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Characterization of the nuclear ribosomal DNA units and phylogeny of Beta L. wild forms and cultivated beets *

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Summary. The nuclear rDNA units of species belonging to the genus *Beta* were characterized using heterologous probes of flax (entire unit and 25S) and sunflower (6.1-kb *Eco* fragment containing the 18S, the entire intergenic spacer (IGS) and a small piece of the 25S). The physical maps of one species from each section of the genus was constructed by localization of the *Eco*RI, *Bam*HI, *Hind*III, *Kpn*I and *Sac*I restriction sites. For each species a single individual was used to obtain total DNA. The major unit length is 11 kb, but variant length units at 10.4, 10.7 and 11.3 kb were found as minor forms. However, some individuals carried the 10.4-kb or the 10.7-kb variant length unit as the major form. For the variant length units of one species the restriction sites were conserved, so that the variation in length occurred in the IGS. The *Eco*RI fragment corresponding to the intergenic spacer appeared to be the best indicator of variation. The variable sequence in the IGS sometimes generated new restriction sites for the *Corollinae* and mainly, did so, for the *Vulgares* relative to the *Procumbentes*. The variable sites were able, to differentiate the three sections and species within the sections. *Corollinae* species belong to two different groups according to the absence or the presence of the *Bam*HI (B4) site. The *Vulgares* species contain several unit types. We proposed that all the unit types derived from a unique unit, V-11-2.3, by unequal crossing-overs or conversion. We also supposed a homogenization mechanism because we found individuals homogeneous for every unit type. Among the cultivated beets, all the root beets contain only one rDNA unit type, V-11-2.9. Thus, we supposed that the common unit type of cultivated beets either brings a physiological advantage or is strictly linked to a favorable allele. It is likely that the rDNA unit of *B. maritima* were eliminated from sugar beet by the breeding process since they were not recovered. Whatever the process, we deduced that all the cultivated forms of beets likely originated in a unique plant ascendant.

A phylogenic tree of the genus is proposed, based on the nuclear rDNA maps, and subsequently discussed relative to the systematic tree and other molecular phylogenies.

Key words: *Beta* – Nuclear rDNA unit – Variability – Intergenic spacer (IGS)

Introduction

The cultivated beets are important crops, either for industrial purposes (sugar beet) or as fodder for, animals (fodder beet). The table beet and Swiss chard are used as vegetables worldwide. The systematics of genus *Beta* is still evolving since several sections may be acknowledged, to subdivide the genus (for review see Stanescu (1990)). The most developed classification by Frese and Van Hintum (1989) subdivided the *Beta* genus into 15 species over four sections.

Recently, several attempts to clarify the systematics have been made by studying organelle DNAs. Chloroplast DNA enables phylogenetic relationships between the species and the sections to be established (Fritzche et al. 1987; Bonavent et al. 1989). The mitochondrial DNA evolution has appeared too fast to be used in such a phylogenetic tree. In contrast, mitochondrial DNA can

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be used to differentiate closely related cultivars or varieties (Boutin et al. 1987).

A first assay of nuclear gene variability was carried out by Nagamine et al. (1989). However, as yet no attempt to assay the nuclear molecular evolution of the *Beta* species has been made, even though sugar beet is interesting material, to molecular biologists because of comprehensive breeding programs to disease resistance and technology.

rRNA genes are generally arranged in long tandem repeats. It is not known for sugar beet or any other beet species just how many chromosomes carry rRNA genes, but Ingle and al. (1975) estimated the number of genes to be about 1,000. To initiate our work we used heterologous probes from flax and sunflower. We studied the organization of the nuclear ribosomal RNA genes (rDNA) because, first, no *Chenopodiacae* has been studied until now (Rogers and Bendich 1987), and secondly, the variation expected for the rDNA organization has frequently and successfully been used to build up molecular phylogenetic trees fitting with the taxonomic trees (Appels et al. 1980; Appels and Dvorak 1982; Choumane and Heizmann 1988). In this work the ribosomal DNA physical maps of most of the species of three sections have been defined. We studied the structural variations of the unit among other species of the sections and among cultivated forms. We constructed a phylogenic tree of the genus, which we compared with the taxonomies proposed by Barocka (1966) and Bosemark (1969) and the phylogeny proposed by Fritzche et al. (1987).

Material and methods

Plant material

Plant material was obtained from the collection of the Beet Breeding laboratory of Dijon. When certain species or accessions were not available, we used dry seeds to prepare the DNA. Wild species of the *Procumbentes* section and the *Corollinae* section were obtained from Dr. Mesken, IVT Wageningen. The *Vulgares* section was largely represented by several accessions or ecotypes per species. The cultivated forms of table beet and Swiss chard were obtained from private companies. All the plant sources are listed in Table 1. The plants were grown in a greenhouse on a 20 °C day/15 °C night cycle. Leaves were picked from an at least 3-month-old individual plant; these were then either rinsed with cold water before freezing for conservation at −20 °C or below or were ground immediately.

