Theor Appl Genet (1995) 91: 1271–1278

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Distribution of novel and known repeated elements of *Solanum* and application for the identification of somatic hybrids among *Solanum* species

Received: 19 May 1995 / Accepted: 18 August 1995

Abstract Species-specific repetitive DNA probes are a useful tool for the molecular identification of somatic hybrids. Therefore, the distribution of three repetitive DNA elements of *Solanum* was investigated in *Solanum* wild species, *Solanum* breeding lines, and in more distantly related species of the genera *Lycopersicon*, *Nicotiana*, and *Datura*. The clone pSCH15, obtained from *S. circaeifolium*, represents a new 168-bp repetitive element; it shows 73–79% sequence similarity to repetitive elements of *S. brevidens* and *Lycopersicon* species. The 163-bp element in pSBH6, cloned from *S. bulbocastanum*, turned out to be very similar (95% sequence homology) to the *Lycopersicon* element pLEG15/TGRI previously regarded to be present only in species of the genus *Lycopersicon* and in *S. lycopersicoides*. Lower sequence similarity of approximately 80% was observed to repetitive elements of *S. brevidens* which are organized differently. The repeats exhibited different degrees of specificity: by Southern hybridization the element represented by the clone pSBH6 could be detected in almost all *Solanum* species investigated here but only after long exposure to X-ray film. The previously described "*Solanum*-specific" element represented by the clone pSA287 was also found, although in a very low copy number, in *Lycopersicon esculentum*. Therefore, detection of the repetitive elements pSA287 and pSBH6 in those species in which the respective repeat is less represented depends on exposure time. In contrast, the element pSCH15 is prominently present only in a small number of *Solanum* wild species and – to some extent – in the diploid breeding lines as revealed after long exposure. Use of these repeated elements for the identification of specific genomes in protoplast-fusion hybrids between *Solanum* wild species and *Solanum* breeding lines, or between two breeding lines, was evaluated.

Key words Hybrid identification · Molecular evolution · Repetitive DNA elements · Satellite DNA · Solanaceae

Communicated by G. Wenzel

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Introduction

The plant family Solanaceae comprises a great number of geographically widespread species. Some agronomically important species, such as potato (*Solanum tuberosum*), tomato (*Lycopersicon esculentum*) and tobacco (*Nicotiana tabacum*), belong to this family. The genus *Solanum* consists of 235 species of which 180 are able to form tubers (Hawkes 1990). Therefore, these species provide a valuable genetic resource for different pathogen resistance traits and for adaptation to extreme climatic conditions (Ross 1986). Only a few of these potato wild species have been crossed directly with the cultivated potato (Ross 1986). However, introduction of genetic material from sexually incompatible wild species is possible by biotechnological methods, e.g. by protoplast-fusion (Keller and Melchers 1973) and regeneration of hybrid plants (Melchers and Labib 1974).

Since the late 1970s a symmetric combination of two diploid potato breeding lines has been successfully carried out (Wenzel et al. 1979; Schilde-Rentschler et al. 1987). In addition, wild species of *Solanum* have been combined with diploid breeding lines by symmetric electrofusion of protoplasts and subsequent regeneration (Pehu et al. 1990; Schilde-Rentschler et al. 1993; Novy and Helgeson 1994; Stelzer et al. 1994). *S. bulbocastanum*, *S. circaeifolium* and *S. pinnatisectum* are of special interest because they all confer resistance to *Phytophthora infestans*, one of the severest potato pathogens.

Identification of those regenerates containing genetic material of both fusion partners requires DNA markers which show a certain genome specificity for the respective fusion partners. Rapid and simple identification of regenerates with a hybrid character at an early stage of regeneration is an economic factor in classical and biotechnological breeding. Repetitive elements fulfil these prerequisites (Schweizer and Hemleben 1988; Hemleben et al. 1992).

Highly repetitive DNA elements or "satellite" DNA in dicots consist of sequences often 160–180 bp or 320–370 bp in length (Hemleben et al. 1992; Hemleben 1993). They are mostly organized in tandem arrays and located at specific sites in the genome (Flavell 1986; Lapitan 1992). The repetitive elements vary in their specificity. Some repeats occur in a large number of members of a given plant genus whereas others are specifically present in one or a few species. The repetitive elements also appear to be amplified to different degrees within wild species and cultivated species (Schweizer et al. 1988, 1993). Satellite DNA elements can show a divergence of 5–10%, or higher, in their sequences within individuals of the same species.

