Rearrangements in sugar beet mitochondrial DNA induced by cell suspension, callus cultures and regeneration

A. E. Dikalova¹, N. A. Dudareva¹, M. Kuhalakova², R. I. Salganik¹

¹ Institute of Cytology and Genetics, Academy of Sciences of the USSR, Siberian Department, Novosibirsk 630090, USSR
² Institute of Experimental Botany, Academy of Sciences of Czechoslovakia, Department of Plant Biotechnology, Olomouc 77200, Czechoslovakia

Received: 24 September 1992 / Accepted: 9 December 1992

Abstract. Structural alterations in mitochondrial DNAs (mtDNAs) from a plant of a sterile sugar beet line, callus derived from it, suspension-cultured cells and plants regenerated from the callus were studied. BamHI restriction analysis revealed that structural alterations between the mtDNAs of the callus and the control plant had occurred. Multiple rearrangements were also demonstrated in the mtDNA from the suspension culture, of which some were similar to those appearing in the callus, and others had arisen de novo. Rearrangements were also identified by means of blot hybridization of BamHI-digested mtDNA from suspension-cultured cells with the genes encoding subunit II of cytochrome oxidase (coxII) and subunit 1 of NADH-dehydrogenase (Ndl). No alterations were observed in the mitochondrial genome of the callus and regenerants. The location of the genes for the \( \alpha \)-subunit of F1-ATPase (atpA) and apocytochrome b (cob) in the mtDNA remained unchanged.

Our salient finding was of a plant with an altered mitochondrial genome as judged by EcoRI and BamHI restriction analysis. This exceptional plant had retained the sterile phenotype like all of the other regenerants and the parent. The set of plasmid-like molecules of mtDNA remained the same as that in the control plant and in all of the regenerants, callus and suspension-cultured cells. The only type of plasmid-like molecule found in all of the DNAs was the 1.6-kbp minicircle, which is a feature of sterile cytoplasms. These structural changes in mtDNA were obviously a consequence of somaclonal variation during the in vitro cultivation of the sugar beet cells.

Key words: Sugar beet – MtDNA – Somaclonal variation – Regenerants

Introduction

It is known that rearrangements in the plant mitochondrial genome can be induced by the in vitro culture of cells. Differences in mitochondrial genome organization between cultured and parent plants have been detected by restriction analysis in such plants as tobacco (Dale et al. 1981), pearl millet (Ozias-Akins et al. 1987), wheat (Hartmann et al. 1989; Rode et al. 1988), Brassica campestris (Shirzadegan et al. 1989) and rice (Chowdhury et al. 1990; Saleh et al. 1990). Structural changes in mitochondrial DNA (mtDNA) have been observed during callogenesis in rice (Oono 1987) and also during regeneration from the callus of plants such as maize (Gegenbach et al. 1981), wheat (Galiba et al. 1986) and sugar beet (Brears et al. 1989).

Structural changes of this kind are attributable to the nuclear genome and are known as somaclonal variation. Similar changes can also be taken advantage of in the construction of new mitochondrial genomes of higher plants and also in the production of structural mutations in the nuclear and mitochondrial genes.

Of interest is the development of new mutant types of mitochondrial genomes in sugar beet with male-sterile cytoplasm. Cytoplasmic male sterility (CMS) is a trait encoded by the mitochondrial genome, and it is economically important in hybrid seed production. To our knowledge, Owen's type of sterility was the only source of the CMS trait in sugar beet. As a result, there arose the hazard of a ubiquitous infection with the same pathogen in all the sugar beet plants having the
same modified mtDNA organization and their eventual elimination. In fact, this was observed for maize with the CMS-T cytoplasm (Leaver and Gray 1982).

The aim of the work presented here was to study the structural alterations in the mitochondrial genome of sterile sugar beet that occur during cell cultivation and regeneration from callus. We attempted to answer the question of whether or not plants with the mutant genotype retain their original phenotype. Another question was whether a new type of CMS can arise as a consequence of rearrangements in the mtDNA of sugar beet.

Materials and methods

Plant material

A diploid CMS sugar beet line no. 22003 (supplied by the Kralice na Hane Breeding Station, Czechoslovakia) was used. The primary culture was derived in 1985 from axillary buds isolated from a flowering plant with confirmed pollen sterility. The cultivation continued on LS medium (Linsmaier and Skoog 1965) with one-half concentration of mineral salts, 20 g/l sucrose and 5 μg/ml kinetin or BAP supplement. The culture was kept at 23 ± 2 °C under a 16-h photoperiod. Shoot tips propagated this way were used as explants for the induction of embryogenic callus (Kubalakova 1990). The callus was cultivated on either standard MS (Murashige and Skoog 1962) or PGo (DeGreef and Jacobs 1979) medium without growth regulators. A suspension culture was induced from friable callus and cultivated on MS or PG medium. Plants, which were propagated in vitro, and regenerants from callus culture were planted in a sterile soil substrate. Rooted plants were transplanted first to a greenhouse and finally to an experimental field. All of these plants had the sterile phenotype.

The sugar beet plants of the sterile msSOAN-31 and fertile SOAN-31 lines were kindly provided by Dr. S. Maletsky (collection of the Population Genetics Laboratory of the Institute of Cytology and Genetics).

Isolation of mtDNA

Mitochondria from callus and suspension-cultured cells were isolated in principle as previously described for sugar beet seedlings (Dadareva et al. 1988a, b); the lysis of the mitochondria and further preparation of mtDNA were carried out according to Rogers and Bendich (1985). MtDNA was isolated from sugar beet roots as described elsewhere (Rogers and Bendich 1985).

Electrophoresis and Southern blotting

Samples of mtDNA were digested with BamHI, EcoRI and SalI restriction endonucleases under standard conditions and then fractioned in horizontal 0.8% agarose gels in TAE buffer (Maniatis et al. 1982). Gel electrophoresis of the undigested mtDNA was carried out in 1.5% agarose gels. The gels were stained with 0.5 mg/ml ethidium bromide, photographed and transferred to nitrocellulose filters according to Southern (Maniatis et al. 1982). The products of HindIII and EcoRI restriction of phage lambda DNA were used as molecular weight standards.

Hybridization analysis of mtDNA

Southern blots were probed with the cloned mitochondrial genes. Genomic clones of maize subunit II of cytochrome c oxidase (coxII), alpha subunit of F1-ATPase (atpA) and apocytochrome b (cob) were gifts from Dr. C. J. Leaver, Edinburgh University, Scotland. The clone of subunit 1 of the Oenothera NADH-dehydrogenase gene (Ndh1) was provided by Dr. A. Brennicke.

Minicircle c from sugar beet mtDNA was kindly provided by Dr. C. Thomas, John Innes Institute, Norwich, UK. Integrated minicircle c and mitochondrial gene sequences were recovered from the recombinant plasmids by electroelution and then labelled by random priming (Maniatis et al. 1982). The hybridization and filter washings were done under standard conditions (Maniatis et al. 1982). The filters were autoradiographed on X-ray film at -70 °C.

Results

Restriction analysis of callus and suspension-cultured cells mtDNAs

MtDNA was isolated from a plant of a sterile line, callus and in vitro-cultured cells derived from the sterile sugar beet plant lines. BamHI restriction analy-