Total DNA preparation

Total DNA was prepared according to Dellaporta et al. (1983) with the following modifications. The leaves (5–50 g) were frozen in liquid nitrogen and then ground until a fine homogeneous powder was obtained. The homogenization buffer TEN (50 m*M* TRIS HCl pH 8, 25 m*M* EDTA Na_2, 0.6 *M* NaCl) plus 1.5% (w/v) SDS, 1% (w/v) Sarkosyl, 1% (v/v) Diethylpyrocarbonate (DEPC) and 1% (v/v) Beta mercaptoethanolamine was then added (2.5 ml/g fresh weight), and the homogenate was incubated at 65 °C for 10 min, after which it was cooled in ice for 30 min. Cold potassium acetate was added to the homogenate to a final concentration of 1.25 *M* and then mixed gently by inversion. The proteins were eliminated at 8,000 *g* for 30 min at 4 °C. The supernatant was further deproteinized by the chloroform phenol treatment. Solid CsCl was added to the last homogenate to a final refractive index of 1.392 at 20 °C. The final concentration of ethidium bromide was adjusted to 5 µg/ml. After ultracentrifugation in the TFT 80.13 rotor (Kontron) at 65,000 RPM at 20 °C for 20 h, the DNA band was withdrawn and diluted twice in water. The DNA was precipitated with ethanol and resuspended in TE (5 µg DNA/10 µl TE) for further use. For the preparation of DNA from dry seeds we used the method described by Santoni et al. (1991) for 10 g of seeds, i.e., 300 seeds. The DNAs were further CsCl purified as those from leaves.

Total DNA was restricted with ten units of enzymes per microgram DNA at 37 °C for 2.5 h according to the manufacturer's conditions. Submarine agarose (0.5–1.5%) electrophoresis took place in TAE (Maniatis et al. 1982). The DNAs were transferred to nylon membranes (Amersham, N^+) according to Southern (1975).

Probes

As heterologous probes, we used the 6.1-kb *Eco*RI fragment from sunflower (6.1-kb probe) kindly provided by P. Heizmann (Choumane and Heizmann 1988). It carries an almost complete 18S fragment, the IGS and a small part of the 25S fragment. A sub-fragment *Bam*HI - *Eco*RI of 1,050 bp corresponds to the coding sequence of the 18S fragment. We also used the entire flax rDNA unit pBG35, (Goldsbrough and Cullis 1981). A subclone of the unit corresponds to a part of the 25S fragment (pRH 83). They were kindly provided by N. Ellis. The probes were prepared from the squeeze-freeze method of Maniatis et al. (1982) in order to obtain inserts free of most of the cloning vector.

Hybridization conditions

Each probe (25 ng) was labelled using the Random Primed DNA labelling kit of Boehringer with 20 µCi ^{32}PαdCTP (3,000 Ci/mmol). The nylon membranes (Amersham N^+) 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.

Estimation of genetic distances

For the estimation of genetic distances, we used the method described by Nei and Li (1979). The phylogenic tree was constructed according to the UPGMA method (Sneath and Sokal 1973).

Results

Vulgares section

I. Construction of physical maps

1. Beta vulgaris sugar beet (Figs. 1 and 2). The enzymes *Kpn*I or *Hind*III led to a unique fragment of 11 kb whatever probe was used. Therefore, the unit type length is 11 kb. With *Eco*RI, fragments of 4.4, 3.7, and 2.9 kb were hybridized with the entire unit as a probe. The 2.9-kb fragment was recognized as corresponding to the IGS. This unit type is further designated as V-11-2.9 (V

Table 1. List of the plant material. The numbers refer to the INRA collection. The origin refers to Institutes and Botanical gardens where the seeds were obtained or to persons who collected the seeds during prospect

Section	Species or variety	Author	Reference or common name	Origin
Procumbentes	*B. procumbens*	Chr. & Sm.	H 2148	Rosenhof, Germany
	B. patellaris	Moq.	F 3977	Rosenhof, Germany
	B. webbiana	Moq.	F 3981	Rosenhof, Germany
Corollinae	*B. corolliflora*	Zoss.	WB 12	East Germany
	B. trigyna	Waldst.	WB 8	Turkish
	B. lomatogona	Fisch.	G 2362	Beltsville, MD
	B. intermedia	Bunge.	F 3971	Turkish
	B. macrorhyza	Stev.	G 2365	Turkish
Vulgares	*B. maritima* var 'maritima'	Arcang.	H 2151	INRA Colmar, France
			F 3997	INRA Dijon, France
			F 4006	INRA Dijon, France
			F 4007	INRA Dijon, France
			F 4008	INRA Dijon, France
			F 4009	INRA Dijon, France
			F 4010	INRA Dijon, France
			L 1000	Lille, France
	B. maritima var 'atriplicifolia'	Rouy	F 3968	Leningrad, SSSR
	B. maritima var 'macrocarpa'	Guss.	F 3957	INRA Dijon, France
			F 3958	INRA Dijon, France
	B. orientalis	Roth	H 2145	Leningrad, SSSR
	B. cicla var 'cicla' (Swiss chard)	Linn.	BL 63 Poirée à carde rouge	BL, The Netherland
			TZ 38501 Swiss chard spinach	Tézier, France
	B. cicla var 'flavescens' (Swiss chard)	Linn.	TZ 31509 Poirée verte à carde blanche	Tézier, France
			TZ 38514 Poirée blanche à carde blanche	Tézier, France
	B. vulgaris var 'conditiva' (table beet)	Linn.	TZ 31509 Plate d'Egypte	Tézier, France
			TZ 31527 Globe Lorette	Tézier, France
	B. vulgaris var 'crassa' (fodder beet)	Linn.	I 1706	INRA Le Rheu France
	B. vulgaris var 'altissima' (sugar beet)	Linn.	059	INRA Dijon, France
			59	INRA Dijon, France
			E5039	INRA Dijon, France
			01012	INRA Dijon, France
			Marina	Maribo, Danmark
			Corum F 3904	Desprez, Turkish
			F 3905	Desprez, Poland
			F 3906	Desprez, China

for *Vulgares*) according to its length and the *Eco*RI fragment in the IGS. Several minor bands of 3.5 and 3.8 kb were obtained with *Eco*RI. These corresponded to minor variant length units of 11.6 and 11.9 kb respectively, and were subsequently designated as V-11.6-3,5 and V-11.9-3.8. The *minor Eco*RI fragment of 2.6 kb corresponds to a 11-kb unit type. Consequently, we supposed that a new *Eco*RI site (E4) appeared 0.3 kb away from site E3 which defined the 2.9 kb fragment (Fig. 4). The unit type was named V-11-2.6.