For the Solanaceae, several repeated elements have been characterized. One 162-bp element, pLEG15/TGRI, was found to be prominently present in subtelomeric regions of *Lycopersicon* species and in *Solanum lycopersicoides* and was therefore regarded as "*Lycopersicon*-specific" (Ganal et al. 1988; Schweizer et al. 1988; Lapitan et al. 1989). The 183-bp repetitive element pSA287 is amplified in most species of the genus *Solanum* (Schweizer et al. 1988, 1993) whereas another repeat type (370 bp in length), represented by the clone pSDT382, is characteristic only for some *Solanum* species (Schweizer et al. 1993). In *S. brevidens*, specific elements were found which turned out to be approximately 75% similar to the *Lycopersicon* repeats pLEG15/TGRI (Pehu et al. 1990; Preiszner et al. 1994). Species-specific repetitive elements have been described for *N. tabacum* (Koulakova et al. 1989). So it may be possible to isolate more sequences which exhibit a high species specificity for members of the genus *Solanum*.

Therefore, we searched for new repetitive elements in the wild species, *S. circaeifolium* and *S. bulbocastanum*, selected for protoplast-fusion experiments which allow for the detection of the respective genome parts. In this paper, we also present a survey of the known and novel repetitive DNA elements of *Solanum* with respect to their distribution among some species of Solanaceae. The suitability of the repetitive elements for hybrid identification of potato fusion regenerates will be discussed.

Material and methods

Plant material

Wild species and some breeding line material (*S. tuberosum* K7, B15/H256/1, R1, R2) of *Solanum* as well as of *L. esculentum*, *N. tabacum*, and *Datura stramonium*, were from the sources described by Schweizer et al. (1993), Borisjuk et al. (1994) and Zanke et al. (1995). New diploid *S. tuberosum* breeding lines (2n=2x=24) were kindly provided by Fa. Bioplant, Ebstorf, Germany (BP 2, 89/2; BP 7, 89/7; BP 86, 86/622/3; BP 1076, 1076/1; BP 15, B 15), to Dr. L. Schilde-Rentschler, University of Tübingen. Plants were cultivated as shoot cultures under sterile conditions on MS medium (Murashige and Skoog 1962).

Protoplast-fusion, regeneration, and a first molecular characterization of the fusion products was carried out in the laboratory of Prof. H. Ninnemann and Dr. L. Schilde-Rentschler (University of Tübingen) as described by Schilde-Rentschler et al. (1993).

Plasmids

pLEG15, a 162-bp repeat cloned from *L. esculentum* (Schweizer et al. 1988), is similar to TGRI (Ganal et al. 1988); pSA287, is a 183-bp repeat cloned from *S. acaule* (Schweizer et al. 1988); pSBH6, is a 163-bp repeat cloned from *S. bulbocastanum* (this paper); pSCH15, is a 168-bp repeat cloned from *S. circaeifolium* (this paper); pSDT382, contained part of a 370-bp repeat cloned from *S. demissum* (Schweizer et al. 1993).

DNA isolation and characterization

Total genomic DNA was isolated from sterile, 3–6 week-old, shoots according to Gebhardt et al. (1989). DNA concentration was determined by photometric measurement at 260 nm; 8–10 µg of genomic DNA were subjected to restriction enzyme cleavage according to the supplier's instructions (10–20 units of enzyme were used per sample). All samples loaded on one gel contained equal amounts of DNA per lane. Gel electrophoresis for separation of the DNA fragments was performed on 1% agarose gels in standard electrophoresis buffer at 100 mA. The gels were Southern-blotted onto positively charged nylon membranes (Hybond N^+; Amersham) under alkaline conditions (0.4 N NaOH for approximately 4 h). Radioactive labelling and hybridization conditions followed standard procedures as described by Maniatis et al. (1982) and by Schweizer et al. (1993). Hybridization was carried out under stringent conditions at 65°C. The most stringent post-hybridization wash was carried out in 0.1×SSC/0.1%SDS at 65°C for 10 min. Stripping of hybridized filters was carried out at 45°C in 0.4 M NaOH for 30 min followed by an incubation in 0.1×SSC/0.1% SDS at the same temperature.

Cloning of repeated DNA, screening procedures, and nucleotide sequencing

DNA of the respective wild species were cleaved with the 4-bp-recognition enzyme *Hae*III. Two different cloning strategies were applied: cleaved DNA was randomly cloned into pUC 18/19 (Yanisch-Perron 1985). Colony filters were hybridized with ^{32}P-labelled total genomic DNA cleaved with the cloning enzyme. Colonies containing repetitive DNA should produce a stronger hybridization signal than single-copy clones. In order to detect new repetitive elements, selected clones were miniscreened and, after Southern-blotting, hybridized with known repeats, e.g. pSA287 (Schweizer et al. 1988) and pSDT382 (Schweizer et al. 1993), in order to exclude fragments already characterized.

