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Molecular analysis of highly repeated genome fractions in *Solanum* and their use as markers for the characterization of species and cultivars

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Summary. Highly repeated DNA of potato (*Solanum* sp.) was characterized by cloning various major repeated elements of the nuclear genome. The percentage of the nuclear genome of the specific fractions and the restriction enzyme patterns were determined in order to show the distribution and organization of the respective repeats in the genome of *Solanum tuberosum* cultivars, dihaploid breeding lines and in wild species of *Solanum*. Several of the clones obtained were represented in a high copy number but showed no informative RFLP patterns. More information was gained from 'restriction satellite' repeats. The clone pR1T320 was found to contain satellite repeats (360 bp in length) that are proportionally present in the genome of all *Solanum* species at frequencies, between 0.5% and 2.6% and which are differently organized. This repeat was also found in the genera *Lycopersicon*, *Datura* and *Nicotiana*. With various restriction enzymes characteristic RFLP patterns were detected. A more or less genus-specific element for *Solanum* was the 183-bp repeat (clone pSA287; between 0.2–0.4% of the nuclear genome) that was present in the majority of the *Solanum* species analyzed except *S. kurtzianum*, *S. bulbocastanum* and *S. pinnatisectum*. In a few wild species (prominently in *S. kurtzianum*, *S. demissum* and *S. acaule*) a specific repeat type was detected (clone pSDT382; repeat length approximately 370 bp) that could be used to trace the wild species introduced into *S. tuberosum* cultivars. The repeats analyzed together with the 18S, 5.8S and 25S ribosomal DNA (1.9–5.2%, corresponding to 1800–5500 rDNA copies) comprised approximately 4–7% of the *Solanum* genome.

Key words: Repeated DNA – Restriction satellite – RFLP – Solanaceae – Species-specificity

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Introduction

Eucaryotic nuclear genomes, especially those of higher plants, consistently contain a relatively large amount of repeated DNA (Wenzel and Hemleben 1982) composed of different element members. The main components are middle repetitive DNA interspersed into unique sequences and highly repeated, tandemly arranged DNA repeats characterized by specific restriction enzyme sites. This latter DNA component has been called 'restriction satellite' DNA (Pech et al. 1979). Hybridization of digested and Southern-blotted DNA with the respective repeat probe results in a ladder-like structure of bands that extend from monomer to multimers depending on point mutations and/or methylation of the restriction site. The size of the monomers appears to be rather conserved; in dicotyledonous plants satellite repeats between approximately 160 and 180 bp or 320 and 360 bp occur (Hemleben et al. 1992). Nucleotide sequences vary enormously, even between closely related species, and different molecular mechanisms act on the evolution of this genome fraction (Flavell 1985; Bostock 1986).

In order to characterize the genome organization of tuber-bearing *Solanum* species (Hawkes 1990) in

more detail, we have investigated some of the major repeated elements of the nuclear genome (Schweizer et al. 1990). Previous investigations had already shown the existence of a *Solanum* restriction satellite repeat (183 bp in length) that does not occur in tomato (Schweizer et al. 1988). A species-specific repeat has been described for *Solanum brevidens* (Pehu et al. 1990). For *Lycopersicon* another repeat type, 163 bp in length, appears to be typical (Schweizer et al. 1988; Ganal et al. 1988); this repeat is localized in clusters of tandem arrays mostly at the telomers of the 12 chromosomes. Two other major repeated elements have been described in *Lycopersicon* that cross-react only with *S. lycopersicoides* but not with other *Solanum* species (Ganal et al. 1988). Of the *Nicotiana tabacum* genome 2% consists of a 184-bp tandem repeat characterized by a *Bam*HI site (Koukalova et al. 1989); two middle-repetitive DNA sequences have been found to be characteristic of the *N. tomentosiformis* component of the allotetraploid *Nicotiana* genome (Kuhrova et al. 1991).

Nuclear DNA of dihaploid *S. tuberosum* breeding lines and of the wild species *S. demissum* was cloned and the clones obtained were screened for highly repeated DNA. During this screening program repeated DNA elements were found, some of which could distinguish wild species or cultivars of *Solanum* (Schweizer et al. 1990). Additionally, the percentage of the respective repeated elements and of ribosomal DNA was determined.