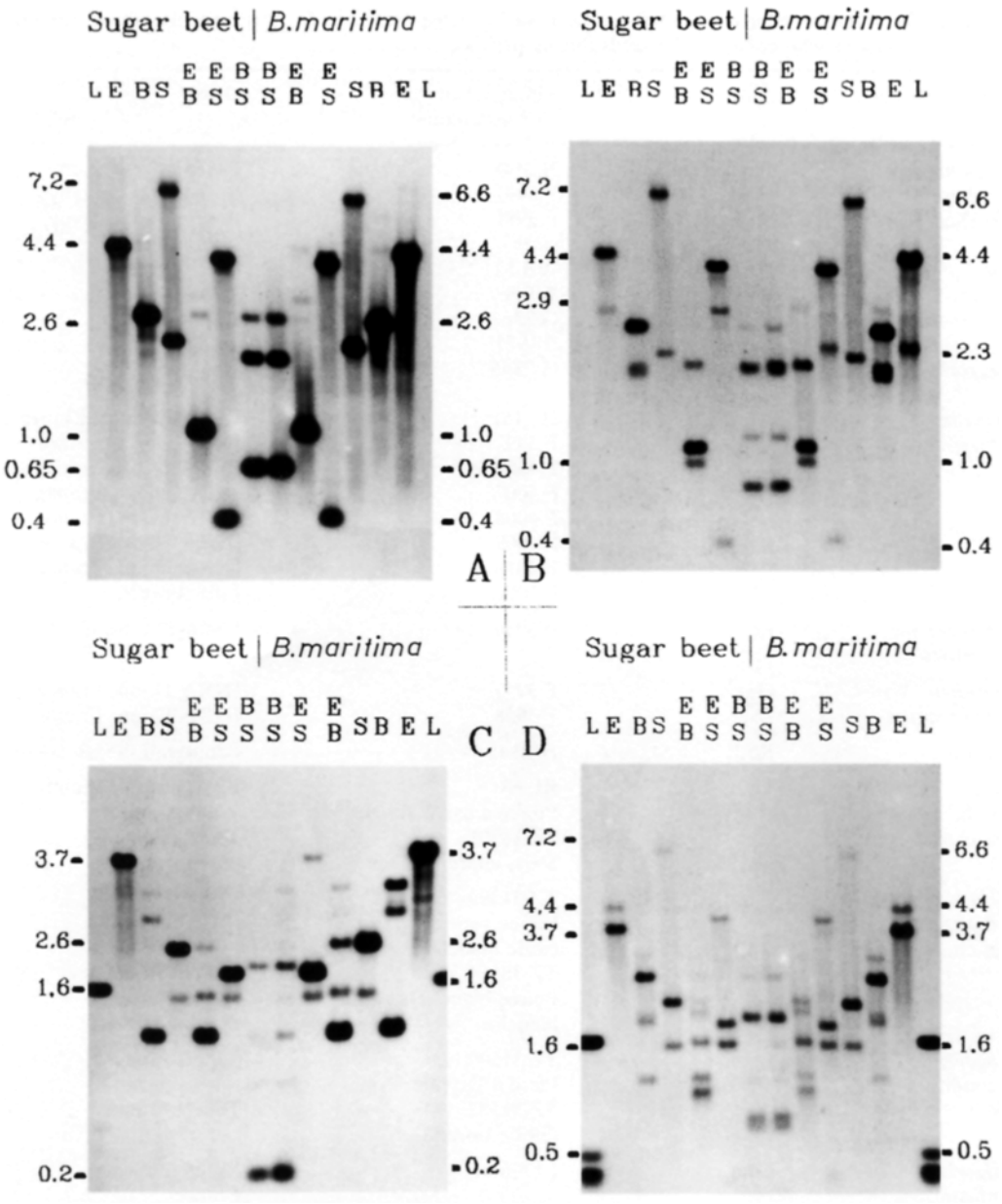

Fig. 1 A–D. Hybridization patterns of restricted nuclear DNA from *Beta maritima* and the sugar beet 059 with the 18S 1050 EB probe (**A**), the 6.1-kb probe (**B**), the 25S pRH83 probe (**C**), the flax entire unit pBG35 probe (**D**). *Eco*RI, *B Bam*HI, *H Hin*dIII, *K Kpn*I, *S Sac*I. L 1-kb ladder from B.R.L.

2. Beta maritima (Figs. 1 and 2). Several individuals from various accessions displayed variations in rDNA unit types.

– Accession F 3968: with *Hind*III or *Kpn*I, a unique fragment of 10.4 kb was hybridized with each of the probes. With *Eco*RI, the 6.1-kb probe hybridized fragments of 4.4 and 2.3 kb. Therefore, this individual only carried one unit type 10.4 kb long. It was named V-10.4-2.3.

