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Comparison of chloroplast and mitochondrial DNA from five morphologically distinct *Beta vulgaris* cultivars: sugar beet, fodder beet, beet root, foliage beet, and Swiss chard

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Summary. Two cytoplasms, N and S, are used in the breeding of sugar beet, *Beta vulgaris* var. *altissima*. These cytoplasms can be distinguished by their mitochondrial DNA. In an attempt to detect new cytoplasms, we compared the restriction profiles of chloroplast and mitochondrial DNA from five different cultivars of *Beta vulgaris*. All restriction patterns of chloroplast DNA were identical. With the exception of sugar beet with S-cytoplasm, all cultivars studied showed the same restriction profile of mitochondrial DNA, indicating that these cultivars all contain the N-cytoplasm. These results are discussed with regard to the large morphological differences of the cultivars and the cytoplasmic variability found in natural populations of the wild beet, *Beta maritima*.

Key words: *Beta vulgaris* cultivars – Mitochondrial and chloroplast DNA – Restriction endonuclease fragment comparisons

Introduction

The species *Beta vulgaris* comprises several cultivated varieties that represent classical examples of morphological changes. These cultivars include sugar beet, fodder beet, beet root, foliage beet, and Swiss chard (for review see Barocka 1985). In sugar beet (*Beta vulgaris* var. *altissima*) two different cytoplasms, N and S, can be distinguished by the restriction profiles of mitochondrial DNA (Powling 1982; Powling and Ellis 1983) and chloroplast DNA (Mikami et al. 1984b). The S-cytoplasm (Owen 1945) is correlated with cytoplasmic male sterility (CMS) and is extensively used in hybrid seed production. Differences in mitochondrial DNA restriction profiles and gene expression, sometimes correlated with CMS, have been observed in many higher plant species (reviewed in Newton 1988; Ecke et al. 1989; Levings and Brown 1989).

In this work we were interested to see if the other cultivars of *Beta vulgaris* contain additional cytoplasms, and if the large morphological differences between certain cultivars are correlated with differences in the organization of the mitochondrial or the plastid genome. We isolated mitochondrial and plastid DNA from five *Beta vulgaris* cultivars and compared their restriction profiles.

Materials and methods

Plant material

Sugar beets (*Beta vulgaris* var. *altissima*) of line 0049Y1 with N-cytoplasm and line 0052A1 with S-cytoplasm were kindly provided by Kleinwanzlebener Saatzucht AG (Einbeck, FRG). Seeds of fodder beet – "Futterrübe Rota" (*Beta vulgaris* var. *crassa*), beet root – "Rote Bete Rote Kugel" (*Beta vulgaris* var. *conditiva*), foliage beet – "Blattmangold Lukullus" (*Beta vulgaris* var. *vulgaris*), and Swiss chard – "Stielmangold Glatter Silber" (*Beta vulgaris* var. *flavescens*) were purchased from a local seed shop. The plants were grown in the experimental garden of the University of Düsseldorf.

DNA isolation and restriction digestion

Isolation of mitochondrial and chloroplast DNA, restriction digestion, and gel electrophoresis of DNA were done as previously described (Boutin et al. 1987). For separation of DNA fragments, 0.7% agarose gels were used and the fragments were visualized by ethidium bromide staining. We encountered certain problems in restricting beet root mitochondrial DNA, even when this DNA was purified by CsCl density gradient centrifugation. From several enzymes tested, only SalI was found to produce complete digestions.

Results

Chloroplast DNA was isolated from leaf material of the five cultivars listed in "Materials and methods". For

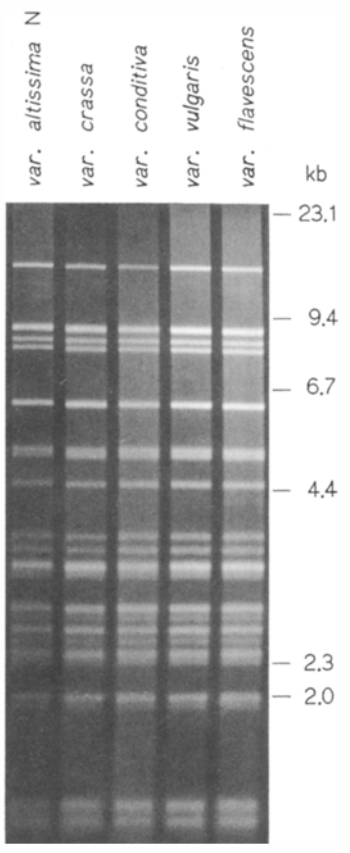

Fig. 1. BamHI restriction patterns of chloroplast DNA from different cultivars of *Beta vulgaris*. N: N-cytoplasm; var. *altissima:* sugar beet; var. *crassa:* fodder beet; var. *conditiva* beet root; var. *vulgaris:* foliage beet; var. *flavescens:* Swiss chard

