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Two different satellite DNAs in *Beta vulgaris* L.: evolution, quantification and distribution in the genus

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Summary. Two highly repeated *Eco*RI (0.45×10^6) and *Bam*HI (0.17×10^6) fragments per haploid genome were found in sugar beet genomic DNA. Both fragments were located by 6% acrylamide-gel electrophoresis, purified and cloned in pUC18. Four of the inserts corresponding to each family were chosen for further study. Both fragment families display the main characteristics of the satellite DNA of animals and plants. The *Eco*RI and *Bam*HI fragment families are arranged in long tandem arrays. Fragments of the *Eco*RI family (pBVE) were analyzed. They vary both in sequence and in length (158–160 nt) in comparison with the consensus sequence of 159 nt. Both families are A-T rich; pBVE is 59% rich while pBVB is 69% rich. The BVESAT family is present in all the members of the section *Vulgares*. It is conserved in the section *Procumbentes* with 80% homology and the same length, but is not detectable in the *Corollinae*. The sequence variation rate and the variation in length (330 ± 5 nt) are of the same order in comparison with those of the BVESAT family. However, the BVBSAT family is present in species of the section *Vulgares* only. As regards other plant satellite DNAs, the BVESAT family shares homology with *Allium cepa* satellite DNA, with three of the yeast centromeric sequences, and with three *Arabidopsis thaliana* sequences. The BVBSAT family is unique to the *Vulgares* and does not share any homology with other plant or animal satellite DNAs sequences so far.

Key words: Satellite DNA – Highly repeated sequence – Tandem arrangement – Sugar beet – Sequence analysis

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Introduction

Satellite DNAs are organized as long arrays of tandemly repeated elements. Each family differs by either the length of the array or its sequence. Those repeats estimated to be amplified 10^3 to 10^5 times at each site have been shown to be clustered at a few loci per genome (Barnes et al. 1985; Nakaseko et al. 1986; Lapitan et al. 1989).

Satellite DNAs in animals are now well characterized (Pages and Roizes 1988). In plants, satellite DNAs have been characterized only for about ten species belonging to both Monocots (Gramineae and Liliaceae) and Dicots (Cruciferae, Leguminosae, Papilionatae, Solanaceae, Cucurbitaceae, and Hesperideae). Plant satellite DNAs have so far been studied both for structural and evolutionary aspects of the families concerned.

The cultivated beets, *Beta vulgaris* L., are of interest commercially as vegetables, for feeding animals, and for sugar production. Beets and their wild relatives are studied by breeders, biotechnologists and molecular biologists with the intention of improving the breeding process. The *genus Beta* is divided into three main sections (*Vulgares, Corollinae*, and *Procumbentens*) each including several species. The evolution of beet species has already been studied from botanical (Stanescu 1990) and molecular points of view (Fritzsche et al. 1987). Recently, both aspects have been consolidated in a phylogenetic tree (Santoni and Bervillé 1992).

In this article we report the characterization of two satellite DNA families cloned from the sugar beet genome. The BVESAT family is present in all *Vulgares* and shares homologies with the *Procumbentes*. The BVBSAT family appears unique to the *Vulgares*. Evolutionary rates and phylogenetic implications are discussed for both families of satellite DNAs.

Table 1. List of origins and reference number of the plant accessions

Section	Species or variety	Author	Reference or common name	Origin
Procumbentes	*B. procumbens*	Chr. and Sm.	H2148	Rosenhof, Germany
	B. patellaris	Moq.	F3977	Rosenhof, Germany
	B. webbiana	Moq.	F3981	Rosenhof, Germany
Corollinae	*B. lomatogona*	Fisch.	G2362	Beltsville, MD
	B. intermedia	Bunge.	F3971	Turkish
	B. macrorhyza	Stev.	G2365	Turkish
Vulgares	*B. maritima* var. *maritima*	Arcang.	H2151	INRA Colmar, France
			F3997	INRA Dijon, France
			F4006	INRA Dijon, France
			F4008	INRA Dijon, France
			F4009	INRA Dijon, France
			F4010	INRA Dijon, France
	B. maritima var. *macrocarpa*		F3958	INRA Dijon, France
			TZ38501 Swiss chard spinach	Tézier, France
	B. vulgaris var. *conditiva*	Linn.	TZ31509 Plate d'Egypte	Tézier, France
	B. vulgaris var. *crassa* (fodder beet)	Linn.	I1706	INRA Le Rheu, France
	B. vulgaris var. *altissima* (sugar beet)	Linn.	059	INRA Dijon, France
			59	INRA Dijon, France
			Marina	Maribo, Danmark
			Corum F3904	Desprez, Turkish
			F3905	Desprez, Poland
			F3906	Desprez, China

Materials and methods

Plant material

B. vulgaris var altissima, a tetraploid cultivar Marina (from Maribo), was used as a source of purified nuclear DNA for cloning satellite DNA. The other plant sources are listed in Table 1. The origins and sources of plant accessions have already been described in Santoni and Bervillé (1992) The plants were cultivated in a glasshouse at 25 °C for 16 h (day) and 15 °C (night). Leaves were harvested on at least 3-month old plants. Seeds from accessions unable to germinate were used to prepare DNAs.