Following another strategy, cleaved DNA was separated on 5% polyacrylamide gels. Repeated elements often can form visible bands in the gel after ethidium bromide staining. For cloning the *S. circaeifolium* repeated element, prominent bands of a *Hae*III cleavage of genomic DNA were cut out of the gel, cloned into pUC 18/19, and characterized as described above.

Nucleotide sequencing was carried out in an automatic sequencer (A.L.F.; Pharmacia), using the Pharmacia "autoread sequencing kit" according to the supplier's instructions. Computer analysis and alignment of the sequences was performed with Align Plus, Sequence Alignment Program, Version 2.0, from Scientific and Educational Software (Myers and Miller 1988).

Results

Cloning and characterization of a repetitive element of *S. circaeifolium*

The known repetitive elements of *Solanum* did not allow an identification of the *S. circaeifolium* genome parts in fusion hybrids (Table 1). Therefore, we cloned a new element as described in Materials and methods. After screening, the clone pSCH15 exhibited a certain species specificity. The sequence of the insert of pSCH15 is shown in Fig. 1. It is 168 bp in length and, interestingly, shows some sequence similarity to other repeated elements characterized for *L. esculentum* and *Solanum brevidens*.

After hybridization with the insert of this clone to filters with *Hae*III-cleaved total genomic DNA of different wild species and diploid *Solanum* breeding lines the characteristic ladder structure of a satellite DNA repeat with a monomer length of 168 bp can be detected (Figs. 2A, B), confirming that the clone pSCH15 contained a complete repeat unit.

In addition to *S. circaeifolium*, this repeat is prominently present in *S. neorossii*. Weaker hybridization signals can be found with *S. acaule*, *S. demissum*, *S. gourlayii*, and *S. leptophyes* DNA, while no hybridization occurred with *S. bulbocastanum*, *S. pinnatisectum* and *S. sparsipilum* (see Table 1). To some extent, this repetitive element is present in all the breeding lines examined here though exhibiting hybridization signals of different intensity: *S. tuberosum* R2 delivers a strong signal whereas *S. tuberosum* B15 (H256/1), for example, shows only a weak reaction. However, hybridization occurred most prominently with *S. circaeifolium* DNA.

Therefore, a selection of somatic hybrids between a "weak-signal" breeding line and a "strong-signal" wild species is possible applying short exposure times (Table 1). Figure 2B shows the identification of a fusion hybrid between *S. circaeifolium* and *S. tuberosum* B15 (H256/1) by hybridization with pSCH15. Actually, after a relatively short exposure to X-ray film (12 h) only DNA of *S. circaeifolium* (lane 2) and of the hybrid (lane 4) shows hybridization signals with this probe. For *S. tuberosum* B15 (H256/1) specific probes will be developed.

Cloning and characterization of a repetitive element of *S. bulbocastanum*

In order to find a species-specific repetitive element in the wild species *S. bulbocastanum*, the DNA was digested with *Hae*III, and a 163-bp element was cloned (clone pSBH6) and sequenced following the same strategy as for the pSCH15 repetitive element. Surprisingly, the insert of the clone turned out to be nearly identical (with a 95% similarity of the sequences; see Fig. 1) to a 162-bp repeated element, previously described as pLEG15/TGRI, specifically occurring in *L. esculentum* and other *Lycopersicon* species (Ganal et al. 1988; Schweizer et al. 1988). Until now, this element was assumed to be absent in species of the genus *Solanum* (except for *S. lycopersicoides*); therefore, it is probably much less represented in *S. bulbocastanum*.

Table 1 Distribution of the repetitive elements among Solanaceae

	pSA287	pSBH6	pSDT382[a]	pSCH15
S. acaule	+++	+	+++	(+)
S. andigena	+++	n.e.	n.e.	n.e.
S. phureja	+++	+	–	n.e.
S. kurtzianum	+++	++	+++	n.e
S. juzepczuckii	+++	+	–	n.e
S. etuberosum	–	+++	–	n.e.
S. vernei	++	+	–	n.e.
S raphanifolium	+++	++	–	n.e.
S. maglia	++	++	+++	n.e.
S. bukasovii	+	++	–	n.e.
S. gourlayi	++	+	–	+
S. neorossii	+++	++	–	+++
S. sparsipilum	+++	+	–	(+)
S. spegazzinii	+++	+	–	n.e.
S. leptophyes	+++	+	–	+
S. demissum	+++	+++	+++	(+)
S bulbocastanum	–	++	–	–
S. circaeifolium	++	+++	–	+++
S. pinnatisectum	(+)	+++	–	–
S. tuberosum R1	+++	+++	+++	n.e.
S. tuberosum R2	++	+++	+++	+++
S. tuberosum B15	++	++	–	+
S. tuberosum BP 2	++	++	–	(+)
S. tuberosum BP 7	+	++	n.e.	n.e.
S. tuberosum BP 86	+	++	+++	n.e
S. tuberosum BP 1076	+	++	+++	n.e.
S. tuberosum BP 15	+	++	+++	+
S. tuberosum K7	+	++	n.e.	(+)
Lycopersicon esculentum	+	+++	–	–
Petunia hybrida	–	–	–	n.e.
Datura species	–	+	–	n.e.
Nicotiana tabacum	–	+	–	n.e.