Material and methods

Plant material

Most of the plant material used (Table 1) was cultivated as shoot cultures under sterile conditions on MS medium (Murashige and Skoog 1962).

Nicotiana tabacum and *Atropa belladonna* were obtained from the Institute of Cell Biology and Genetic Engineering, Kiev, Ukraine; *Solanum nigrum*, *Solanum dulcamara*, *Capsicum annuum* and *Physalis alkekengi*, from the collection of the Botanical Garden, Tübingen. The DNA of *Datura minoxia* and *D. stramonium* was kindly provided by Dr. M. Meixner (Institute für Angewandte Genetik, Freie Universität, Berlin).

DNA isolation, digestion and hybridization

DNA was prepared from isolated nuclei according to the method of Hemleben et al. (1982) or, alternatively, by the method described by Shure et al. (1983). The procedures used were those described by Maniatis et al. (1982). Sample preparation for the pulsed-field gel electrophoresis (PFGE) was carried out as described by Ganal and Tanksley (1989) with slight modifications. Restriction enzyme cleavage was done according to the instructions of the suppliers. Labelling of the DNA fragments with [^{32}P] or digoxigenin was by the random hexamer priming method (Feinberg and Vogelstein 1983).

Radioactive hybridization was performed according to 'Amersham Protocols' using Hybond-N$^+$ membranes and high stringency washing procedures. In some experiments hybridization procedures used by Ganal et al. (1986) were applied. Non-radioactive hybridization with digoxigenin-labelled DNA probes was carried out according to 'Boehringer's Protocol' using a Hybond-N membrane (Amersham).

Slot-blot hybridization assays for determining the percentage of DNA hybridizing with a specific probe was carried out according to Maniatis et al. (1982) using a 'Minifold' apparatus (Schleicher and Schuell, Göttingen).

Cloning of repeated DNA

Nuclear DNA of the breeding lines *S. tuberosum* R1 and R2 or of the wild species *S. demissum* was digested with the restriction endonuclease *Taq*I and randomly cloned into pUC 18/19 (Yanisch-Perron et al. 1985). Clones containing repetitive DNA were identified by colony hybridization (Grunstein and Hogness 1975) and hybridization of miniscreen preparations (Birnboim and Doly 1979) to *Taq*I-digested and [^{32}P]-labelled total genomic DNA of the respective breeding line or wild species. Only repetitive DNA gave a strong hybridization signal in this test. The clone pSA287 has been described by Schweizer et al. (1988) and the clone pRZ52 containing 25S rDNA sequences by Torres (1985); pR1T320 was cloned from the *S. tuberosum* breeding line R1, and pSDT382 originated from *S. demissum*; the clones pR1T206, pR1T335, pR2T334 and pR2T383 were obtained from the breeding lines R1 or R2, respectively.

Results

Characterization of repeated DNA elements

Total nuclear DNA of *S. tuberosum* R1 and R2 and *S. demissum*, respectively, was digested with *Taq*I and cloned; six clones representing repeated DNA were selected for further analyses. The frequency of each

