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Distribution and evolution of two satellite DNAs in the genus *Beta*

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Summary. EcoRI monomers of a highly repetitive DNA family of *Beta vulgaris* have been cloned. Sequence analysis revealed that the repeat length varies between 157–160 bp. The percentage of AT-residues is 62% on average. The basic repeat does not show significant homology to the BamHI sequence family of *B. vulgaris* that was analyzed by us earlier. Both the EcoRI and BamHI sequences are investigated and compared to each other with respect to their genomic organization in the genus *Beta*. Both repeats were found to be tandemly arranged in the genome of *B. vulgaris* in a satellite-like manner. The EcoRI satellite DNA is present in three sections (*Beta, Corollinae* and *Nanae*) of the genus, whereas the BamHI satellite DNA exists only in the section *Beta*. The distribution of the EcoRI and BamHI satellite families in the genus is discussed with respect to their evolution.

Key words: *B. vulgaris* – Wild beet – Satellite DNA – Genome organization – Sequence analysis

Introduction

Repetitive DNA represents a considerable component of the higher plant genome. During the last few years a number of studies on plant repetitive sequences have been performed (Bedbrook et al. 1980; Hemleben et al. 1982; Kato et al. 1984; Grellet et al. 1986; Simeons et al. 1988), and these have provided detailed information about their structure, genomic organization, and evolution. Nevertheless, the function of highly repeated DNA remains still obscure.

Short repetitive DNA sequences organized as tandemly arranged elements are defined as satellite DNA (Hemleben 1990), whereas other repetitive sequences can show a dispersed widespread distribution over the whole genome. The divergence of the members of a satellite DNA by the accumulation of mutations combined with their amplification by mechanisms like unequal crossing over, gene conversion, or extrachromosomal replication leads to species-specific satellite DNA.

The genus *Beta* contains important cultivars like sugar beet, fodder beet, foliage beet, and swiss chard (for review see Barocka 1985). It is divided into four sections *Beta, Nanae, Corollinae* and *Procumbentes* that show a considerable variability regarding their morphology and geographical distribution. The heterogeneity of the plastome (Mikami et al. 1984; Fritzsche et al. 1987) and the chondrome (Boutin et al. 1987; Ecke and Michaelis 1990) has already been described. The first satellite DNA family in the nuclear genome of *Beta vulgaris* was isolated as a BamHI sequence family (Schmidt and Metzlaff 1991).

In this paper we report a second sugar beet satellite DNA isolated as a EcoRI sequence family that shows no homology to the BamHI family. The distribution of both satellite DNA sequences in the genus *Beta* is described.

Material and methods

Plant material

The plant material involved in our experiments is listed in Table 1. *Spinacea oleracea* and *Chenopodium bonus-henricus* were included as *Beta*-related species. We used *B. maritima* of three geographically distinct origins. Species characterized by a BGRC number were kindly provided by L. Freese, CGN Wageningen. *B. intermedia, B. nana,* and *B. webbiana* were obtained from Prof. G. Wricke, University of Hannover. *C. bonus-henricus* was obtained from the Botanical Garden, University of Halle. The remaining wild species and *Beta* cultivars were kindly

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Table 1. Species used in hybridization experiments

Genus	Species	Subspecies	Cultivar	BGRC No.	Section
Beta	*vulgaris*	*vulgaris*	*crassa*		
	vulgaris	*vulgaris*	*altissima*		
	vulgaris	*vulgaris*	*cicla*	053656	
	vulgaris	*orientalis*		056779	
	atriplicifolia			056775	*Beta*
	patula			056782	
	vulgaris	*maritima*		054842	
	vulgaris	*maritima*		057719	
	vulgaris	*maritima*		056651	
	vulgaris	*vulgaris*	*flavescens*		
	vulgaris	*vulgaris*	*conditiva*		
	corolliflora				
	lomatogona				
	trigyna				*Corolli-*
	macrorhiza				
	intermedia				*nae*
	nana				*Nanae*
	patellaris				*Procum-*
	webbiana				*bentes*
	procumbens				
Spina-cea	*oleracea*				
Cheno-podium	*bonus-henricus*				

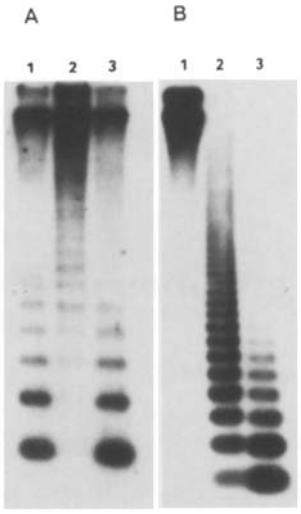

Fig. 1 A, B. Genomic organization of the BamHI and EcoRI satellite DNA monomer. Total DNA of sugar beet was digested with BamHI (*1*), EcoRI (*2*), and BamHI/EcoRI (*3*), electrophoresed and transferred onto nylon membrane filters. The filters were hybridized with **A** pBV1 and **B** pEV1.

provided by Prof. R. Melzer, Institute for Beta Research, Klein Wanzleben. Spinach (*S. oleracea*), swiss chard (*B. vulgaris* cv *flavescens*), and beet root (*B. vulgaris* cv *conditiva*) were purchased from a local vegetable shop.