+++, very strong signal
++, strong signal
+, weak signal
(+), very weak signal
–, no signal
n.e., not examined
[a] The data for clone pSDT382 were published by Schweizer et al. (1993)

The distribution of pSBH6 among *Solanum* wild species, breeding lines, and some other Solanaceae, was investigated by Southern-blot hybridization. In fact, after a short exposure of the filter to X-ray film it was detected only in *L. esculentum* (Fig. 3A). This might explain the genus specificity of the clones pLEG15 and TGRI described by Ganal et al. (1988) and Schweizer et al. (1988), respectively. However, after long exposure, this repetitive element was found in all the *Solanum* wild species and breeding lines investigated here (Fig. 3B) exhibiting variations in hybridization-signal intensity; even with the *Nicotiana* and *Datura* species weak hybridization signals are visible though *Petunia* did not react with this clone.

Evidently, application of the clone pSBH6 is not suitable for the identification of genome parts of *S. bulbocas-*

Fig. 1 Nucleotide sequence of the 168-bp insert of pSCH15 cloned from *S. circaeifolium* containing the monomer of a new repetitive potato element. The sequence is compared with a tomato-specific repeat pLEG15 (Schweizer et al. 1988), which is similar to TGRI (Ganal et al. 1988), with the new clone pSBH6 cloned from *S. bulbocastanum*, and with a repeated region in the clone pSB4/2 (SBREPDNA) of *S. brevidens* spanning the region from nucleotide 1016 to 1186 (Preizner et al. 1994). The nucleotide sequences of the clones appeared in the EMBL data subs@EBI.AC.UK under the following accession numbers: pSCH15: X83883; pSBH6: X87234, pLEG15: X87233

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                                ALIGNED SEQUENCE

                      170        180        190             200
                        *          *          *               *
pSCH15.SEQ      CC---CGTGAGGGCAAATTGGCTACTTAG-----GTCTTAACGGACGTTA
pLEG15          ..AAC...AT.----C..A.A.A..---------.....G.....A..CC
pSBH6           ..AAC...AT.----C..A.A.A..---------.....G........CC
SBREPDNA        ..---....G..A.C..CC....C.A...CTAAG.......A......CC

                  130        140        150        160        170
                    *          *          *          *          *
pSCH15.SEQ      AGAATAAATTTTAGAAAAAATGAAGTCAGAATTCCGGATCACCGAAAAAA
pLEG15          .CG.AC..A...G.C.TTTT...C...-.G.A...T.......C.....-
pSBH6           .CG.AC..A...G.C.TTTT...C..-T.G.A...C.......C.....-
SBREPDNA        .TG.A......CG.C....T...C...G..............A....TC

                   80         90        100        110        120
                    *          *          *          *          *
pSCH15.SEQ      GATGGTGAACTATAACATATGAAAATTGGTAAAAGAGAGGGTTAACCT-G
pEG15          -..A...TG.A...G..C.C.......-..C...AT....CG..TG..-C
pSBH6           -......TG.A...G..C.C.-...A.C..C...AT....CG..TG..C.
SBREPDNA        CTA.----....C.G..C.C.........A....TG.G.....TG.T.-A