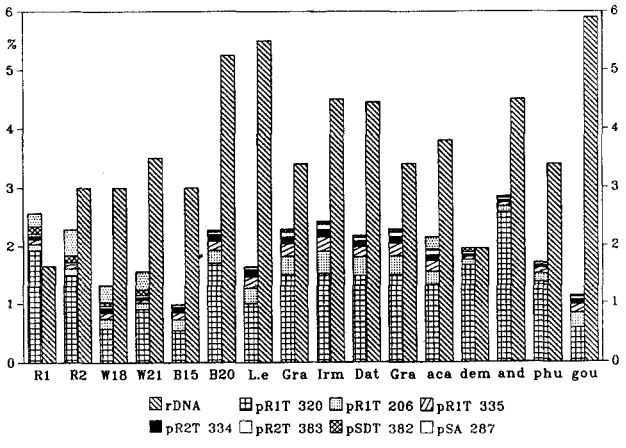

Fig. 1. Summary of the percentage of the nuclear genome of various repeated genome fractions in cultivars, dihaploid breeding lines and wild species of *Solanum*. The percentage was determined by slot-blot hybridization with the respective [^{32}P]-labelled insert of the clones indicated. *R1*, *R2*, *W18*, *W21*, *B15*, *B20*, *S. tuberosum* breeding lines; *L.e.*, *L. esculentum*; *Gra Irm Dat Gra*, *S. tuberosum* cvs *Granola*, *Irmgard*, *Datura*, *Grata*, respectively; *aca*, *S. acaule*; *dem*, *S. demissum*; *and*, *S. tuberosum* ssp. *andigena*; *phu*, *S. phureja*; *gou*, *S. gourlayi*

Table 1. List of potato wild species and cultivars used (names and classification according to Hawkes 1990)

Plants	Accession	Source[a]
Subsection *Potatoe* G. Don		
Series II *Bulbocastana* (Rydb.) Hawkes		
S. bulbocastanum	BGRC N 008006	GDC
Series III *Pinnatisecta* (Rydb.) Hawkes		
S. pinnatisectum Dun.	BGRC N 008168	GDC
Series VI *Circaeifolia* Hawkes		
S. circaeifolium Bitt. ssp. *quimense*	BGRC N 027036	GDC
Series IX *Yungasensa* Corr.		
S. chacoense Bitt.	Acc B2	MPI
Series XVI *Tuberosa* (wild species)		
S. canasense Hawkes	Acc A3	MPI
S. berthaultii Hawkes	Acc C3	MPI
S. gourlayi Hawkes	5.6	GFP
S. kurtzianum Bitt. et Wittm.	Acc A1	MPI
S. leptophyes Bitt.	8.27	GFP
S. neorossii Hawkes et Hjerting	11.42	GFP
S. sparsipilum (Bitt.) Juz. et Buk.	14.9	GFP
S. spegazzinii Bitt.	17.45	GFP
Series XVI *Tuberosa* (cultivated species)		
S. curtilobum Juz. et Buk. cv Luke	702455	CIP
S. juzepczukii Buk. cv Yurac Kaipii	702078	CIP
cv Pinaza	702445	CIP
S. phureja Juz. et Buk.	IVP 101	CPBR
S. stenotomum Juz. et Buk.	–	CIP
S. tuberosum L. ssp. *tuberosum*	Acc A2	MPI
S. tuberosum L. cv Lorch	–	UPRI
S. tuberosum L. cv NOS	117 cl	NBS
S. tuberosum L. cv Datura	–	SAKA
S. tuberosum L. cv. Granola	–	SAKA
S. tuberosum L. cv Grata	–	StSa
S. tuberosum L. cv Irmgard	–	WM
S. tuberosum L. R1	HB 6304/82	RAGIS
S. tuberosum L. R2	6186/79	RAGIS
S. tuberosum L. K7	AH 79.5503	MPI
S. tuberosum L. B15	H 256/1	BLBP
S. tuberosum L. B20	H 387/3	BLBP
S. tuberosum L. W18	H 82 3.02	SAKA
S. tuberosum L. W21	H 78.2022/9	SAKA
S. tuberosum L. ssp. *andigena* Hawkes		
cv Ccompis	700921	CIP
Series XVII *Acaulia* Juz.		
S. acaule Bitt.	–	CIP
Series XIX *Demissa* Buk.		
S. demissum Lindl.	–	CIP

[a] GDC, German-Dutch Curatorium for Plant Genetic Resources, Braunschweig; CIP, Centro Internacional de la Papa, Lima, Peru; GFP, Gesellschaft zur Förderung der Pflanzenzüchtung, Bonn; CPBR Center for Plant Breeding and Reproduction Research CPRO, Wageningen, The Netherlands; NBS, Nordostbaverischer Saatbauverband, Marktredwitz; RAGIS, RAGIS-Zuchtstation Heidehof, Heidehof; MPI, Max-Planck-Institut für Züchtungsforschung, Köln; UPRI, Ukrainian Potato Research Institute, Nemeshaevo, Ukraine; SAKA, SAKA Pflanzenzuchtbetrieb, Windeby; StSa, Stader Saatzucht, Niedersachsen; WM, Wallmüller, Saatzucht Moosbach, Bayern; BLBP, Bayerische Landesanstalt für Bodenkultur und Pflanzenbau, Freising