DNA preparation and cloning of satellite DNA sequences

Total DNA from *Beta* species was isolated as described previously (Schmidt et al. 1990). The total sugar beet DNA was digested to completion with EcoRI and fractionated on a 1.3% agarose gel. The restriction profile obtained shows a ladder of bands corresponding to multimeric DNA fragments of a basic repeat approximately 160 bp in length. This monomer was eluted from the gel and cloned into the EcoRI site of M13mp19. Recombinant DNA was used to transform *E. coli* JM103Y cells (Mandel and Higa 1970). The resulting phages were screened for the integration of the EcoRI monomer in phage dot hybridization experiments (Messing 1983) with ^{32}P-labelled total DNA of *B. vulgaris*. Recombinant plasmid DNA of clones that gave strong hybridization signals was prepared using the rapid alkaline extraction method (Birnboim and Doly 1979).

Sequence analysis

The repetitive inserts were sequenced by the dideoxynucleotide chain termination procedure (Sambrook et al. 1989) using ^{35}S-dATP and the M13 universal primer.

Radioactive labelling and Southern blot hybridization

Recombinant plasmids were ^{32}P-labelled by the random primer labelling technique (Feinberg and Vogelstein 1983). For preparation of genomic Southern filters total DNA was completely digested and fractionated by gel electrophoresis on horizontal agarose gels. DNA fragments were transferred onto nylon membranes (PALL). Hybridization with repetitive sequences was carried out at 65 °C overnight according to Sambrook et al. (1989). Filters were washed for 20 min in 2 × SSC/0.1% SDS at the same temperature.

Results

Cloning, genomic organization, and sequence analysis of sugar beet satellite DNA

Total DNA from cultivars and wild species of the genus *Beta* was digested to completion with BamHI and EcoRI, respectively, and separated on 1.3% agarose gels. The resulting restriction patterns of all species of the section *Beta* show the typical ladder pattern of tandemly repeated sequences.

The cloning of the EcoRI monomer sequence of *B. vulgaris* resulted in recombinant M13 phages designated pEV1, pEV2, pEV3, etc.. The cloned monomer pBV1 of the BamHI sequence family (Schmidt and Metzlaff 1991) and the EcoRI basic repeat pEV1 were used in all further experiments. To investigate the genomic organization of both satellite DNAs the labelled plasmids pEV1 and pBV1 were hybridized to genomic Southern blots of EcoRI, BamHI, and double-digested sugar beet DNA, respectively. When pBV1 is used as probe, a ladder pattern is present both in the BamHI and in the double-digestion. From this we assume that the BamHI repeats are organized in tandemly arranged clusters. A ladder of bands is also visible in the high molecular range of the EcoRI digestion indicating the existence of a fraction of

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A
          10          20          30          40          50          60
GGATCCACAC AAACCTCTAT TTGTAAGTTC AATGCAACGA ACTCGAATTG TTAAATTAAA

          70          80          90         100         110         120
ATACACAAAC AGTTGATTTG AGGGAATTAG ACTTACAAAC ATAATAGGAC CCCCATATCA

         130         140         150         160         170         180
TTAAAAAACA AAAAAAAAAA ATTATAAAGC AACTGATGTG TTTGTGGATA GTAAAATCAA

         190         200         210         220         230         240
TAACAAGTCT CAGAACACTC GAATCTCACT TCTACTTGTT GAAAGATTCG ATCTTTATAT

         250         260         270         280         290         300
GAAATAGGGC ATAAAGATTG AATCTTTATA TTGAATAGGC TTTGTTGTTT GCTCGAGAAC

         310         320
TAGAGGATCT TTCATTAACC TCTATTA

B
          10          20          30          40          50          60
GAATTCGTTA AAGTTCGATT TTTTGCGTCT GTGTCGTGCT CATCTTGGCC CAAACTCGTA

          70          80          90         100         110         120
ATTTAGGCCT AAATCCTGCT CGGACATCAG AACTGATCTA TTCAAAAGCA TGAAATGTTT

         130         140         150         160
TTTTTTTTAC CATTTCTAAG AGGTTCGTGA GCTCAAATCT
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Fig. 2A, B. Nucleotide sequences of the BamHI (**A**) and EcoRI (**B**) monomer of satellite DNAs of *B. vulgaris*

this satellite DNA family that has emerged EcoRI recognition sites. The pBV1 sequence also hybridized to BamHI relic and EcoRI relic DNA (Bedbrook et al. 1980) suggesting either methylation or sequence divergence of the concerned recognition sites (Fig. 1 A).