                   30                  40         50
                    *                  *          *                Homology
pSCH15.SEQ      CCTAGGG---T---CT--TTTGACCTTGAAAATGG   (168 bps)    100%
pLEG15          G..CC..---.GCT.G--.........CC...C..   (162 bps)     73%
pSBH6           ...CC..GGC.---.G--.........CC......   (163 bps)     75%
SBREPDNA        .TCT...---G---..CG.............A..   (171 bps)     79%
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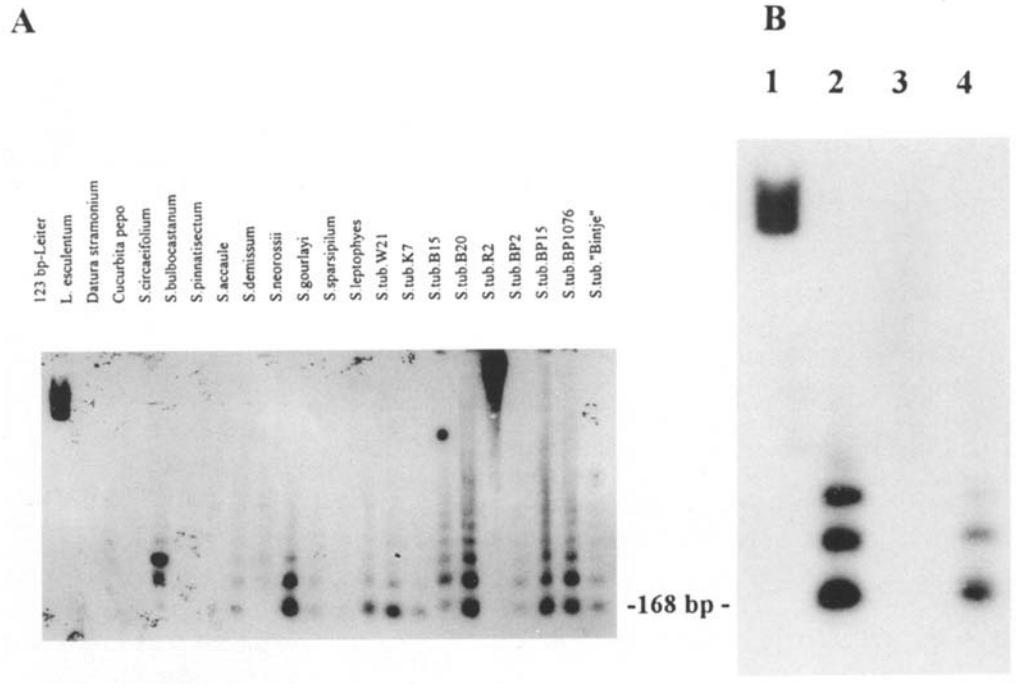
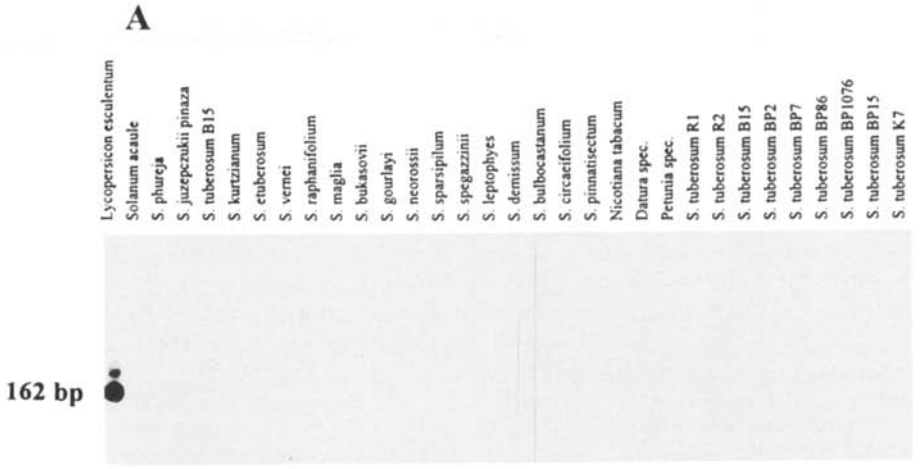


Fig. 2 Distribution of the repetitive DNA element pSCH15 cloned from *S. circaeifolium*. **A** Southern blot of DNA from wild species and breeding lines of *Solanum* and other Solanaceae. Each lane contained 6 µg of *Hae*III-digested DNA of the respective species; the filter was hybridized with a [^{32}P]-labelled insert of pSCH15; the exposure time to X-ray film was 3 days. **B** Identification of a hybrid between *S. circaeifolium* and *S. tuberosum* B15 with the labelled pSCH15 DNA element. *Lane 1*: 123-bp ladder as a length standard; *lanes 2–4*: 6 µg of *Hae*III digested DNA of *S. circaeifolium* (*lane 2*), *S. tuberosum* B15 (*lane 3*) and the potential hybrid (lane 4); the exposure time for X-ray film was 12 h

tanum in protoplast-fusion hybrids between this species and diploid *S. tuberosum* breeding lines.

