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Plastid DNA diversity in natural populations of *Beta maritima* showing additional variation in sexual phenotype and mitochondrial DNA

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Summary. Plants of two natural populations of *Beta maritima,* characterized by high percentages of male-sterile plants, have been investigated for organelle DNA polymorphism. We confirm the two classes of mitochondrial DNA variation previously described: (i) mitochondrial DNA (mtDNA) type N is associated with male fertility, whereas mtDNA type S can cause cytoplasmic male sterility (CMS); (ii) the 10.4-kb linear plasmid is observed in both types of mitochondria and is not correlated with the cytoplasmic male sterility occurring in this plant material. A third polymorphism is now described for chloroplast DNA (ctDNA). This polymorphism occurs within single populations of *Beta maritima.* Three different ctDNA types have been identified by HindIII restriction analysis. Among the plants studied, ctDNA type 1 is associated with N mitochondria and type 2 with S mitochondria. Chloroplast DNA type 3 has been found both in a fertile N plant and in a sterile S plant. This finding suggests that the chloroplast DNA polymorphism reported is not involved in the expression of male sterility. A comparison with *Beta vulgaris* indicates that ctDNA type 3 of *Beta maritima* corresponds to the ctDNA of fertile sugar beet maintainer lines. The three types of *Beta maritima* ctDNA described in this study differ from the ctDNA of male-sterile sugar beet.

Key words: Wild beet (*Beta maritima*) – Chloroplast DNA – Cytoplasmic male sterility (CMS) – Mitochondrial DNA – Population diversity

Introduction

Cytoplasmically inherited male sterility (CMS) in the sugar beet *Beta vulgaris* L. was described by Owen (1942, 1945), and this source of CMS, called S, is the only one used worldwide for the production of hybrid varieties. New sources of cytoplasmic male sterility, derived from the wild beet *Beta maritima*, are being studied in several laboratories (Boutin et al. 1987; Halldén et al. 1988; Mann et al. 1989; Mikami et al. 1985). Classical genetic and molecular analyses of these new sources of CMS indicate that they differ from Owen's type S of *Beta vulgaris.* Populations of *Beta maritima* growing along the French Atlantic coast have attracted interest, because high proportions of male-sterile (female) plants were found to coexist with their male-fertile (bisexual or hermaphroditic) counterparts. Sex expression in these gynodioecious populations consisting of female and hermaphroditic individuals is dependent upon both nuclear and cytoplasmic genetic factors. Segregating and nonsegregating plants can be distinguished by genetic experiments (Boutin et al. 1987; Boutin-Stadler et al. 1989). Nonsegregating plants produce only hermaphroditic offspring, whereas the progeny of segregating plants consists of female, intermediate, and hermaphroditic individuals. Detailed studies of a high number of plants revealed that (i) the segregating and nonsegregating characters are maternally inherited, and (ii) the proportion of sexual phenotypes in segregating progenies is determined by the presence of nuclear restorer alleles within the population.

The genetic classification of segregating and nonsegregating plants was confirmed by the analysis of mitochondrial DNA. Two different restriction patterns of mitochondrial DNA were observed, and the results indicated a correlation between the type of mitochondrial DNA and the presence or the absence of segregation in the progenies (Boutin et al. 1987, 1988; Saumitou-Laprade 1989). An additional variation of mitochondrial DNA is caused by the presence or absence of a linear 10.4-kb mitochondrial plasmid that has no connection with male sterility (Saumitou-Laprade et al. 1989).

Due to this peculiar heterogeneity of mitochondrial DNA, additional plants were analyzed and special attention was directed towards a detailed study of chloroplast DNA. In this article, variation of chloroplast DNA within single natural populations of *Beta maritima* is described. In addition to the variability of mitochondrial DNA and the mitochondrial plasmid, this chloroplast DNA polymorphism represents a third type of organelle DNA variation within a single population.

Materials and methods

Plant material

Beta vulgaris (L) ssp. maritima Arcang (wild beet). The original seed stock was collected from two natural populations along the French Atlantic coast. These two populations, Canche A and Canche B, have been previously analyzed by Boutin et al. (1987) and Saumitou-Laprade et al. (1989). The plants studied in this work are listed in Table 1.

Beta vulgaris (L) ssp. vulgaris (sugar beet). The clones 5A 3031 (male sterile; S) and 5B 3031 (fertile; N) were kindly provided by Kleinwanzlebener Saatzucht AG, Einbeck (Germany).

Inheritance of sexual phenotype

Details are described in Boutin-Stadler et al. (1989) and Saumitou-Laprade (1989).

Isolation of mitochondrial DNA

The procedure of Boutin et al. (1987) was followed.