DNA preparations

The methods employed to obtain total DNA have been previously described using, either dry seeds (Santoni et al. 1991) or total DNA from leaves (Santoni and Bervillé 1992). The DNAs were further purified by CsCl.

To prepare nuclear DNA, fully expanded leaves (50 g) were rinsed, cooled at 4 °C, and sliced before grinding with a waring blender in 250 ml of homogenization buffer TB1 (50 mM Tris-HCl pH 7.5, 0.3 M sucrose, 5 mM $MgCl_2$). The homogenate was filtered through two layers of cheesecloth (40 μm mesh) and then centrifuged at 2,500 *g* for 5 min. The pellets were resuspended in a half volume of TB1 plus 2% (w/v) Triton-X 100 and incubated at room temperature for 10 min. After centrifugation at 2,500 *g* for 5 min, the pellets were resuspended in 1/20 of TB1. The suspension was loaded onto a 2.2 M sucrose layer in 50 mM Tris-HCl pH8, 10 mM $MgCl_2$ and centrifuged at 38,000 *g* for 45 min using a A8-24 rotor (Kontron). The green supernatants were carefully drained and the white pellets resuspended in lysis buffer [10 mM Tris-HCl pH8, EDTA Na_2 10 mM, 0.1 M NaCl, 1.25% (w/v) Sarkosyl] and then incubated at 65 °C for 5 min. The suspensions were deproteinized with phenol-chloroform and the DNAs were ethanol-precipitated.

Restriction and electrophoresis conditions

Total DNA was restricted with ten units of enzyme per μg DNA at 37 °C, according to the manufacturer's instructions, for 2.5 h. Four micrograms of DNA were restricted in every lane unless otherwise specified. Submarine agarose (0.8–1.5%) electrophoresis or vertical 6% polyacrylamide gels were used in TAE buffer (Maniatis et al. 1982). The DNAs were transferred to nylon membranes (Amersham, N^+) according to Southern (1975).

Cloning of purified DNAs recovered from acrylamide gels

The DNA fragments were recovered by diffusion overnight from the polyacrylamide gel according to Maniatis et al. (1982). The *Eco*RI and *Bam*HI fragments were inserted into the *Eco*RI or the *Bam*HI site of pUC 18 vector, respectively. The recombinant vectors were propagated in DH5α *E. coli* cells. For each cloning experiment 2 × 96 colonies, out of about 500 obtained, were blotted and hybridized with nuclear DNA as a probe, to determine those containing highly repeated genomic sequences.

Labelling of probes and hybridization conditions

Every probe (25 ng) was labelled using the Random Primed DNA labelling kit of Boehringer with 20 μCi of ^{32}P αdCTP (3,000 Ci/m mol). Nuclear DNA (200 ng), used as a probe, was labelled by the nick translation DNA labelling kit of Boehringer with 20 μCi of ^{32}P αdCTP (3,000 Ci/m mol). The nylon mem-

branes were prehybridized and hybridized according to the manufacturer's instructions in 6 × SSC, 5 × Denhardt, 0.1% SDS, and 1 mg/ml of salmon sperm at 62 °C overnight. The membranes were rinsed with 0.1 SSC unless otherwise specified.

Quantification of hybridization signals

Was performed with an image analyzer (SAMBA 200S Alcatel TITN) on autoradiographs with different controls and standards.

Sequencing and sequence analysis

Sequences of the chosen cloned monomers were obtained by the enzymatic chain-termination procedure with ^{35}S γdATP and the Multiwell Sequencing kit from Amersham (Sanger et al. 1977).

Sequence recovery from GenBank and EMBL databases employed the GCG program (Devereux et al. 1984). Sequence analysis was performed according to Wilbur and Lipman (1983).

Results

Characterization and cloning of highly repeated fragments

Figure 1A displays restricted sugar beet nuclear DNA electrophoresed in a 6% polyacrylamide gel stained with ethidium bromide. With *Eco*RI, *Sac*I and *Stu*I an intense discontinuous band appears at 160 bp, while with *Bam*HI an intense discontinuous band appears at 330 bp. The *Eco*RI and *Bam*HI bands were purified and cloned in pUC18.