Distribution of the repetitive element pSA287

Since we noticed the presence of the "*Lycopersicon*" element pSBH6/pLEG15/TGRI in all species of the genus *Solanum* investigated here, it was of interest to examine whether the "*Solanum*-specific" 183-bp repeat represented by the clone pSA287 (Schweizer et al. 1988, 1993) also exhibits a cross reaction with DNA of *Lycopersicon* and other Solanaceae not described so far. Consequently, more wild species (see Schweizer et al. 1993) were tested with this clone under long-exposure conditions. The filters already used for hybridization with pSBH6 were, after extensive stripping, hybridized with the repetitive DNA element pSA287 (Fig. 4). Surprisingly again, after long exposure, pSA287 was detected in *L. esculentum* with a repeat length of approximately 180 bp; therefore, the "*Sola-*



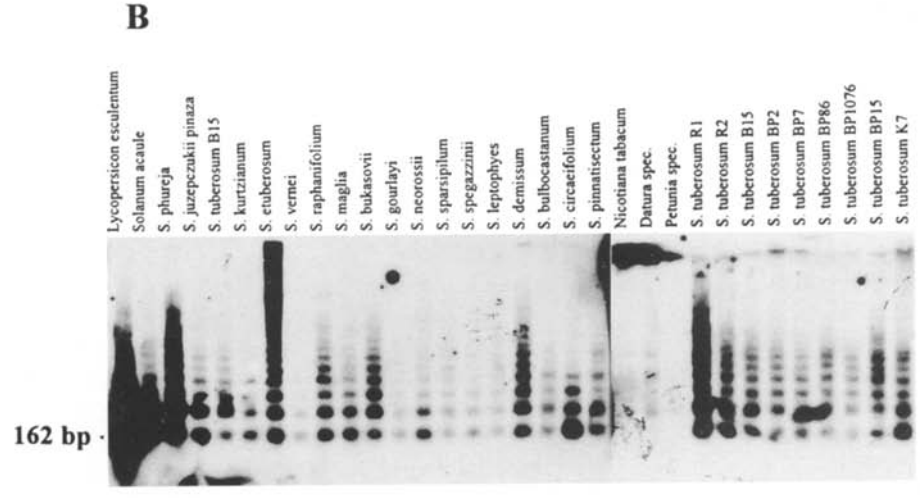


Fig. 3A, B The repetitive DNA element pSBH6 cloned from *S. bulbocastanum*. **A** short exposure of 10 µg of *Hae*III-digested DNA of different *Solanum* wild species, breeding lines, and other Solanaceae which were blotted and hybridized with the *S. bulbocastanum* repetitive element pSBH6, this being 95% homologous to pLEG15/TGRI; the exposure time was 3 h. **B** same filter as in A; the exposure time was 3 days

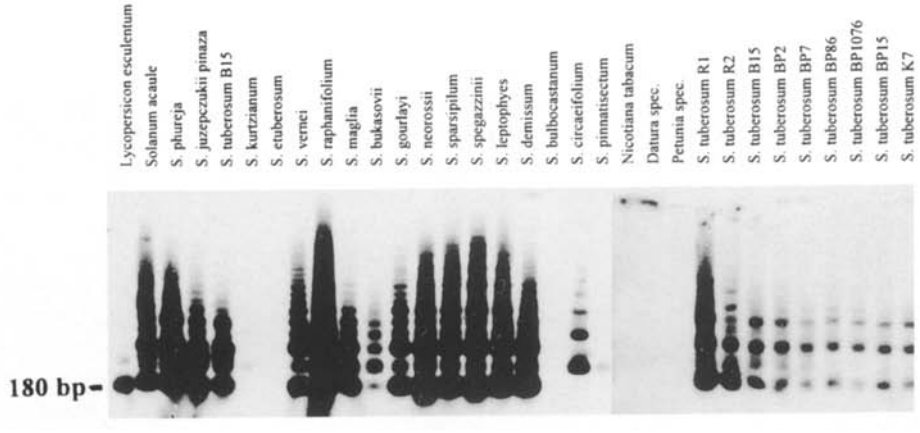


Fig. 4 Distribution and organization of the repetitive element pSA287 cloned from of *S. acaule* among the Solanaceae. For hybridization, the filter used for the experiments in Fig. 3 was stripped and hybridized with pSA287; the exposure time was 3 days. The clone pSA287 (Schweizer et al. 1988) appeared in the EMBL data subs@EBI.AC.UK under the accession number X87235

num specificity" described by Schweizer et al. (1988) depends on exposure time. As previously shown, this repeated element reacted with the DNA of most of the investigated *Solanum* species (Schweizer et al. 1993). It is also present in all the breeding lines studied here. In *S. pinnatisectum* and in *S. kurtzianum* only a weak signal was detected. *Solanum circaeifolium, S. pinnatisectum* and *S. kurtzianum* showed a repeat length of approximately 360 bp, i.e. twice as long as in other *Solanum* species (see Table 1). Only two species, *S. etuberosum* and *S. bulbocastanum,* did not react with the pSA287 repeat.