– Accession F 3997: with *Hind*III and *Kpn*I, two fragments of 11 and 10.4 kb were hybridized with the entire unit as a probe. With *Eco*RI, the probe hybridized fragments of 4.4 and 2.3 kb. Therefore, two unit types were present in equal proportion, each characterized by the 2.3-kb *Eco*RI fragment in the IGS. The first unit type is V-11-2.3, while the second type is V-10.4-2.3. With respect to V-11-2.9, the unit was likely obtained because of a new *Eco*RI site (E5) 0.6 kb away from site E3 and

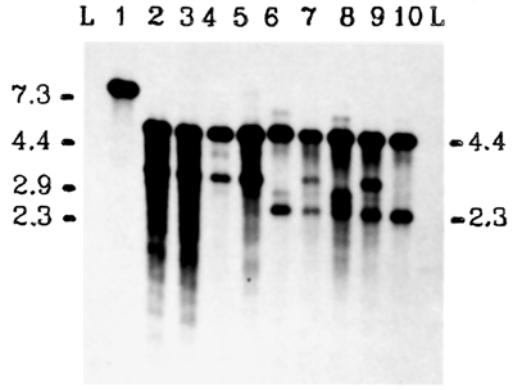

Fig. 2. Hybridization patterns of *Eco*RI-restricted DNA of *Beta* species with the 6.1 *E-E* kb probe. *Lane 1 B. patellaris*, *lane 2 B. macrocarpa* F 3957, *lane 3 B. macrocarpa* F 3958, – *B. vulgaris* sugar beet, *lane 4* 01012, *lane 5* 059, – *B. maritima*, *lane 6* F 4010, *lane 7* F 4009, *lane 8* F 4006, *lane 9* F 4007, *lane 10* F 3997, *lane* L 1-kb from B.R.L.

0.3 kb away from site E4. Consequently, we supposed that the number of sub-repeats between E3 and E5 had been increased by steps of 300 bp (Rogers and Bendich 1987), while for the V-10.4-2.3 unit, a deletion of two sub-repeats likely occured.

– Accession F 4008: with *Eco*RI, two fragments of 2.6 and 2.3 kb were hybridized with the 6.1-*E-E* kb as a probe. Each fragment characterizes one unit type. With *Hind*III and *Kpn*I, two fragments of 11 and 10.4 kb were hybridized with the entire unit as a probe. Therefore, two unit types were present in equal proportions: the V-11-2.6 type already found and the V-10.4-2.3 type.

II. Variability in the *Vulgares* section (Fig. 2, Table 2)

A large sample of beet varieties and old sugar beet varieties was studied. Most of the RFLP were due to variant length unit types revealed with *Eco*RI, *Hind*III, *Kpn*I and

Table 2. rDNA unit types, accessions, varieties and wild and cultivated forms of *Vulgares*

B. vulgaris		Accessions unit types			
		V-11-2.9	V-11-2.6	V-11-2.3	V10.4-2.3
ssp *vulgaris* var Sugar beet	059	++	–	–	–
	59	++	–	–	–
	E 5039	++	–	–	–
	01012	++	–	–	–
	Marina	++	–	–	–
	F 3904	++	–	–	–
	F 3905	++	–	–	–
	F 3906	++	–	–	–
var Table beet	TZ 31509	++	–	–	–
	TZ 31527	++	–	–	–
var Forage beet	J 1706	++	–	–	–
ssp *cicla*	BL 63	+	+	–	–
var Swiss chard	TZ 38501	+	+	–	–
	TZ 38510	+	+	–	–
	TZ 38514	+	–	+	–
ssp *orientalis*	H 2145	+	–	+	–
ssp *maritima*	H 2151	+	–	+	–
	F 3997	–	–	+	+
	F 4006	–	+	–	+
var 'maritima'	F 4007	+	–	–	+
	F 4008	–	+	–	+
	F 4009	+	–	–	+
	F 4010	–	–	–	++
	L 1000	–	–	–	++
ssp *macrocarpa*	F 3957	++	–	–	–
	F 3958	++	–	–	–
ssp *atriplicifolia*	F 3968	–	–	–	++

+: One of the main types; ++: only type present

*Sac*I with the 6.1-kb probe. Among the cultivated beets, the varieties of sugar beet, fodder beet and table beet plus the old varieties of sugar beet all contained only the V-11-2.9 unit type. All of the Swiss chards studied four carried two unit types: three of them contained the V-11-2.9 and the V-11-2.6 while the other one contained the V-11-2.9 and the V-11-2.3 unit type. The situation for the accessions corresponding to the wild forms is much more complex since for the 12 wild accessions studied we found that most of the individuals carried two unit types in equal proportion: three accessions contained the V-10.4-2.3 unit type; the two *B. macrocarpa* were homogeneous for the V-11-2.9; one accession contained the V-11-2.9 and the V-11-2.3 types; two accessions contained the V-11-2.9 and the V-10.4.-2.3; two accessions contained the V-11-2.6 and the V-10.4-2.3; one accession contained the V-11-2.3 and the V-10.4-2.3.

We looked at the controlled crosses between *B. maritima* Lille × 059. The hybrids displayed all of the bands present in each parent. The 2.9-kb band and the 2.3-kb band appeared in equal proportions in the hybrids. Consequently, when the beets contained two unit types in equal proportion we were able to suppose that they were hybrids between two individuals, each carrying one type of the units.

Corollinae section

I. Construction of physical maps

1. Beta corolliflora (Figs. 3 and 4). The *Hind*III enzymes led to one fragment of 11 kb, while *Kpn*I led to two fragments of 9.5 and 2 kb when hybridized with the 6.1-kb probe. The 25S probe hybridized with the 9.5-kb fragment. In this species we considered the length unit to be conserved but that a new *Kpn*I site (K2) is present 2 kb away from the one named K1 in *B. vulgaris*. The other restriction sites were located on the basis of the fragment sizes observed through single or double digests probed whith the set of probes (not shown).