However, after hybridization of the pEV1 probe to the same genomic filter, strong signals were found in the high molecular weight and relic DNA of the BamHI digestion, clearly demonstrating that this cloned EcoRI repeat belongs to a different type of satellite DNA. A ladder of repeats with a monomeric unit of 160 bp can be observed after hybridization to EcoRI and double-digested sugar beet DNA. Fragments up to 15 mers of the pEV1 repeat were detected in EcoRI digestions even after incubation with an excess of restriction enzyme. This means that at least 15 repeats are tandemly arranged (Fig. 1 B). The chain termination procedure was used to sequence several repeats from both sugar beet satellite DNA families. Figure 2 presents the sequences of the repetitive inserts of pEV1 and pBV1 in which there are some interesting structural features. Both sequences contain a high number of AT-residues, with AT contents of 61.9% and 69.1%, respectively. The analyzed EcoRI monomers have a repeat length of 157–160 bp, whereas the length of the repeats of the BamHI satellite DNA is 327–328 bp. All repeats show sequence divergence mostly due to randomly distributed point mutations. An inverted repeat of 7 bp exists in all pEV clones at position 61–74. The 27-bp duplication of two imperfect inverted repeats in pBV1 and the assumed putative precursor sequence have been described in a previous publication (Schmidt and Metzlaff 1991).

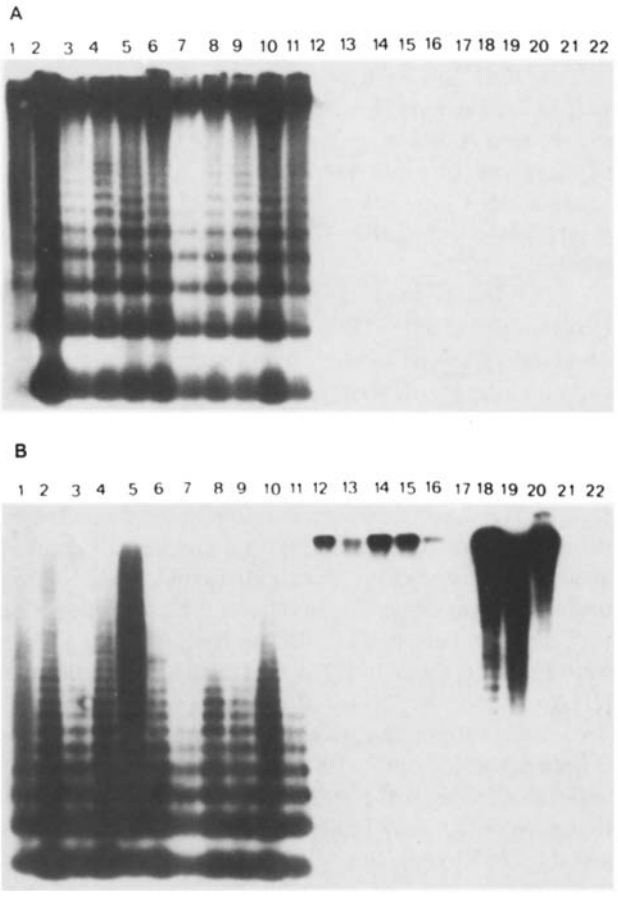

Fig. 3A, B. Distribution of different satellite DNA types in the genus *Beta* and related species. DNA of *1 B. vulgaris* cv *crassa*, *2 B. vulgaris* cv *altissima*, *3 B. vulgaris* cv *cicla*, *4 B. vulgaris* subsp. *orientalis*, *5 B. atriplicifolia*, *6 B. patula*, *7 B. vulgaris* subsp. *maritima*, *8 B. vulgaris* subsp. *maritima*, *9 B. vulgaris* subsp. *maritima*, *10 B. vulgaris* cv *flavescens*, *11 B. vulgaris* cv *conditiva*, *12 B. corolliflora*, *13 B. lomatogona*, *14 B. trigyna*, *15 B. macrorhiza*, *16 B. intermedia*, *17 B. nana*, *18 B. patellaris*, *19 B. webbiana*, *20 B. procumbens*, *21 S. oleracea*, *22 C. bonus-henricus* was digested with BamHI (**A**) and EcoRI (**B**), electrophoresed, and transferred onto nylon membranes. The filters were hybridized with pBV1 (**A**) and pEV1 (**B**).