Detection of the 10.4-kb mitochondrial plasmid

The 10.4-kb mitochondrial plasmid was visualized by agarose gel electrophoresis and ethidium-bromide staining (Saumitou-Laprade et al. 1989).

Isolation of chloroplast DNA

Isolation of chloroplasts and purification of chloroplast DNA were performed as described previously (Boutin et al. 1987).

Restriction and gel electrophoresis of DNA

Restriction endonuclease digestions were carried out under conditions suggested by the suppliers. DNA was electrophoresed on 0.7% agarose slab gels buffered with 40 m*M* TRIS-HCl, 30 m*M* Na-acetate, 2 m*M* EDTA, 18 m*M* NaCl, pH 8.0.

Results

We have continued to analyze progeny plants from the two well-characterized *Beta maritima* populations A and B of the Canche estuary. Each family studied is composed of the progeny of a single, open-pollinated plant (generation G_0) from the original natural populations A or B. *Beta maritima* progeny plants from Mont St. Michel and sugar beet (*Beta vulgaris*) material were included for comparison (Table 1). The inheritance of pollen sterility, the restriction profile of mitochondrial DNA, and the occurrence of the 10.4-kb linear plasmid were determined. For the characterization of chloroplast DNA, we chose 12 families with different combinations of the three characters mentioned (Table 1).

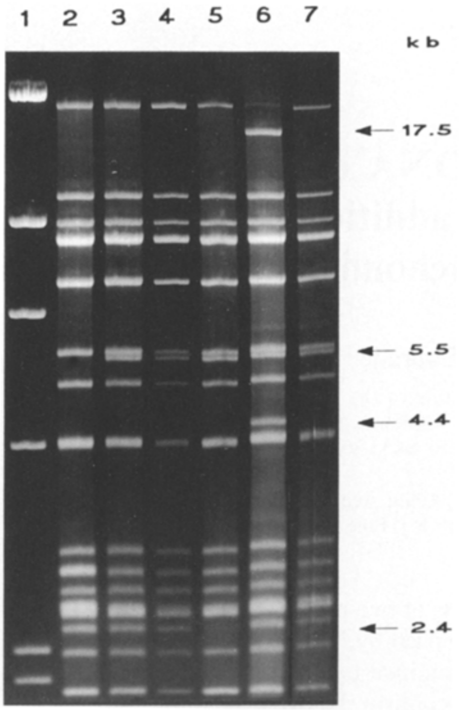

Fig. 1. HindIII digests of the chloroplast DNA from various *Beta maritima* (*B.m.*) and *Beta vulgaris* (*B.v.*) plants. *Lane 1:* Lambda DNA digested with HindIII as marker. *Lane 2: B.v.* cytoplasmic male-sterile (S_{vulgaris}) clone 5A 3031. *Lane 3: B.v.* male-fertile O-type (N_{vulgaris}) clone 5B 3031. *Lane 4: B.m.* from Canche B population – hermaphrodite offspring of the non segregating hermaphrodite B5. *Lane 5: B.m.* from Canche B population – hermaphrodite offspring of the nonsegregating hermaphrodite B16. *Lane 6: B.m.* from Canche A population – female offspring of the female plant A103. *Lane 7: B.m.* from Mont St. Michel population – female offspring of the female plant M1. *Arrows* indicate main differences between the four types of restriction profiles

Chloroplast DNA was extracted and digested with restriction endonucleases. The resulting fragments were separated by agarose gel electrophoresis. *Beta maritima* plants with N or S cytoplasm and *Beta vulgaris* plants showed the same restriction pattern of chloroplast DNA when BamHI or SmaI was used for cleavage (data not shown). However, different restriction profiles of chloroplast DNA were obtained with HindIII (Fig. 1). The restriction profile type 1 characterized by the lack of a 2.4-kb fragment (lane 5 of Fig. 1) was observed in plants from populations Canche A and Canche B. In the same two populations, type 2 of chloroplast DNA (lane 6 of Fig. 1) occurred. In this DNA, the largest HindIII fragment of about 21 kb was replaced by two fragments of 17.5 and 4.4 kb, respectively. Type 3 (lanes 3, 4, and 7 of Fig. 1) was represented by the O-type of sugar beet and the two *Beta maritima* families B5 and Mont St. Michel.