Compared with *B. vulgaris*, the *Eco*RI site (E4) and the two *Bam*HI sites (B4, B5) were conserved in the unit. The *Hind*III site (H2) and the *Kpn*I site (K2), however, were unique to the *Corollinae* (Fig. 4). Minor *Eco*RI fragments hybridized by the 6.1-kb probe corresponded to minor unit types of the same length, 11 kb. The minor unit types were named C-11-2.3 and C-11-2.45, while the common unit type was C-11-2.6.

II. Variability in the *Corollinae* section (Figs. 3 and 4)

*1. Beta trigyna, Beta macrorhyza. Eco*RI, *Kpn*I and *Bam*HI led to hybridization patterns identical to those of *B. corolliflora* with the 6.1 kb as a probe. Consequently, we could not reveal any RFLP between the three species.

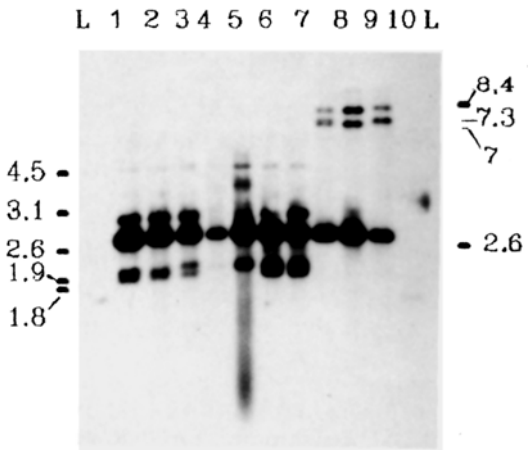

Fig. 3. Hybridization patterns of *Bam*HI-restricted nuclear DNA of *Beta* species with the 6.1-kb probe. *Lane 1 B. maritima* F 4008, *lane 2 B. vulgaris* sugar beet 059, *lane 3 B. macrorhyza, B. lomatogona, lane 5 B. intermedia, lane 6 B. maritima* H 2151, *lane 7 B. vulgaris* table beet TZ 31509, *lane 8 B. procumbens, lane 9 B. webbiana, lane 10 B. patellaris, lane* L 1-kb ladder from B.R.L.

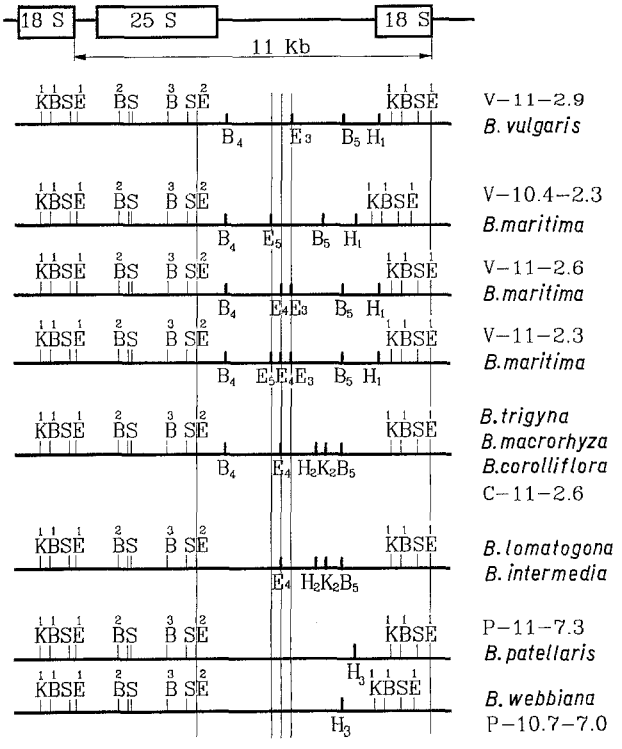

Fig. 4. rDNA physical maps of the *Beta* species. *E Eco*RI, *B Bam*HI, *H Hind*III, *K Kpn*I, *S Sac*I. B1 and B3 sites were often partially hydrolyzed; therefore fragments corresponding to the sum of shorter fragments were detected

2. Beta lomatogona and Beta intermedia (Figs. 3 and 4). The hybridiziation patterns were identical to those of *B. corolliflora* with *Eco*RI and *Kpn*I. With *Bam*HI, the pattern of fragment sizes looked different from the one of *B. corolliflora*. We deduced that the *Bam*HI site B4 in the IGS was absent in *B. lomatogona* and *B. intermedia*.

Section Procumbentes

I. Construction of physical maps

1. B. patellaris (Figs. 3 and 4). The *Kpn*I or *Hind*III enzymes led to a unique fragment of 11 kb whatever probe was used. Therefore, the unit was only cut once by *Kpn*I. The sites were established relative to K1, and the new site (H3), respectively. The sites of other restriction enzymes, *Bam*HI, *Sac*I and *Eco*RI, were located on the basis of the hybridization experiments with the set of probes. Single and double digests enabled us to locate the restriction sites relative to each other (Fig. 4).

II. Variability in the *Procumbentes* section (Figs. 3 and 4)

1. Beta webbiana. Results were obtained with DNAs restricted with *Bam*HI, *Hind*III and *Eco*RI and hybridized with the 6.1-*E-E* kb probe. A 10.7-kb-long unit was constructed according to the data. The plant was homogeneous for this unit type length. The other restriction sites – *Kpn*I, *Sac*I and *Hind*III – were conserved relative to *B. patellaris.*

2. Beta procumbens. The probes hybridized fragments corresponding to variant length units of 11 and 10.7 kb present in one individual. Moreover, as the hybridization signals were equivalent, the unit quantities were supposed to be equivalent.

