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

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Received July 15, 1990; Accepted September 5, 1990

Communicated by R. Hagemann

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 beets.

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 hermaphrodite) 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 indicate 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 mM TRIS-HCl, 30 mM Na-acetate, 2 mM EDTA, 18 mM 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 *Gn*) 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).

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 Smal 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.