Four colonies from each cloning experiment were chosen because they displayed the most intense signals when hybridized with nuclear sugar beet DNA as a probe (data not shown). The *Eco*RI family of fragments was designated pBVE with clone number 1 as the type; the *Bam*HI family was designated pBVB with clone number 1 as the type. The pBVE1 insert, used as a probe, was hybridized to transfers of restricted sugar beet total DNA. Figure 1B shows the typical hybridization patterns obtained. With the four *Eco*RI inserts similar patterns were produced. We observed strong hybridization signals at 160 bp, 320 bp, 480 bp and 640 bp with *Eco*RI, *Stu*I, and *Sac*I, while no signal was detectable in the *Bam*HI lane (except in the well). Fig. 1 C shows the typical hybridization pattern with the pBVB1 insert used as a probe. With the five *Bam*HI insert similar patterns were obtained. We observed strong hybridization signals at 330 bp, 660 bp, and 990 bp with *Bam*HI and *Xho*I while only faint signals are visible at the same positions for the other lanes, except for *Sac*I. We also observed a second scale of less intense signals at 360 bp, 720 bp and 1,080 bp and concluded that this was due to a 360 bp element which shares strong homologies with the BVB-SAT family.

Organization of tandem arrays

Both hybridization patterns are typical of hybridized elements arranged in tandem arrays. The scales are due to

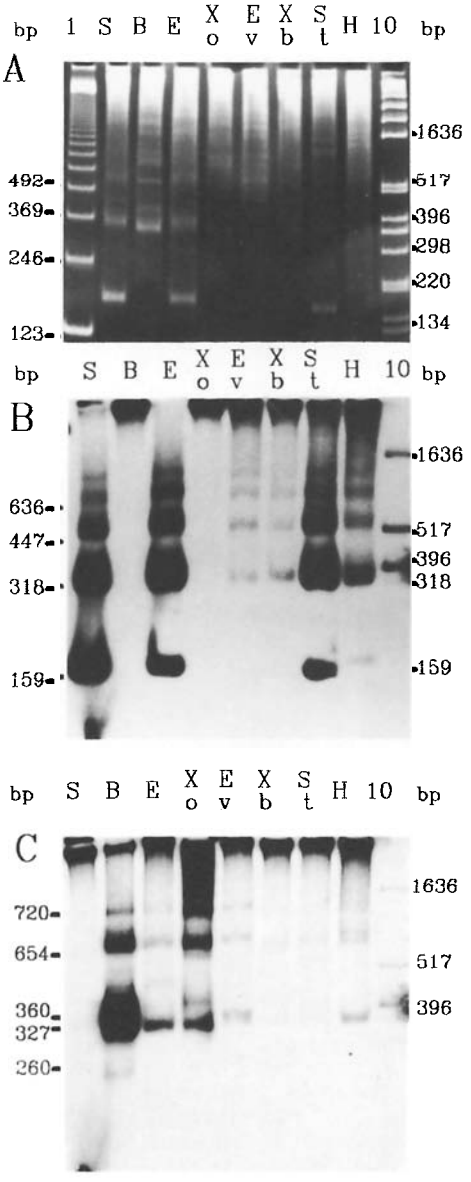

Fig. 1A–C. Characterization of highly repeated fragments. **A** Electrophoregram of restricted sugar beet nuclear DNA electrophoresed in a 6% polyacrylamide gel stained with ethidium bromide. *Lane 1*, 123 bp ladder from Bethesda Research Laboratories (BRL); *lane 10*, 1 kb ladder from BRL. Twelve micrograms of DNA per lane were hydrolyzed with 60 u of restriction enzymes. Every other lane corresponds to restriction by the indicated enzymes. *S*, *Sac*I; *B*, *Bam*HI; *E*, *Eco*RI; *Xo*, *Xho*I; *Ev*, *Eco*RV; *Xb*, *Xba*I; *St*, *Stu*I; *H*, *Hin*dIII. **B** Autoradiogram of the Southern transfer probed with the pBVB1 fragment

uncleaved restriction sites because of base substitutions at these sites. In order to verify such an organization of elements, a monomer fragment of each family was sequentially hybridized on sugar beet total DNA, restricted and then electrophoresed in agarose gel. The pBVE1 insert used as a probe, hybridized a scale of fragments corresponding to an association of 160 bp monomers (Fig. 2A). In accordance with Horz and Zachau (1977)