clone in the genome as a percentage was estimated by slot-blot hybridization of the respective clone (Fig. 1). In general, potato cultivars and the primitive cv *S. tuberosum* ssp. *andigena* exhibited a higher amount of repetitive DNA than most of the wild species investigated. Some of the dihaploid breeding lines (B15, B20, R1, R2, W18, W21; Table 1) derived from different *S. tuberosum* cultivars and used for protoplast fusion (Schilde-Rentschler et al. 1987; Schweizer et al. 1990) also contained a high percentage of repeated DNA.

In order to determine the amount of a middle repetitive genome fraction, the ribosomal DNA (rDNA) coding for the 18S, 5.8S and 25S ribosomal

RNA components (Hemleben et al. 1992), slot-blot hybridizations were also carried out with a [^{32}P]-labelled ribosomal DNA probe (2900-bp insert of clone pRZ52; Torres 1985) representing approximately 30% of a rDNA repeat. Calculations based on an average potato rDNA repeat length of 9 kb (Gruber 1991) gave percentages ranging between 1.9% and 5.2% rDNA (Fig. 1). If we assume a genome size of 0.9 pg/1C for potato (Arumuganathan and Earle 1991) the number of ribosomal DNA genes varies between 1800 and 5500 copies in the cultivars, breeding lines and wild species analyzed so far.

The presence and organization of the repeated DNA in *S. tuberosum* cultivars (4n), dihaploid breeding lines (2n) and different wild species of *Solanum* were analyzed by hybridizing Southern blots of restricted nuclear DNA. Digestion with various restriction endonucleases was carried out in order to detect specific RFLP patterns among different lines or species.

The clone pR1T320 appeared to be interesting and useful as a molecular marker. The genome component represented by this clone comprised 0.5–2.6% of the nuclear genome depending on the species, cultivar or breeding line (Fig. 1). The restriction enzyme patterns of the DNA obtained with *Hae*III and *Dra*I demonstrated rather informative RFLPs whereas after digestion with *Sau*3A a clear ladder-like structure with a basic monomer size of approximately 360 bp occurred (Fig. 2). Since this repeat type could also be detected in relatively low amounts in *Lycopersicon*, *Datura* and