The repeating units of both satellite DNA families do not show any significant homology to one another. From the results of Northern experiments it can be concluded that the members of both satellite DNA families are noncoding (results not shown).

Distribution of the EcoRI and BamHI satellite DNA in the genus Beta

For investigating the distribution of both satellite DNAs in the genus we used pBV1 and pEV1 to probe genomic Southern filters of digested total DNAs of 18 *Beta* species (20 origins) and two releated species.

When the monomer pBV1 of the BamHI DNA sequence family was hybridized to BamHI-digested DNA,

the typical ladder pattern was once again observed (Fig. 3A). Hybridization occurs only with species of the section *Beta*. Strong hybridization is also found in the relic DNA. No hybridization was detected in the remaining sections (*Corollinae, Nanae, Procumbentes*) nor in *S. oleracea* and *C. bonus-henricus* which suggests that this satellite DNA type either does not exist or exists only in a very low copy number in the nuclear genome of these species.

After the labelling and hybridization of pEV1 to EcoRI-digested total DNA a different distribution was observed. The satellite-like hybridization pattern indicates that the EcoRI monomer sequence is also tandemly organized in all species of the section *Beta*. However, hybridization was also found in two other sections of the genus. Hybridization signals in the five species of the section *Corollinae* that were examined were restricted to the relic DNA, demonstrating that the pEV1 repeat is arranged in large clusters without EcoRI sites. No hybridization was detectable in *B. nana*, the only species of the section *Nanae* studied. Strong hybridization signals were found in the relic DNA and in the high molecular DNA of the three species of the section *Procumbentes*. The observed banding pattern in *B. procumbens* is to a certain extent identical with the hybridization in the high molecular range of the section *Beta*. It can be assumed that most of the EcoRI repeats in the *Procumbentes* have lost the EcoRI sites; this would result in the DNA fragments of higher molecular weight that were detected. No hybridization was observed in *S. oleracea* and C. *bonus-henricus*.

Discussion

In this paper we describe the genomic organization and sequence of two different types of satellite DNA in *B. vulgaris* and their distribution in the genus *Beta*.

Because of the high copy number of both sequences in the section *Beta* it is possible to detect the typical ladder pattern after ethidium bromide staining of electrophoretically separated digestions of total DNA. The BamHI and EcoRI sequence families do not show any significant homology to each other, which suggests that no evolutionary relationship exists between both sequences. Therefore, we assume that both satellites have evolved from different ancestral sequences. The occurrence of different non-homologeous satellite DNA sequences have been also described in *Arabidopsis thaliana* (Martinez-Zapater et al. 1986; Simeons et al. 1988) and in the genus *Brassica* (Hallden et al. 1987, Gupta et al. 1990), whereas the existence of related satellite DNA families has been reported for *Cucumis sativus* (Ganal et al. 1986; Ganal and Hemleben 1988). With a length of 160 bp and 327 bp for the EcoRI and BamHI monomer, respectively, both sequences agree with the observed preferred repeat size of approximately 160–180 bp (Grellet et al. 1986; Simeons et al. 1988; Schweizer et al. 1988) and 350–370 bp (Leclerc and Siegel 1987, Ganal and Hemleben 1988). This size seems to be characteristic for plant satellite DNA.

Both satellite DNA sequences are specific to the genus *Beta* since no hybridization was observed in the related species *S. oleracea* and *C. bonus-henricus*. The BamHI repeat is present only in section *Beta*. It is noteworthy that this satellite DNA sequence was not found in the section *Corollinae*. The *Corollinae* are relatively closely related to the species of the section *Beta* as revealed by phylogenetical analysis of plastid DNA (Fritzsche et al. 1987). Therefore, we assume that the BamHI sequence family is relatively young and that their amplification occurred after the separation of the sections *Beta* and *Corollinae*.

In contrast, the EcoRI satellite DNA sequence of *B. vulgaris* shows a different distribution in the genus. It seems to be older because it is found in three of four sections (*Beta, Corollinae, Procumbentes*) of the genus. We assume that the ancestral sequence of the EcoRI sequence family was already present in the progenitor of all *Beta* species. After separation of the four sections of the genus the amplification of the ancestor took place in a different manner in each section, resulting in the described genomic organization and in different copy numbers. However, the absence of the EcoRI satellite in the only species of the section *Nanae, B. nana*, clearly demonstrates that the amplification of the precursor sequence probably did not take place to the same extent.

The genomic organization of the EcoRI satellite DNA is similar in all species of one specific section with respect to the remaining sections of the genus. Thus, hybridization experiments with the EcoRI repeat can contribute to the resolving of questions concerning *Beta* taxonomy on a molecular level.

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