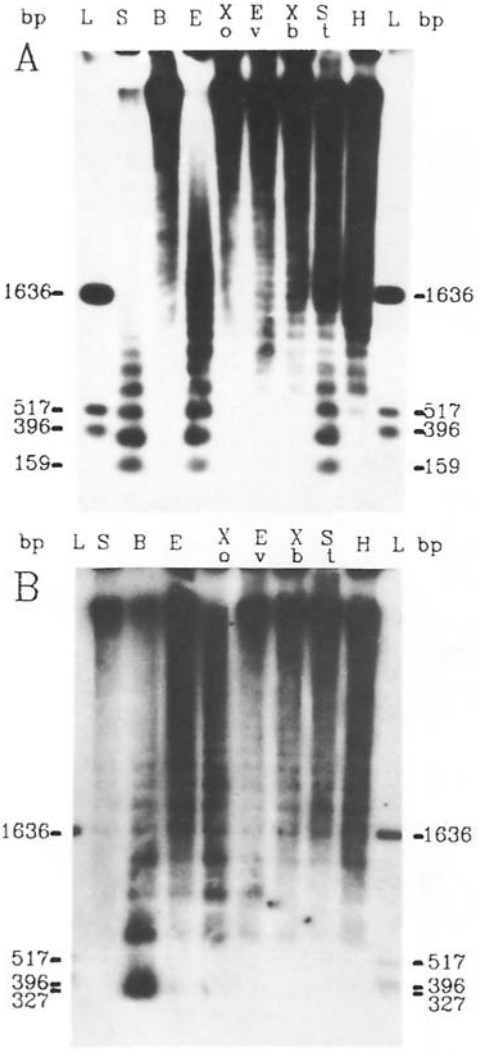

Fig. 2 A, B. Autoradiograms of the Southern transfer of restricted sugar beet total DNA electrophoresed in an 0.8% agarose gel. **A** Probed with the pBVE1 fragment. **B** Probed with the pBVB1 fragment. *Lanes 1 and 10*, 1 kb ladder from BRL. 4 µg DNA per lane were hydrolyzed with 20 u of restriction enzymes. Every other lane corresponds to restriction by the indicated enzyme. *S*, *Sac*I; *B*, *Bam*HI; *E*, *Eco*RI; *Xo*, *Xho*I; *Ev*, *Eco*RV; *Xb*, *Xba*I; *St*, *Stu*I; *H*, *Hin*dIII

we observed a type A profile, corresponding to a complete restriction of adjacent units, with *Sac*I or *Eco*RI. By contrast a, type B profile was obtained when a substantial proportion of the restriction sites were not cut in an array of adjacent units, as was the case with *Eco*RV, *Xba*I, *Stu*I and *Hin*dIII. Figure 2B displays the hybridization patterns with the pBVB1 insert used as a probe. The type A profile was observed with *Bam*HI while the type B profile was observed with *Eco*RI, *Xho*I, *Xba*I and *Stu*I. They both correspond to satellite DNA families which do not cross-hybridize.

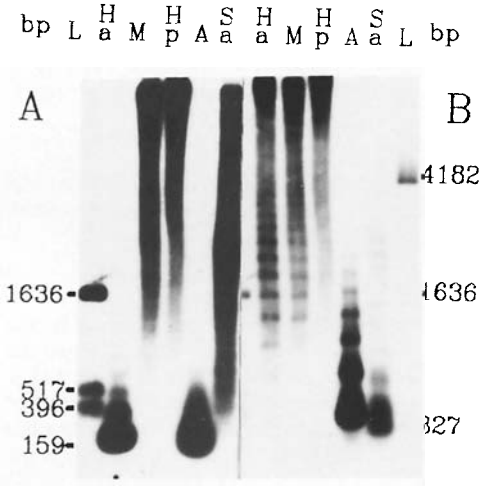

Fig. 3 A, B. Autoradiograms of the Southern transfer of restricted sugar beet total DNA, electrophoresed in 0.8% agarose. **A** Hybridized with the pBVE1 insert. **B** Hybridized with the pBVB1 insert. *Lane 1*, 1 kb ladder from BRL; *lane 7*, 123 bp ladder from BRL. Every other lane corresponds to restriction by the indicated enzyme. *Ha*, *Hae*III; *M*, *Msp*I; *Hp*, *Hpa*II; *A*, *Alu*I; *Sa*, *Sau*3A

Methylation of satellite DNA families

Hydrolyses with enzymes recognizing a tetranucleotide sequence were performed. *Hae*III or *Alu*I cuts the monomer of the BVESAT family into two, or more, subfragments, while there is no site for *Msp*I, *Hpa*II or *Sau*3A (Fig. 3A). *Sau*3A cuts the BVBSAT monomer leading to a scale of sub-fragments. The *Alu*I restriction profile is of the A type while *Hae*III displays a B-type profile (Fig. 3B). The *Msp*I profile contains more bars than the *Hpa*II profile, due to the more frequent methylation of the internal C of the CCGG sequence. But an important proportion of unrestricted DNA is due to the methylation of both C residues in the restriction site.

Estimation of copy numbers

The dot-blot assays allow a broad estimation of copy number (Fig. 4A, B). The BVESAT family corresponds to 0.4×10^6 units ($\pm 0.05 \times 10^6$ units), while the BVBSAT family corresponds to 0.155×10^6 units ($\pm 0.015 \times 10^6$ units), per haploid genome of 1.35 pg (Ingles et al. 1975).