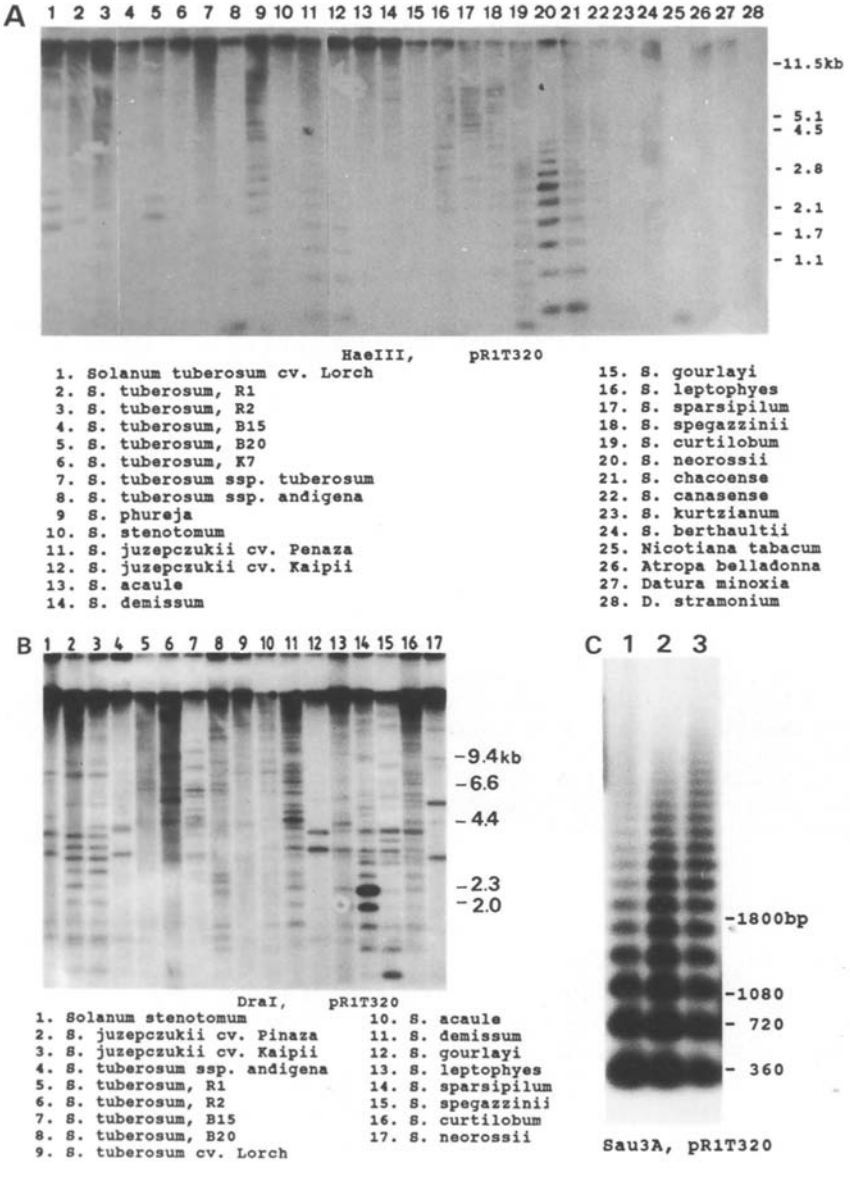

Fig. 2A–C. Distribution and restriction fragment length polymorphism (RFLP) of the repeated DNA component represented by the clone pR1T320 in various cultivars, breeding lines and wild species of *Solanum* and some other Solanaceae (autoradiograms). Each lane contained 2 µg DNA (**A** and **B**) that was digested with *Hae*III (**A**) and *Dra*I (**B**), respectively, separated on a 1% agarose gels, Southern blotted and hybridized with the [^{32}P]-labelled insert of the clone pR1T320. In **C** 5 µg DNA *S. tuberosum* cv 'Irmgard' (*lane 1*) *S. phureja* (*lane 2*) and the dihaploid breeding line B20 (*lane 3*) were digested with *Sau*3A and treated as described for **A** and **B**

Nicotiana, it may be used as an indicator for at least several genera of the Solanaceae.

The percentage of nuclear DNA represented by clone pSA287, which has been sequenced and described by Schweizer et al. (1988), was estimated to be between 0.2% and 0.4% of the genome (see Fig. 1). It was found to be present in all of the *Solanum* species investigated except for *S. kurtzianum* (Fig. 3), *S. bulbocastanum* and *S. pinnatisectum* (not shown). *Hae*III digestion resulted in a ladder-like structure with a basic monomer of approximately 180 bp in length. Interestingly, the restriction patterns of the four *Solanum* wild species, *S. gourlayi*, *S. leptophyes*, *S. sparsipilum* and *S. spegazzinii* showed a regular distribution of prominent multimers alternating with less prominent bands. This repeat type occurred in most of the