With respect to these results, the repetitive element pSA287 can serve as a marker in symmetric fusion experiments with diploid breeding lines and with those wild spe-

cies in which this repeat is absent or rather under-represented (Table 1), e.g. for somatic hybrids between *S. bulbocastanum* or *S. pinnatisectum*, respectively, with *S. tuberosum* diploid breeding lines (Stelzer et al. 1994; Zanke et al. 1995).

In Table 1 the hybridization results obtained with the different repeated elements are summarized. [Data for the clone pSDT382 already published by Schweizer et al. (1993), which has not yet been sequenced, are included]. Notably, for the identification of hybrids those repeats have to be selected as hybridization probes which allow for the detection of the respective genomes of the fusion partners. For some combinations the specificity of the reaction depends on exposure times.

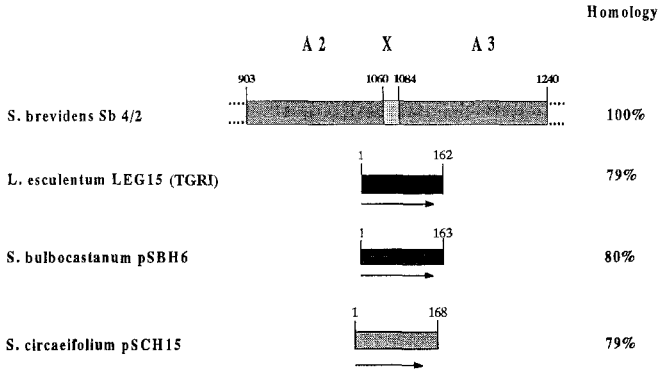


Fig. 5 Schematic drawing and sequence similarity of a region within the clone pSB4/2 described by Preizsner et al. (1994; see Fig. 1) and the related sequences pLEG15 (similar to TGRI; Ganal et al. 1988; Schweizer et al. 1988), pSBH6, and pSCH15

Discussion

This paper deals with the investigation of the differential distribution of repetitive DNA elements within various wild species and breeding lines of *Solanum* and other Solanaceae with the aim of looking for rather specific DNA sequences allowing a rapid identification of the genomes of the respective fusion partners in symmetric protoplast-fusion hybrids.

The novel repetitive elements showed different degrees of genome specificity: pSCH15 was detected only in a few *Solanum* wild species whereas pSBH6 was found to be present in nearly all the wild species investigated here but only after long exposure suggesting that only a relatively small array size of this element is represented by the pSBH6 repeat in *Solanum* species (see Table 1).

The repetitive element pSCH15 was found to be differentially represented in the *Solanum* wild species and breeding lines (Fig. 2; Table 1). The sequence of this element showed 73–79% similarity with the *Lycopersicon* repeat (LEG15/TGRI) and a region within the *S. brevidens* clone pSB 4/2 (Preizsner et al. 1994) which shows an approximately 75% sequence similarity among each other (see Figs. 1 and 5). Thus we have found a new sequence with a good degree of species specificity, because a sequence divergence of approximately 25% is sufficient to discriminate satellite DNA sequences under stringent hybridization conditions. Remarkably, in *S. brevidens* the approximately 180-bp repetitive element described by Preiszner et al. (1994) belongs to the same repeat family but exhibits a different organization. The repeat shows an insertion of 19 bp in comparison to pSBH6/TGRI and seems not to be strictly organized in a clustered tandem arrangement, resulting in a different hybridization pattern. Sequence similarity among repeated elements of 25–30% in related species were also observed in the genus *Cucurbita* (King et al. 1995). Species-specific repetitive elements have been described for *Nicotiana tomentosiformis* (Koulakova et al. 1989) and for the different genome components of *Oryza sativa* (Wu and Wu 1992), for example. Using this element as a hybridization probe, identification of certain genome fractions of *S. circaeifolium* in hybrid genomes requires stringent hybridization and short exposure time, e.g. in protoplast-fusion hybrids as demonstrated in Fig. 2B.