Sequence determination of the BVESAT family

Four clones were sequenced in both strands (Fig. 5A). We observed a slight variation between the four sequences. These modifications randomly affected restriction sites for elements in one array, causing modifications in restriction enzyme sites, either allowing or not allowing cuts, and leading to a typical ladder of signals. The consensus sequence displays two palindromic inverted

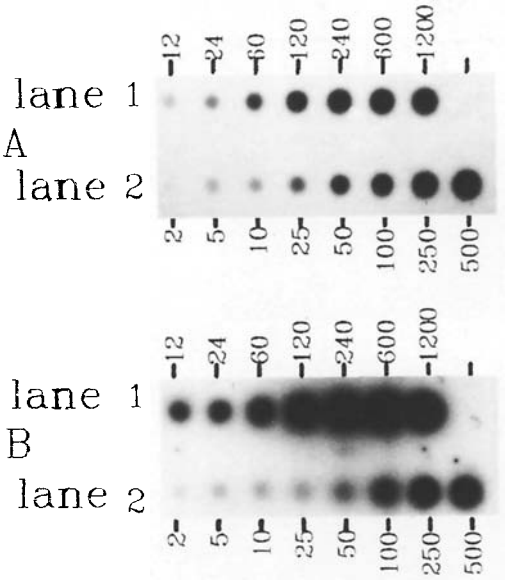

Fig. 4 A, B. Determination of copy numbers for the BV-SAT families. **A** BVESAT: *lane 1*, dots of total DNA (ng); *lane 2*, dots of the pBVE1 insert (pg). Dots were hybridized with the pBVE1 insert. **B** BVBSAT: *lane 1*, dots of total DNA (ng); *lane 2*, dots of the pBVB1 insert (pg). Dots were hybridized with the pBVB1 insert. Two other similar experiments have been performed

repeats at nucleotides (nt) 61 and the 146, of 14 nt and 8 nt respectively.

A sequence determination of the BVBSAT family was recently performed by Schmidt and Metzlaff (1991). The BVESAT and the BVBSAT families are 59% and 69% A-T rich, respectively.

Comparison with other plant satellite DNAs

Significant homologies (Fig. 5B) have been found with the *Allium cepa* satellite DNA (Barnes et al. 1985). Eighteen nucleotides share 83% homology. Moreover, 26 nt of the BVESAT sequence, between nt 17 and nt 43, are 81% homologous to three yeast centromeric DNAs (Yanagida, unpublished). The 25 nt sequence of BVESAT (nt 22–47) displays 68% homology with 25 nt (126–149) of *Arabidopsis thaliana* satellite DNA (Simoens et al. 1988). Thus, it is possible that the BVESAT family might play a role as a centromeric satellite DNA in the sugar beet genome.

Distribution of pBVE- and pBVB-like families in the genus Beta

(1) The *Vulgares* section. Both BVESAT and BVBSAT families are present in all the *Vulgares* accessions examined (Figs. 6, 7). According to the intensity of ethidium bromide staining (Figs. 6A) and the hybridization signals (Figs. 6B, 7B) we inferred that all the accessions carry equivalent copy numbers of each family. Moreover, the

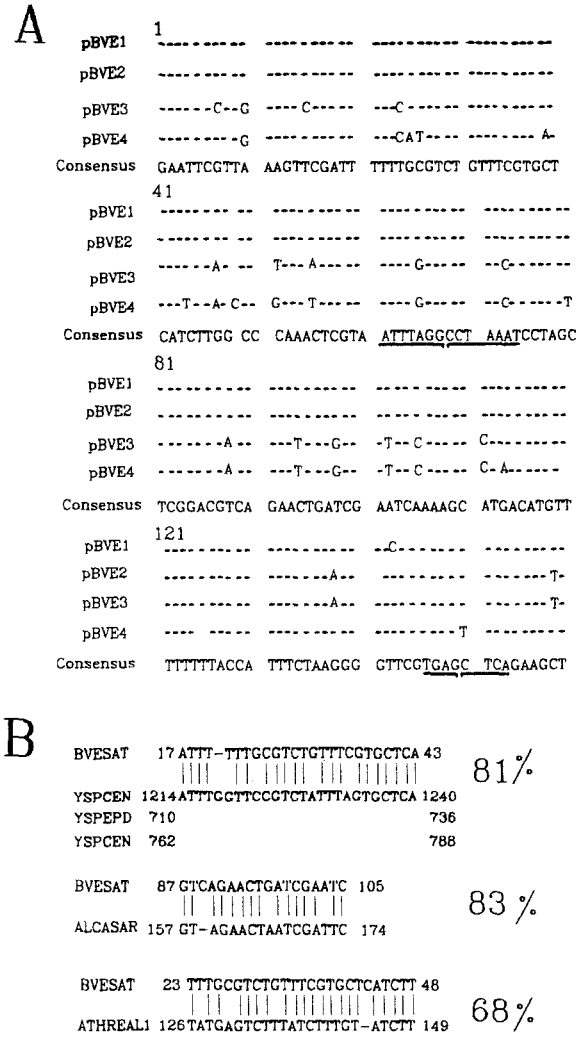

Fig. 5 A, B. Sequences of four members of the BVESAT family. The nucleotide changes are indicated (*letter*). A *blank* indicates a deletion. The *thick lines* indicate the two palindromic sequences. **B** Homologies between the pBVE family and other plant satellite DNAs. Designations are according to Table 1. The three yeast sequences (YSP CEN, YSP EPD and YSP CEN are identical). The percentages of homology are indicated on the right