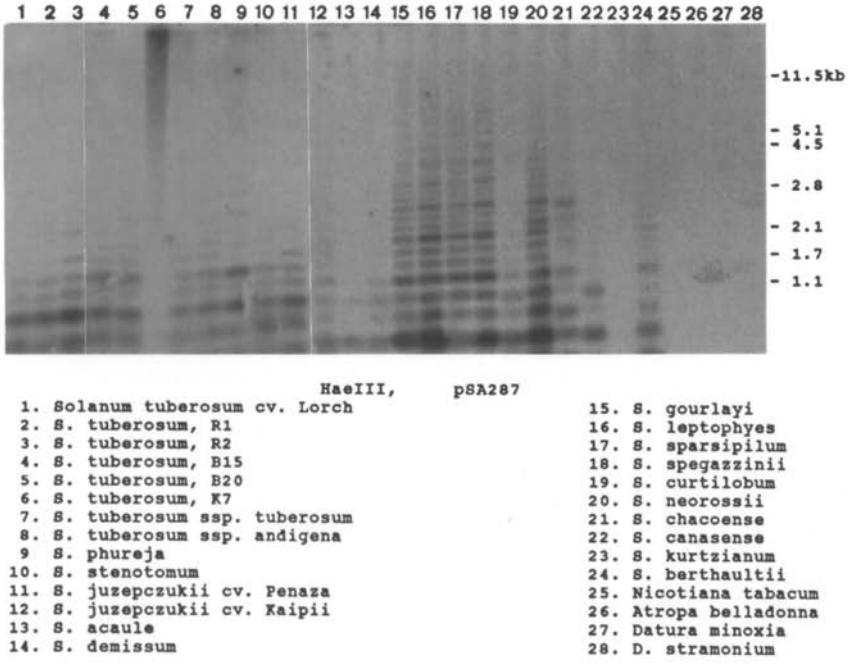

Fig. 3. Distribution and organization of the repeated DNA component represented by the clone pSA287 in various cultivars, breeding lines and wild species of *Solanum* and some other Solanaceae (autoradiogram). Each lane contained 2 µg DNA that was digested with *Hae*III, separated on a 1% agarose gel, Southern blotted and hybridized with the [^{32}P]-labelled insert of clone pSA287 (Schweizer et al. 1988).

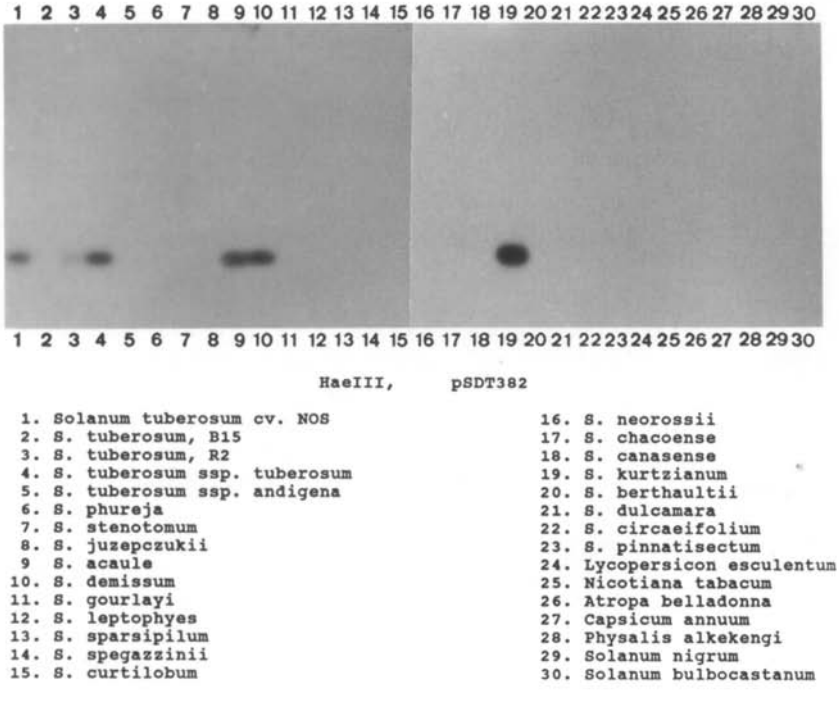

Fig. 4. Presence of the repeated DNA component represented by the clone pSDT382 in various cultivars, breeding lines and wild species of *Solanum* and some other Solanaceae. Each lane contained 2 µg DNA that was digested with *Hae*III, separated on 1% agarose gel, Southern blotted and hybridized with the [^{32}P]-labelled insert of the clone pSDT382

Solanum species analyzed in this investigation and can, therefore, be used more or less as a genus-specific probe.