The element pLEG15/TGRI was first found to be genus specific for *Lycopersicon* species (Ganal et al. 1988; Schweizer et al. 1988). Now, however, the occurrence of this DNA element in *Solanum* species has been demonstrated by pSBH6 cloned from *S. bulbocastanum*; it showed a 95% sequence similarity to pLEG15/TGRI. The fact that after stringent hybridization and long exposure on X-ray film this repetitive element was found to be present in all the *Solanum* species studied here (Fig. 3B) indicates a small array size of this repeat in *Solanum* species in contrast to *L. esculentum*. Even species of the genera *Nicotiana* and *Datura* exhibited a slight cross reaction with this DNA element. As already mentioned above, in *S. brevidens* relatively similar repetitive elements have been described. One element, pSB7 (Pehu et al. 1990), displayed a sequence homology of 76% to the *S. bulbocastanum* repeated sequence (clone pSBH6) with a monomer repeat length of 167 bp. A similar sequence is represented in the clone pSB4/2 which contains several repeated sequences (Fig. 5; Preizsner et al. 1994). Though under-represented, the pSBH6/pLEG15/TGRI repetitive element occurs in all the *Solanum* species investigated here. Therefore, it can be used as a marker for *Lycopersicon* genome parts (in which this repeat is prominently amplified) in intergeneric fusion hybrids between *Lycopersicon* species and *Solanum* species, as described by Schweizer et al. (1988), by applying short exposure times.

The former "*Solanum*-specific" element pSA287 (Schweizer et al. 1988) also appeared to be present in *Lycopersicon* species after long exposure of the filters to X-ray film. However, the widespread element pSA287 can still serve as a marker for breeding lines in fusion experiments with those wild species under-representing or lacking this repeat (e.g. fusions between *S. pinnatisectum* or *S. bulbocastanum* and *S. tuberosum* B15; Table 1, Stelzer et al. 1994; Zanke et al. 1995). Obviously, this marker is

located at several positions in the genome of potato as shown by RFLP analysis (Gebhardt et al. 1995). This might be an advantage for the identification of symmetric hybrids in contrast to single-copy probes which identify only specific regions of the genome (Gebhardt et al. 1989).

The presence of pSA287 ("*Solanum* specific") and pSBH6/pLEG15/TGRI ("*Lycopersicon* specific") in a wide range of species seems to point to elements which were established early in the evolution of the *Solanaceae*. Absence of pSA287 in some *Solanum* wild species, namely *S. bulbocastanum* and the nontuber-bearing *S. etuberosum* which belong to different subsections of the genus *Solanum* (Hawkes 1990), could mean the loss of this repetitive element as a secondary event. Notably, a different organization of pSA287 in *S. circaeifolium*, *S. pinnatisectum* and in *S. kurtzianum* was observed with a greater repeat length of 360 bp. The sequence of this longer repeat is not yet known.

The pSBH6/pLEG15/TGRI repetitive element can be detected in even more distantly related species and genera. Such a widespread element has possibly undergone a long evolutionary process with changes in structure and sequence. It may have its origin in a common ancestor of the genera *Lycopersicon* and *Solanum* and the more distantly related genera *Datura* and *Nicotiana*. Obviously, *Lycopersicon* species amplified the repeat whereas *Solanum* species kept it at a low level. The lower sequence similarity between the *Lycopersicon*-specific repetitive element and repeated sequences in the nontuber-bearing *S. brevidens* (74–78%, Pehu et al. 1991; Preizsner et al. 1994) and in *S. circaeifolium* indicates the molecular evolution of an ancestral repeat type which appears modified within different species. The nontuber-bearing *S. brevidens* belongs to the *Solanum* subsection of *Etuberosa* while *S. bulbocastanum* forms a series within the subsection *Potato* (Hawkes 1990). Whether a closer relationship of species of the genus *Lycopersicon* to the tuber-bearing potatoes than to the nontuber-bearing species of the genus *Solanum* can be derived from this study is not clear. Nevertheless, the taxonomic position of *Lycopersicon* species needs to be reconsidered (see Borisjuk et al. 1994).

The problems of using satellite DNAs for the elucidation of evolutionary relationship of the species of a plant family was discussed in detail for the genus *Cucurbita* (King et al. 1995). In the *Cucurbita* species, two main types of repeated elements can be found. In certain species, one or both repeat types have undergone amplification whereas in other species only one type can be detected after PCR amplification. For the Solanaceae, evolutionary relationships have to be investigated in more detail by cloning and sequencing the repeats of a greater number of wild species followed by computer analysis. Furthermore, if application of Southern-blot analysis fails to detect a repeat in a species, the very sensitive method of PCR amplification has to be applied.

In general, repetitive DNA elements can support the elucidation of evolutionary relationships among members of a plant family. The fact that the sequences and the amount of specific repeats can vary among relatively related species provides a valuable tool for the identification of specific genome parts in protoplast-fusion hybrids and enables a rapid and simple screen, e.g. by dot-blot analysis; however, the hybridization conditions and the exposure times have to be considered.

Acknowledgements Financial support granted by the Federal Minister for Research and Technology (BMFT) is gratefully acknowledged (03919456A). H.-J. Zanke was very helpful in correcting the manuscript.

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