*Alu*I sub-fragments of the BVESAT family are conserved for all accessions (Fig. 8A, B) as judged by both the ethidium bromide staining and the intensity of the hybridization signals. For all accessions, the BVBSAT family displays two basic motives of 327 bp and 360 bp, each one leading to a ladder. The stoichiometric ratio was estimated to be 200 327 bp-fragments for one 350 bp-fragment (data not shown). We have also verified the presence of the two satellite DNA families on forage beet, Swiss chard, and several table beets (Santoni and Bervillé 1992).

(2) The *Corollinae* and *Procumbentens* sections. Although, all the *Corollinae* species were checked the BVESAT family cannot be detected in the *Corollinae*

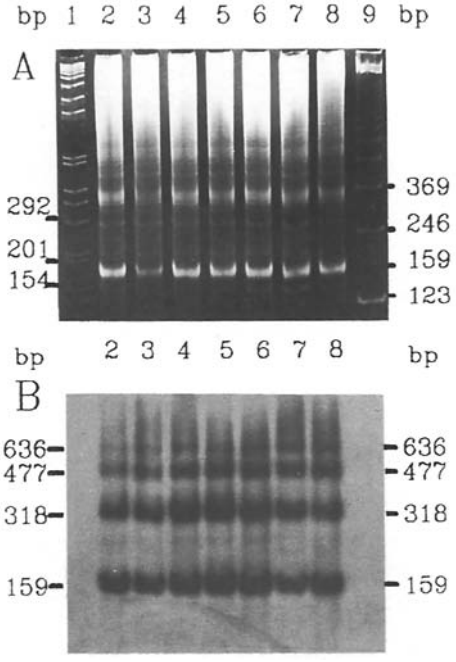

Fig. 6 A, B. Distribution of the BVESAT family in the section *Vulgares*. **A** Electrophoregram in a 6% polyacrylamide gel, stained with ethidium bromide, of *Eco*RI (60 u)-restricted total DNAs (12 µg per lane) from *B. maritima*: *lane 2*, F4009; *lane 3*, F3977; *lane 4*, F4006; *lane 5*, F4010. *B. vulgaris* sugar beet: *lane 6*: 59; *lane 7*: n° 01012. *B. maritima* var. *macrocarpa*: *lane 8*, F3958. *Lanes 1 and 9* are the 1 kb and the 123 bp ladders from BRL, respectively. **B** Autoradiogram of the Southern transfer probed with the pBVE1 fragment and rinsed at 0.1 × SSC

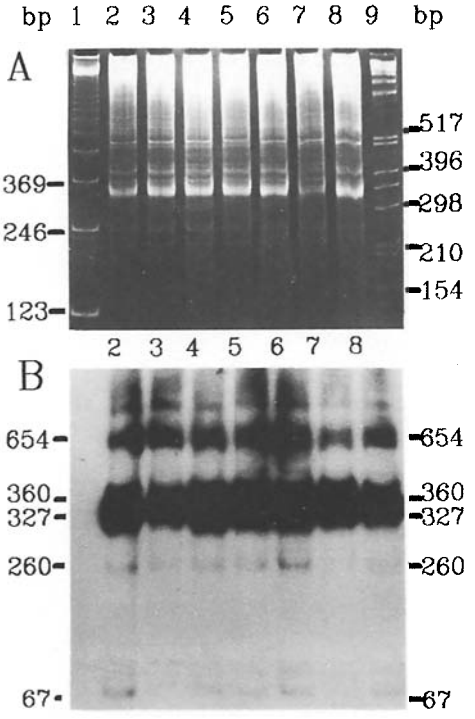

Fig. 7 A, B. Distribution of the BVBSAT family in the section *Vulgares*. **A** Electrophoregram in a 6% polyacrylamide gel, stained with ethidium bromide, of *Bam*HI (60 u)-restricted total DNAs (12 µg per lane) from *B. maritima* var. *macrocarpa*: *lane 2*, F3958. *B. maritima*: *lane 5*, F4010; *lane 6*, n° F4006; *lane 7*, F3997; *lane 8*, F4009. *B. vulgaris* sugar beet: *lane 3*, 01012; *lane 4*, 59. *Lanes 1 and 9* are the 123 bp and the 1 kb ladders from BRL, respectively. **B** Autoradiogram of the Southern transfer probed with the pBVB1 fragment and rinsed at 0.1 × SSC

whatever rinsing stringency is employed (data not shown). But a family homologous to BVESAT was detected in the *Procumbentens* (Fig. 9) after rinsing at medium stringency (2 × SSC). The monomer of the *B. patellaris* (*Procumbentens*) satellite DNA is about 160 bp according to a size determination obtained from an acrylamide gel (data not shown). In comparison with the *Vulgares* the *Eco*RI sites are not conserved, whereas the *Sac*I sites are conserved, in the *Procumbentes* satellite DNA family.