According to the types of rDNA physical maps we can summarize the main features:

- the entire IGS fragment was not cleft by *Bam*HI for the *Procumbentes*;
- the second *Kpn*I site (K2) appeared to be unique to the *Corollinae*;
- in the *Vulgares*, *Eco*RI revealed a wide polymorphism.

Discussion

Because of the lack of green plants from some species – *B. lomatogona*, *B. macrorhyza*, *B. intermedia* – and the sugar beet E 5039, we prepared the DNA from about 300 dry seeds. The hybridization profiles of E 5039 resembled those of other sugar beets. We also compared the hybridization profiles obtained from leaves and dry seeds for line 059: they looked identical to those of one individual. Consequently, we concluded that the profile from one individual is representative of most of the unit types of the variety, and, further, that profile can be considered to be representative of the species.

In the *Vulgares* section we demonstrated the presence of four unit types organized in long homogeneous arrays. One diploïd individual carries either one or two types. Therefore, we suggest that there is only one pair of chromosomes carrying the long arrays. Progenies were not available to us at the time to verify our hypothesis, but cytological studies have already verified that there is only one nucleolar pair of chromosomes, (Bosemark and Bormotov 1971).

By means of two different levels of mechanisms we were able to explain the presence of different unit types and their homogeneity in the array. The mechanism leading to the variation in sub-repeats in the IGS of both wild forms and cultivated beets is still functional, since for all of the accessions several minor bands, over and above the one or two major unit types, were revealed after a long exposure only. For one individual we estimated that all the variant unit types represented less than 5% of the total rDNA units.

We observed a variability in the unit types of wild beets and cultivated ones. The variant length units always carry all the restriction sites of the conserved regions, but they vary in length in the IGS. Such a variation in the number of tandem sub-repeats has been commonly observed between individuals in the progenies of crosses (Rogers and Bendich 1987). Because of possible rearrangements in the number of sub-repeats in the IGS, we wondered whether or not all these unit types could be generated from a unique unit type. Since the *Procumbentes* and the *Corollinae* carry a 11-kb unit type and since the *Eco*RI site (E3) already existed for the *Corollinae*, we supposed that the V-11-2.6 unit type could be the candidate unit leading the other unit types (Fig. 5). Because of the 0.3 kb difference between the unit types we supposed that the IGS contained sub-repeats of 300 bp. Once the *Eco*RI site (E3) appeared in one of the sub-repeats, we were able to explain other *Eco*RI sites by conversion events conserving the same length of the unit type. Unequal crossing-overs between types of tandem arrays produced rDNA repeat units of different lengths carrying several *Eco*RI sites (E3, E4, E5) in the IGS (Fig. 5). The possible unit length and types produced can be verified according to this model to correspond to those found in the *Vulgares*. Nevertheless, unit types other than those we found could be predicted. We probably did not find them because of the low number of individuals we studied. Furthermore, we suppose that a homogenization process acted to make the tandem arrays homogeneous for a unique unit type (Dover 1982). While we cannot speculate on such a mechanism, we did notice that it functioned in both wild beets and cultivated beets, since both appeared homogeneous for different unit types arranged in arrays. Each array of the major unit type should be due to a unique homogenization event. In particular the V-11-2.9 array type appeared preferably in the cultivated beets.

We observed that all of the beet varieties were devoid of the V-10.4-2.3 unit type although natural and controlled crosses occurred with *B. maritima.* In various, breeding programs, crosses have been done to improve the beet varieties. Consequently, since the V-10.4-2.3 unit

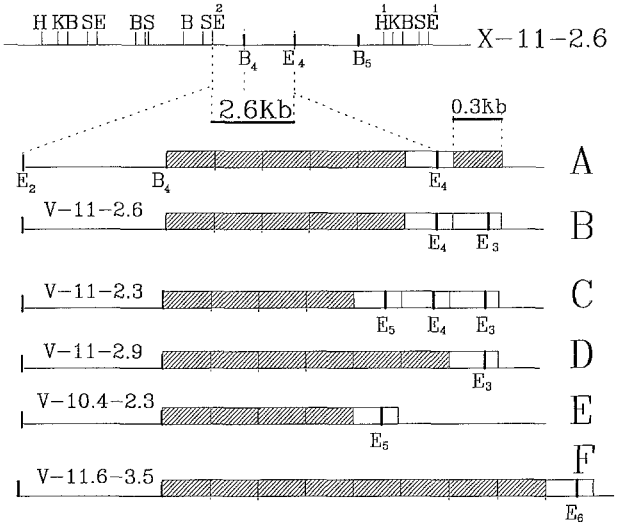

Fig. 5. Diagram for the possible origin of the different unit types found in section *Vulgares*. Unequal crossing-overs or conversion events at the sub-repeat level explain the unit types found in section *Vulgares*. The IGS of the A and B unit types were not differentiable by our technique. The IGS of the A type was found in section *Lomatogonae* and this one of the B type was found in section *Vulgares*. Therefore, it is logical to propose that the B type derived from the A type. Two types of sub-repeats are involved. Type I does not carry and *Eco*RI site, while type II does. *Line A:* the *Eco*RI site E4 appeared by mutation in the type I sub-repeat leading to the type II. *Line B:* a conversion event between two mispaired A units leads to the unit type B with E4 and E3. *Line C:* a conversion event between two mispaired B units types leads ot the C unit type. *Line D:* and opposite conversion event of the one in line C. leads to the unit type D. *Line E:* an asymetric crossing-over between two A units leads to the C units type. *Line F:* the reciprocal event corresponds to the F unit. Other unit type can be producted, but they were not found