During the screening procedure the clone pSDT382 of *S. demissum* was found to contain a repeated DNA element that was prominent in a restricted number of *S. tuberosum* cultivars (Schweizer et al. 1990). The percentage of the genome represented by this element varied from 0.01% to 0.12% (see Fig. 1). Searches made in potato cultivars, various wild species of *Solanum* and in other genera of the Solanaceae for the presence of this element showed that *S. kurtzianum* reacted strongly with this repeat whereas although *S. demissum*, *S. acaule*, *S. tuberosum* ssp. *tuberosum*, *S. tuberosum* cv 'Lorch' and the breeding line R2 also hybridized to it, they did so to a lesser extent (Fig. 4). *Hae*III digestion resulted in a band approximately 340 bp in length, and the restriction enzyme *Mlu*I gave rise to a ladder-like structure with a basic monomer of approximately 370 bp (Fig. 5), confirming the nature of a 'restriction satellite'.

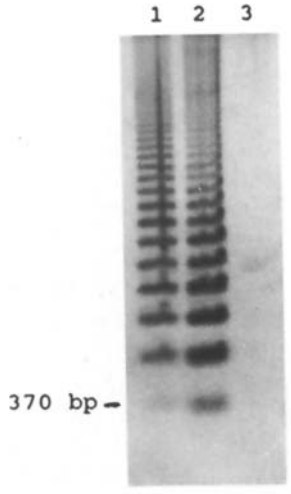

Fig. 5. Organization of the specific repeated genome component (represented by clone pSDT382) in *S. demissum* and two *S. tuberosum* dihaploid breeding lines, R2 and B15 (luminogram). Each lane contained 10 µg DNA of *S. demissum* (*lane 1*), R2 (*lane 2*) and B15 (*lane 3*), respectively, that was digested with *Mlu*I, separated on a 1% agarose gel, Southern blotted and hybridized with the digoxigenin-labelled insert of pSDT382. Signal detection was carried out by the AMPPD-chemiluminescence system

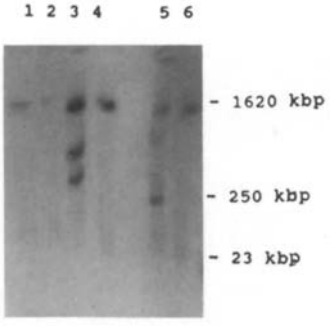

Fig. 6. Organization of the repeated DNA component (represented by the specific clone pSDT382) in the genomes of *S. demissum* and *S. tuberosum* R2 (autoradiogram). DNA of approximately 1×10^6 protoplasts of *S. demissum* (*lanes 1, 3, 5*) and R2 (*lanes 2, 4, 6*) either digested with *Sal*I (*lanes 3 and 4*) and *Xba*I (*lanes 5 and 6*), respectively, or undigested (*lanes 1 and 2*), was separated by pulsed-field gel electrophoresis (PFGE) on a 1% agrose gel, Southern blotted and hybridized with the [^{32}P]-labelled insert of clone pSDT382

Since *S. tuberosum* ssp. *andigena* is assumed to be the ancestor of our cultivated potatoe (Ross 1986; Hawkes 1990) and since this primitive cultivar and some other cultivars did not react with the clone pSDT382, it appeared that the dihaploid breeding lines R1 and R2 derived from *S. tuberosum* cultivars, which obtained this repeat by interbreeding with *S. demissum*. (Approximately 80% of the *S. tuberosum* cultivars used for agricultural purpose were interbred with *S. demissum*.) The organization of this repeat in the genome of *S. demissum* and the dihaploid *S. tuberosum* breeding line R2 was therefore determined. DNA of protoplasts was digested with various restriction enzymes, separated on pulsed-field gels and hybridized after Southern blotting with the [^{32}P]-labelled insert of clone pSDT382 (Fig. 6). With *S. demissum* several large bands reacted with this probe, depending on the restriction enzyme applied, while with the breeding line R2 fewer bands appeared. This result suggested that a certain percentage of the genome of *S. demissum* is represented in the breeding line *S. tuberosum* R2. The largest band detected for *S. demissum* corresponded in size to the band of line R2, indicating that a relatively stable part of the *S. demissum* genome was maintained.

The clones pR1T206, pR1T335, pR2T334 and pR2T383 represented repeated DNA elements that occurred in the genome of all of the *Solanum* species, cultivars and dihaploid breeding lines analyzed and in *Lycopersicon esculentum* at a percentage between 0.04% and 1.2%, respectively (see Fig. 1). They did not give rise to a very informative RFLP pattern, (not shown), and a ladder-like pattern was not obtained using the restriction enzymes *Hae*III and *Taq*I.