The BVBSAT family cannot be detected in either the *Corollinae* or the *Procumbentens* whatever the rinsing stringency (Fig. 10). Therefore, the BVBSAT family is unique to the section *Vulgares*.

Discussion

We have characterized two distinct families of highly repeated fragments which display the main features of satellite DNA. The *Eco*RI or *Bam*HI elements concerned are tandemly arranged in long arrays. Variation of elements belonging to a unique family is one of the characteristics of satellite DNA. The randomly distributed base changes may affect restriction sites leading to a scale of from one to many large-sized fragments because of uncleaved sites. Depending upon the restriction enzyme, hybridization diagrams for both families display either a type A or a type B profile which reflects common or infrequent restriction sites, respectively. Each family represents about 0.5% of beet genomic DNA. Closely related species in the *Vulgares* section carry both satellite families, but the BVBSAT family is unique to the *Vulgares* while the BVESAT family displays hybridization homologies with a satellite DNA family of the *Procumbentes* and also shares sequence homologies with other plant satellite DNAs.

The BVESAT family has one of the smallest units (159 bp) among plant satellite DNAs. The two palindromic inverted repeats might play a role in this satellite family. If there is a function for this DNA family it could be to bind beet (some or all) chromosomes to the mitotic spindle, since the BVESAT family displays homologies with the centromeric satellite sequences of yeast and *Arabidopsis*. The distribution of the BVESAT family in the beet genome has yet be checked to determine whether or

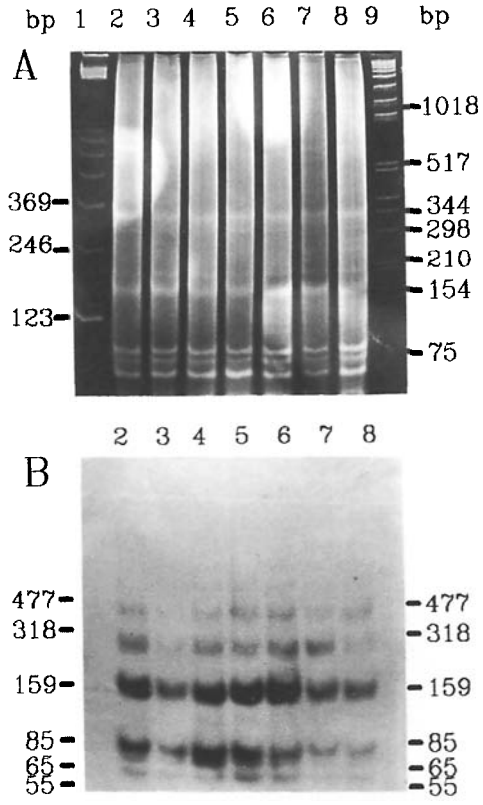

Fig. 8 A, B. Distribution of BVESAT family sub-fragments obtained with *Alu*I in the section *Vulgares*. **A** Electrophoregram in a 6% acrylamide gel, stained with ethidium bromide, of *Alu*I (60 u)-restricted DNAs (12 µg per lane) from *B. maritima*: *lane 2*, F4009; *lane 3*, F3997; *lane 4*, F4006; *lane 5*, F4010. *B. vulgaris* sugar beet: *lane 6*, line 59; *lane 7*, 01012. *B. macrocarpa*: *lane 8*, F3958. *Lanes 1 and 9* are the 123 bp and the 1 kb ladders from BRL, respectively. **B** Autoradiogram of the Southern transfer probed with the pBVE1 fragment

