when the complex repeat is used as a probe. Similar strong signals have not been observed on tomato chromosomes (Ganal et al. 1991; Lapitan 1992). It may be that this region is highly enriched in the Sb4B, but not the Sb4A (TGRI), sequences, and that is why they were not recognized in tomato using the TGRI sequence as a probe, whereas we used a probe (the Sb4/1 fragment) which contained only one A but three B repeats.

The TGRI repeat is present in several *Lycopersicon* species and in *S. lycopersicoides* and *S. brevidens* (according to the present study), but is not present in *S. tuberosum,* other *Solanum* species like *S. acaule* and *S. phureja,* and other more-distantly related solanaceous species (Ganal et al. 1988; Schweizer et al. 1988). These observations are consistent with some former hypotheses concerning the taxonomy and phylogeny of these species. *S. brevidens* (series *Etuberosum*) and *S. lycopersicoides* (series *Juglandifolia*), as non-tuberous species, were grouped together by Hawkes (1990) into the subsection *Estolonifera* within the section *Petota* of the genus *Solanum*, and separately from the subsection *Potatoe* of the tuber-bearing potatoes. On the basis of chloroplast DNA analysis, Hosaka et al. (1984) hypothesized that these species occupy an approximately intermediate position between the genus *Lycopersicon* and *Solanum,* with the former being closer to *Solanum* and the latter to *Lycopersicon*. Hermsen and Taylor (1979) suggested that these species may constitute piers of a bridge between tomato and potato species which can be employed for future gene transfer via crossings. However, recent studies by Spooner et al. (1993), based on detailed chloroplast DNA restriction site and morphological data analysis, placed *Etuberosum* species (*S. brevidens*) as a distinct sister clade to the tuber-bearing members of the section *Petota* (*S. tuberosum, S. acaule, S. phureja*), as well as *Lycopersicon* and the *Solanum* series *Juglandifolia* (*S. lycopersicoides*). According to these investigations the series *Etuberosum* is strongly separated from *Lycopersicon* and the series *Juglandifolia* by 15 chloroplast DNA mutations, while the placement of *Etuberosum* as a separate clade to the tuber-bearing members of the section *Petota* is weak (based on three chloroplast mutations). Concerning this new phylogenetic hypothesis, one could conclude that the Sb4AX (TGRI)-like repeats should be present in the common ancestor of *Etuberosum*, *Petota* and *Lycopersicon* species, and was either lost, or else diverged very much from the original sequence, during the evolution of tuber-bearing potatoes. Alternatively, its parallel appearance in *Etuberosum* and *Lycopersicon* species could be hypothesized. The investigations of the phylogenetic distribution of this sequence, and its organization in more species, should provide a deeper insight into the evolution of repetitive sequence arrays in higher plants as well as the phylogenetic relations of these *Solanaceous* species.

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