Discussion

Major highly repeated DNA elements were characterized in various *S. tuberosum* cultivars and dihaploid breeding lines, and in wild species of *Solanum*. Some of these repeated elements probably represent dispersed repeated DNA fragments that exhibit no species-specificity. These repeated elements also reacted with *Lycopersicon esculentum*, a representative of the genus most related to *Solanum*. This was expected because the genetic linkage maps established for *S. tuberosum* and *L. esculentum* by RFLP mapping with molecular probes are remarkably similar (Bonierbale et al. 1988; Gebhardt et al. 1989). This

result, however, does not exclude the possibility of finding species-specific, dispersed distributed repeats, as shown for *Nicotiana tabacum* (Kuhrova et al. 1991).

The distribution of several 'restriction satellites' showed some interesting features with respect to evolutionary relationships and for their use as molecular markers. A tandemly arranged major component represented by clone pR1T320 was present in variable amounts in all of the *Solanum* species analyzed that exhibited strong RFLP patterns with different enzymes. The basic monomer cut by *Sau*3A is 360 bp in length. A certain percentage of this genome component appears as 'relic' DNA (Metzlaff et al. 1986), indicating some nucleotide sequence divergency between the repeats. This type of DNA can also be detected in species of *Lycopersicon*, *Nicotiana* and *Datura* (see Fig. 2), indicating that a common progenitor of these genera already posessed an ancestor element that diverged but was maintained, differently amplified, in species of the Solanaceae.

Between 0.2% and 0.4% of the highly repeated DNA of various cultivars and species consisted of a *Solanum* specific 183-bp restriction satellite that had been sequenced and described as pSA287 by Schweizer et al. (1988). A rather similar restriction pattern appeared upon cutting the DNA with *Hae*III, especially in the four wild species, *S. spegazzinii*, *S. leptophyes*, *S. sparsipilum* and *S. gourlayi* (Fig. 3), which confirmed the already established close relationship of these species (Hosaka 1986; Debener et al. 1990). The species *S. kurtzianum*, *S. bulbocastanum* and *S. pinnatisectum*, which lack this genome fraction, should be investigated in more detail to clarify whether the loss of a repeated element occurred as a secondary event or whether they are more distantly related.

Of considerable interest for further analyses is the 370-bp repeat type (represented in the clone pSDT382) clearly present only in some species of the genus *Solanum*. It occurred most prominently in *S. kurtzianum*, less distinctly in *S. demissum* and *S. acaule*. Furthermore, some *S. tuberosum* cultivars and some dihaploid *S. tuberosum* breeding lines derived from *S. tuberosum* cultivars were characterized by this repeat. Digestion of total nuclear DNA with *Mlu*I and hybridization with this probe resulted in a ladder-like structure characteristic of a repeat arranged in tandem. At this stage it can be assumed that the cultivars possessing this repeat type derived from *S. tuberosum* ssp. *tuberosum* by the introduction of *S. demissum* (Ross 1986). In the pulsed-field gels that separated the large DNA molecules the *S. tuberosum* breeding line R2 shared distinct common bands with *S. demissum* after digestion with restriction enzymes, suggesting that *S. demissum* was introduced into the cultivar that gave rise to the dihaploid *S. tuberosum* line R2.

It is now possible to search further for species-specific repeated DNA in order to analyze the wild species-specific part of the genome within a cultivated potato. This approach has already helped to trace the nematode resistance gene of *Beta procumbens* in cultivated *Beta vulgaris* (Jung et al. 1990).

In conclusion, major repeated elements within the *Solanum* species were characterized and may be used for species analysis by either RFLP fingerprinting (Hemleben et al. 1992) or by their distribution and occurrence in the genome of different cultivars or wild species. The components analyzed together with the amount of middle repetitive ribosomal DNA amount to 4–7% of the genome. Interestingly, potato cultivars and the primitive cultivar, *S. tuberosum* ssp. *andigena*, contain a larger amount of repeated DNA.

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Note added in proof: The clone pR1T320 was revealed after nucleotide sequencing to contain 5S rRNA coding and spacer sequences.

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