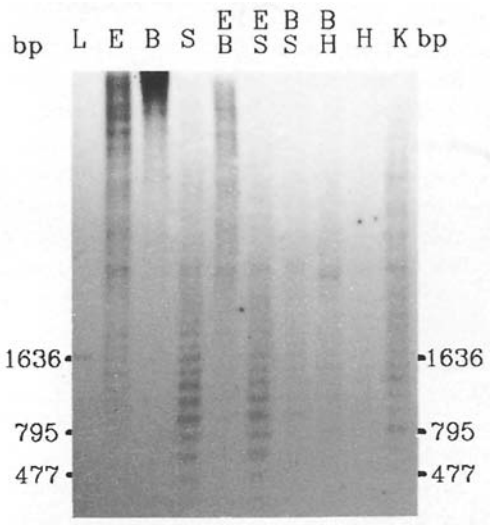

Fig. 9. Autoradiogram of the Southern transfer for restricted electrophoresed *B. patellaris* total DNA (6 µg per lane) in a 1.2% agarose gel probed with the pBVE1 fragment and rinsed at medium stringency (2 × SCC, 65 °C). *Lanes 1 and 12* correspond to the 1 kb ladder from BRL. Thirty units of every enzyme have been used for single or double restriction. Every lane corresponds to the restriction by the indicated enzyme or by a pairwise combination. *S*, *Sac*I; *B*, *Bam*HI; *E*, *Eco*RI; *K*, *Kpn*I; *H*, *Hin*dIII

not they are indeed centromeric sequences. The BVBSAT family contains two variable-length monomers of 327 bp and 360 bp, the former being numerically dominant; the localisation of the two in the beet genome could be different.

For the Brassicaceae the distribution of tandemly repeated DNA sequences has been used to analyze the evolution of the tribe (Halldén et al. 1987). These two satellite DNA families are of particular interest for considering in the genus *Beta* evolution. The BVESAT family is characteristic of the *Vulgares* section, but a homologous family of the same length exists in the *Patellares*. Thus, according to this DNA familiy, the sections *Vulgares* and *Procumbentes* are more closely related to each other than are the *Corollinae* to either of them. We might expect a conservation of the BVESAT family in these two closely related sections on the hypothesis that this sequence might play a functional role. Surprisingly, the sequence was not found in the *Corollinae* as judged by

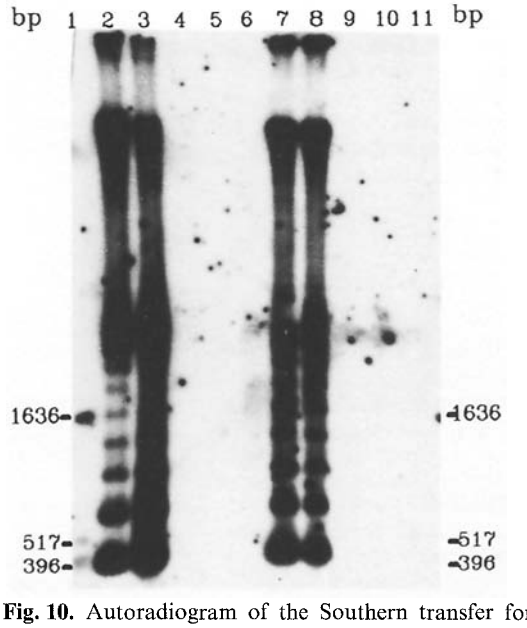

Fig. 10. Autoradiogram of the Southern transfer for *Bam*HI (15 u)-restricted and electrophoresed *Beta* species total DNAs (3 µg per lane) probed with the pBVB1 fragment and rinsed at low stringency (6 × SSC, 65 °C). *Vulgares*; *B. maritima*: *lane 2*; F4008; *lane 7*, H2151. *B. vulgaris* sugar beet: *lane 3*, 059; *lane 8*, table beet TZ 31509. *Corollinae*; *lane 4*, *B. macrorhiza*; *lane 5*, *B. lomatogona*; *lane 6*, *B. intermedia*. *Procumbentens*; *lane 9*, *B. procumbens*; *lane 10*, *B. webbiana*; *lane 11*, *B. patellaris*. *Lanes 1 and 12* correspond to the 1 kb ladder from BRL

hybridization. This is in contradiction with the phylogeny constructed from chloroplast DNA (Fritzsche et al. 1987; Bonavent et al. 1989), RFLP markers (Nagamine et al. 1989), and RFLPs in the nuclear ribosomal DNAs (Santoni and Bervillé 1992), where the *Corollinae* and the *Vulgares* are considered to be more closely related than are the *Patellares* with each of the other two. The BVBSAT family, unique to the *Vulgares*, suggests that satellite DNA families in closely related *Beta* sections might have been amplified from non-homologous basic units (Walbot and Cullis 1985). Consequently, in terms of of the molecular drive mechanism of sequence homogenization (Dover 1982), it would be interesting to determine the distribution of the BVESAT and BVBSAT sequences in the beet genome.

Recently, several highly repeated DNA sequences have been characterized in *B. procumbens* (Schmidt et al. 1990). However, sequence comparisons did not show any homology between PTS (1 to 4) sequences and the BVESAT-homologous sequence found in *B. patellares, B. procumbens* and *B. webbiana*. In the *B. vulgaris* addition line described by Jung et al. (1990) carrying a nematode resistance gene, a *B. procumbens*-specific sequence was characterized but not isolated. Thus, several families of highly repeated sequences coexist in the *Beta* genome. Their distribution and location on the chromosomes could be of value in attempts to improve the beet breeding program.

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