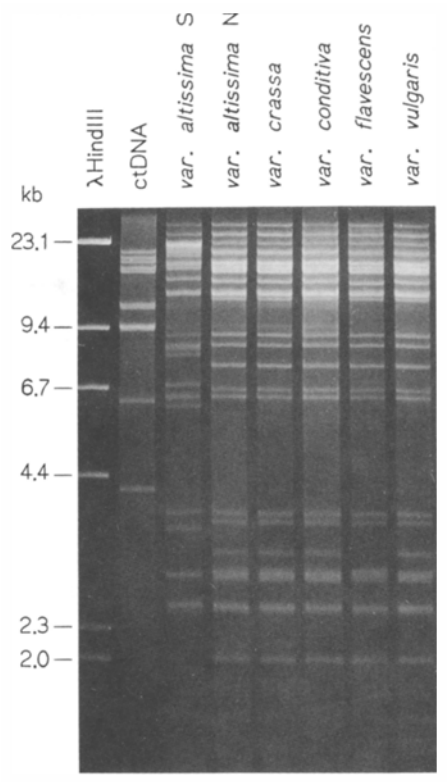

Fig. 2. SalI restriction patterns of mitochondrial DNA from different cultivars of *Beta vulgaris*. λ HindIII: λ DNA digested with HindIII as molecular weight marker. The sizes of the λ fragments are indicated. ctDNA: A SalI digestion of chloroplast DNA was included for comparison to evaluate contamination of the mitochondrial DNAs with chloroplast DNA. N: N-cytoplasm, S: S-cytoplasm; var. *altissima:* sugar beet; var. *crassa:* fodder beet; var. *conditiva:* beet root; var. *vulgaris:* foliage beet; var. *flavescens:* Swiss chard

preparation of mitochondrial DNA, we used leaf material in the case of foliage beet and Swiss chard, and beets of the three other cultivars. BamHI-digested chloroplast DNA from the different cultivars of *Beta vulgaris* is shown in Fig. 1. No differences between the restriction profiles were detectable. A restriction digestion with SalI gave the same result (data not shown). This indicates the absence of major differences in the chloroplast genome organization of the different *Beta vulgaris* cultivars studied.

The corresponding SalI restriction profiles of mitochondrial DNA are shown in Fig. 2. As has been reported earlier, the mitochondrial DNAs from the N- and S-cytoplasm of sugar beet are clearly distinguishable (Powling 1982). The restriction profiles of all the other cultivars look like the profile of sugar beet with N-cytoplasm. Only one variation is apparent: a DNA fragment of 3 kb is missing in the profile of *Beta vulgaris* var. *flavescens*. However, this variation is not specifically correlated with the cultivar *flavescens*. We have previously found this and some other variations regarding one to two fragments between different lines of sugar beet with N-cytoplasm (Ecke 1989).

Discussion

It has been reported that chloroplast DNA differs among several species of the genus *Beta* (Bonavent et al. 1989; Fritzsche et al. 1987; Mikami et al. 1984a). Even within the species of *Beta vulgaris*, a HindIII restriction fragment length polymorphism of chloroplast DNA seems to distinguish N-cytoplasm from S-cytoplasm of sugar beet. Our comparison of the five *Beta vulgaris* cultivars with two endonucleases revealed no difference between the restriction profiles of chloroplast DNA. However, minor differences and base pair substitutions cannot be excluded by our study. Nonetheless, our findings are consistent with the general assumption that closely related taxa

display more similar chloroplast DNA restriction profiles than do more distant ones.

This assumption does not always apply for mitochondrial DNA. In the species *Beta vulgaris* two cytoplasms, N and S, exist that differ greatly in the restriction profiles of their mitochondrial DNAs. Bonavent et al. (1989) have argued that the S-cytoplasm could have evolved within the *Beta vulgaris* species. In this case a major rearrangement of mitochondrial DNA has to be assumed. No additional event of this type emerges from our studies. Apart from the sugar beet with S-cytoplasm, all five *Beta vulgaris* cultivars examined showed the same restriction profiles of chloroplast and mitochondrial DNA, with one minor variation in the mitochondrial DNA. From this result we have to conclude that all the cultivars contain the N-cytoplasm known from sugar beet. The uniformity of the cytoplasms contrasts with the large morphological differences between these cultivars, indicating that the cytoplasm does not play a major role in the development of the distinct morphologies of the different *Beta vulgaris* varieties. The uniformity of the cytoplasms of the cultivars is also in contrast to the cytoplasmic variability reported for a natural population of the ancestral form, the wild beet *Beta maritima* (Boutin et al. 1987; Saumitou-Laprade et al. 1989; Saumitou-Laprade 1989). Plants of a single population have been shown to differ in the restriction pattern of mitochondrial DNA, chloroplast DNA, and by the presence or absence of a 10.4 kb mitochondrial plasmid.

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