Table 1. Variations of organelle DNA in two populations of the wild beet, *Beta maritima*

Plant material species	Origin	Family	Inheritance of sexual phenotype	Mitochondrial DNA	Mitochondrial plasmid	Chloroplast DNA (type)
Beta maritima	Population Canche A	A40	nonsegregating	N	+	1
		A46	nonsegregating	N	+	1
		A78	nonsegregating	N	–	1
		A1	segregating	S	+	2
		A89	segregating	S	+	2
		A95	segregating	S	+	2
		A103	segregating	S	–	2
Beta maritima	Population Canche B	B3	nonsegregating	N	–	1
		B5	nonsegregating	N	–	3
		B13	nonsegregating	N	–	1
		B16	nonsegregating	N	+	1
		B9	segregating	S	+	2
Beta maritima	Mont St. Michel	M1	segregating	S	–	3
Beta vulgaris	O-type/KWS		maternal/fertile	$N_{vulgaris}$	–	3
Beta vulgaris	CMS/KWS		maternal/sterile	$S_{vulgaris}$	–	4

Each plant family of known geographical origin has been characterized by the inheritance of the sexual phenotype, the restriction pattern of mitochondrial DNA (N or S), the presence (+) or absence (–) of the 10.4-kb linear mitochondrial plasmid, and the HindIII restriction pattern of chloroplast DNA

The Owen cytoplasm of sugar beet (*Beta vulgaris* CMS) is known to be connected with a fourth type of chloroplast DNA characterized by the lack of a 5.5-kb fragment and the occurrence of two new bands of about 2.9 and 2.6 kb (lane 2 of Fig. 1).

Discussion

In this study we have demonstrated chloroplast DNA diversity within single gynodioecious populations of *Beta maritima*. This diversity is of interest in itself and extends the characterization of organelle variability that has previously been described in the same populations of wild beets (Boutin et al. 1987). Intraspecific variation observed in chloroplast DNA of higher plants is very low (for review, see Palmer 1987). Nevertheless, intraspecific variation in restriction sites has been reported for different species such as *Lycopersicon* (Palmer and Zamir 1982), *Pisum* (Palmer et al. 1985), *Zea* (Doebley et al. 1987), and *Brassica* (Palmer 1988). In *Lupinus texensis* (Banks and Birky 1985) and in *Dactylis glomerata* (Lumaret et al. 1989), chloroplast DNA variability has been observed within as well as among wild populations. Chloroplast DNA variation within the *Beta* group has been studied by restriction fragment analysis, and was found among plants belonging to different species, subspecies, and cultivars of *Beta* (Fritzsche et al. 1987; Bonavent et al. 1989) or among wild beets from different geographical origins (Mikami et al. 1985).

In the last-mentioned study, three different types of ctDNA are described. With one exception – a wild beet from Turkey – they are identical with the patterns found by Mikami et al. (1984) in the N and S cytoplasms of the sugar beet. These two patterns are designated here as type 3 and type 4. The exceptional pattern observed in the wild beet from Turkey probably corresponds to the chloroplast DNA type 2 from the Canche. The chloroplast DNA type 1 found in the Canche populations has not been described before in the *Beta* group. Thus far, we have not found the chloroplast DNA type 4 characteristic of *Beta vulgaris* CMS lines in the natural populations of *Beta maritima*. Among the plants we have studied, chloroplast DNA type 1 is always associated with nonsegregating plants and N mitochondria. A similar association exists for chloroplast DNA type 2, segregating plants, and S mitochondria. Such strict associations could reflect a causal relationship or, alternatively, a historical association determined by the maternal inheritance of both organelles. However, the detection of ctDNA type 3 in both N and S plants (S-maritima Mont St. Michel, N-maritima Canche and N-vulgaris) strongly suggests that mitochondrial DNA determines cytoplasmic male sterility independent of the type of chloroplast DNA associated with it. This finding also demonstrates that chloroplast DNA should not be used as a strict marker to identify the type of mitochondrial DNA. A careful study of cytoplasmic information requires characterization of both organelle genomes.

The origin of cytoplasmic male sterility in *Beta vulgaris* is still open and has been discussed in several articles (Mikami et al. 1985; Bonavent et al. 1989; Duchenne et al. 1989). Possible models include mitochondrial rearrangements as discussed by Small et al. (1989) and formation of hybrids between different species, subspecies, or genetically distinct lines (for reviews, see Edwardson 1970; Kaul 1988). The discovery of ctDNA type 3 associations with both N and S mitochondria could be an argument that in *Beta maritima* cytoplasmic male sterility is caused intraspecifically by mitochondrial rearrangements.

On the other hand, the observed associations between ctDNA type 1 and N mitochondria and ctDNA type 2 and S mitochondria are compatible with the interspecific or intraspecific hybridization model. In this case, the associations of ctDNA type 3 with N and S mitochondria must be explained by mutation of ctDNA or exceptional paternal transmission of organelles. Biparental transmission of chloroplasts has been described for some angiosperm species (for review, see Hagemann and Schröder 1989), and the paternal transmission of both organelle genomes has recently been reported for the gymnosperm *Sequoia sempervirens* (Neale et al. 1989). Regardless of the molecular bases of organelle DNA change, the described chloroplast DNA variation can be used as an additional marker for analyzing the genetic structure and dynamics of *Beta maritima* populations.

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