type was not revealed in cultivated beets, we concluded that these unit types have been eliminated to a great extent during the breeding steps. However, we also suspected the V-11-2.9 unit type is advantageous in cultivated forms. This could be due to the unit type itself leading to physiological advantages, to genetic drift (founder effect) or to strong genetic linkage with a favorable allele under selection. The selection process for rDNA unit types has already been reported for maize (Rocheford et al. 1990), barley (Shagai-Maroof et al. 1984) and tomato (Levesque et al. 1990). During crosses the recombination events are inhibited in the rDNA arrays making abnormal the sorting out of the favorable allele which cosegregates with the rDNA type (Levesque et al. 1990). For beets, it is the V-11-2.9 type. In the Swiss chards, which have been improved for leaf production and not for root yield, we found three different major unit types. Recently, Fischer (1989) proposed that sugar beet originated in a cross between a Swiss chard and a table beet. We could argue for a specific advantage of the V-11-2.9 unit type linked to root physiology, since the V-11-2.6 and the V-11-2.3 unit types were also present in Swiss chards, but not in the other cultivated beets.

According to our model, the domestication process of beets involved two main events: the appearance of the favorable allele and the homogenization of the rDNA type in the same linkat. Consequently all of the cultivated beets are likely of a unique origin. Nevertheless, we also found that most of the *B. maritima* contain this major unit type. Our simplest explanation is that these individuals were cross-pollinated by cultivated beets. These plants belong to obligate outbreeding species, but the breeding process for sugar beet introduced the self-compatible allele system, which produces homozygosity. However, although table beet varieties are not self-compatible plants, they also appear to be homogeneous for the V-11-2.9 unit type, thus backing up the advantage of the unit type.

For the *Procumbentes*, the restriction sites were conserved in the variant length units of *B. patellaris* and *B. webbiana*. Therefore, we wondered whether or not the mechanism enabling length variation could be still functioning. The *B. procumbens* individual contained both unit types found in the two distinct species. Nevertheless, the cross between *B. Webbiana* and *B. patellaris* has been reported to be incompatible (Bosemark 1969). *B. patellaris* is polyploid (6 ×), while *B. webbiana* is diploid. Our results sustain that *B. patellaris* is autopolyploid but also that *B. webbiana* had been introgressed by the diploid ancestor of *B. patellaris*. The hybridization forms must have been recognized by the taxonomists as *B. procumbens*. Wagner et al. (1989) considered *B. procumbens* and *B. webbiana* to be two forms of the same species. With the hypothesis of frequent rearrangements of the sub-repeats of the *B. procumbens* IGS, the homogenization of the same variant length in *B. webbiana* and in *B. procumbens* has yet to be explained. Thus, the second explanation is less likely.

For the species belonging to the *Corollinae*, the absence of the *Bam*HI site (*B. lomatogona* and *B. intermedia*) or its presence (*B. corolliflora*, *B. trigyna* and *B. macrorhyza*) can be considered to be a major taxonomic difference. Thus, we will divide them further into two subsections. Since the other restriction sites E3 and B5 are present in both subsections and in the *Vulgares*, we considered that the appearance of the B4 site for *B. lomatogona* and *B. intermedia* had been followed by its disappearance. These events resembled those already considered to explain the variation of the sub-repeats for the IGS of the *Vulgares*.

The phylogenic tree of species belonging to the *Beta* genus is displayed in Fig. 6. The genetic distances between the three sections are similar. Each section is characterized by the restriction sites of the conserved rDNA regions. Therefore, the polymorphism due to the restric-

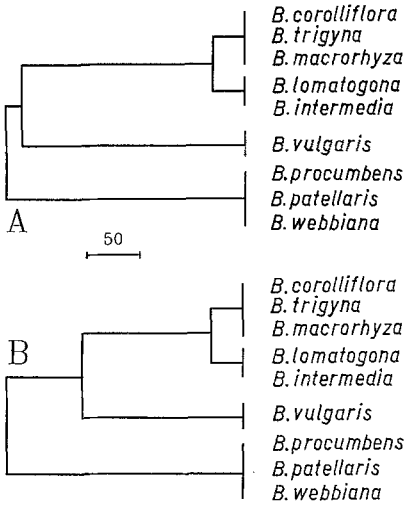

Fig. 6 A, B. Dendrogram of the species belonging to the *Beta* genus built up according to variation in the restriction sites *Eco*RI, *Bam*HI, *Hin*dIII and *Kpn*I in the nuclear rDNA unit types. **A** One *Eco*RI site in section *Vulgares*; **B** Two *Eco*RI sites. The scale corresponds to 50 substitutions per 10^5 nucleotides

tion sites appear to be more characteristic than the one on the unit length.

The three species belonging to the *Procumbentes* are very close since we did not observe any variation in the position of the restriction site. Thus, the phylum towards the two other sections diverged by the appearance of the *Kpn*I (K2) site. The phylum common to *Corollinae* and *Vulgares* diverges from the *Procumbentes* by the appearance of the E3 and B5 sites. The *Corollinae* appeared to be divided into two groups. This subdivision has already been maintained by Barocka (1966) on the basis of phenotypic traits only. More recently, Fritzche et al. (1987) established an equivalent division in the *Corollinae* based on variation in the chloroplast DNA. Furthermore, in the *Vulgares* section several *Eco*RI sites (E3, E4, E5) appeared in the IGS. The *Vulgares* appeared to be clearly divided into two groups: the wild forms and the cultivated ones. *B. macrocarpa*, a wild form, ranged into the cultivated ones, according to the rDNA unit types, but it could be due to an introgression. It is the first time that a unique molecular marker has given a clear difference between the wild species and the cultivated ones. Other molecular markers, plastid DNAs, (Fritzche et al. 1987; Bonavent et al. 1989) and mitochondrial DNAs do not allow such a subdivision to be made in the *Vulgares* (Mikami et al. 1985).

On the basis of the model of Nei and Li (1979) we first took into account the fact that the *Vulgares* originated in a form carrying the V-11-2.6 unit type (Fig. 6A). Then we considered that they originated in a form carrying the V-11-2.3 unit type (Fig. 6B). This did not any cause substantial modification of our conclusion, since only the length of the branches are modified but not the node of the trees (Fig. 6).

In conclusion, our study of the rDNA unit types of the *Beta* genus clearly shows that all the units were connected. The unit of *B. patellaris* is the simplest one; therefore it could be considered to be the ancestor unit. The three *Procumbentes* species are closely related and could correspond to one species only. The *Corollinae* appear to be subdivided into the *Lomatogonae* and the *Trigynae*, as established by Barocka (1966). The *Vulgares* are divided between the wild forms and the cultivated ones. Therefore, the introgression of wild forms into beets for the breeding process could be improved by following the V-11-2.9 rDNA unit type likely linked to a favorable allele under selection for the cultivated forms. The cultivated forms likely originated in a unique ancestor different from all of the present *B. maritima* studied.

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References

Appels R, Dvorak J (1982) The wheat ribosomal DNA spacer region: its structure and variation in populations and among species. Theor Appl Genet 63:337–348

Appels R, Gerlach WL, Dennis ES, Swift H, Peacock WJ (1980) Molecular and chromosomal organisation of DNA sequences coding for the ribosomal RNA in cereals. Chromosoma 78:293–311

Barocka KH (1966) Die Sektion Corollinae der Gattung (Tournef) L. Z Pflanzenzucht 56:379–388

Bonavent JF, Bessone L, Geny A, Bervillé A, Denizot JP, Brian C (1989) A possible origin for the sugar beet cytoplasmic male sterility source Owen. Genome 32:322–327

Bosemark NO (1969) Interspecific hybridization in *Beta*. J Inst Sugar beet Res. 4:1–12

Bosemark NO, Bormotov VE (1971) Chromosome morphology in a homozygous line of sugar beet. Hereditas 69:205–212

Boutin V, Pennenbecker G, Ecke W, Schewe G, Saumitou-Laprade P, Jean R, Vernet P, Michaelis G (1987) Cytoplasmic male sterility and nuclear genes in a natural population of *Beta maritima*: genetical and theoretical aspects. Theor Appl Genet 73:625–629

Choumane W, Heizmann P (1988) Structure and variability of nuclear ribosomal genes in the genus *Helianthus*. Theor Appl Genet 76:481–489

Dellaporta SL, Wood J, Hicks JB (1983) A plant DNA minipreparation: version II. Plant Mol Biol Rep 1:19–21

Dover G (1982) Molecular drive: a cohesive mode of species evolution. Nature 299:11–17

Fischer HE (1989) Origin of the "Weiss Schlesische Rübe" (White Silesian beet) and resynthesis of sugar beet. Euphytica 41:75–80

Frese L, Van Hintum Th (1989) The international data base for *Beta*. In: International crop network, series 3. IBPGR, Rome, pp 17–35

Fritzsche K, Metzlaff M, Melzer R, Hagemann R (1987) Comparative restriction endonuclease analysis and molecular cloning of plastid DNAs from wild species and cultivated varieties of the genus *Beta* (L). Theor Appl Genet 74:589–594

Goldsbrough PB, Cullis CA (1981) Characterization of the genes for ribosomal DNA in flax. Nucleic Acids Res 9:1301–1309

Ingle J, Timmis JN, Sinclair J (1975) The relationship between satellite DNA, ribosomal RNA gene redundancy, and genome size in plants. Plant Physiol 55:496–501

Levesque H, Vedel F, Mathieu C, de Courcel AGL (1990) Identification of a short rDNA spacer sequence highly specific of a tomato line containing Tm-1 gene introgressed from *Lycopersicum hirsutum*. Theor Appl Genet 80:602–608

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Mikami T, Kishima Y, Suguira M, Kinoshita T (1985) Organelle genome diversity in sugar beet with normal and different sources of male sterile cytoplasms. Theor Appl Genet 71:166–171

Nagamine T, Todd GA, McKann KP, Newbury HJ, Ford-Lloyd BV (1989) Use of restriction fragment length polymorphism to fingerprint beets at the genotype and species levels. Theor Appl Genet 78:847–851

Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonuclease. Proc Nat Acad Sci USA 76:5269–5273

Rocheford TR, Osterman JC, Gardner CO (1990) Variation in the ribosomal DNA intergenic spacer of maize population mass-selected for high grain yield. Theor Appl Genet 79:793–800

Rogers SO, Bendich AJ (1987) Ribosomal RNA genes in plants: variability in copy number and in the intergenic spacer. Plant Mol Biol 9:509–520

Santoni S, Faivre-Rampant P, Moreau E, Bervillé A (1991) Rapid control of purity for the cytoplasm of male sterile seed stocks by means of a dot hybridization assay. Mol Cell Probes 5:1–9

Shagai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci USA 81:8014–8018

Sneath PHA, Sokal RR (1973) Numerical taxonomy. Freeman, San Francisco

Southern E (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503–509

Stanescu Z (1990) Un point de vue concernant la classification du genre *Beta*. J Inst Sugar beet, Dijon, Res 25:7–16

Wagner H, Gimbel EM, Wricke G (1989) Are *Beta procumbens* Chr. Sm. and *Beta webbiana* Moq. different species? Plant Breed 102:17–21