Theor Appl Genet (1988) 75: 741–745

Cultivated potato chloroplast DNA differs from the wild type by one deletion – Evidence and implications*

K. Hosaka [1] **, G. A. de Zoeten [2] and R. E. Hanneman, Jr. [3] ***

[1] Department of Horticulture, [2] Department of Plant Pathology, [3] Agricultural Research Service, US Department of Agriculture, Department of Horticulture; University of Wisconsin, Madison, WI 53706, USA

Received April 17, 1987; Accepted November 11, 1987
Communicated by G. Wenzel

Summary. The chloroplast DNA (ctDNA) of *Solanum tuberosum* ssp. *tuberosum* (T type) and *S. chacoense* (W type) yield five different restriction fragment patterns with five different restriction endonucleases. DNA-DNA hybridization tests revealed that these differences were all caused by one physical deletion (about 400 bp in size) in the ctDNA of ssp. *tuberosum*. This suggests that T type ctDNA of the common potato and of Chilean *tuberosum* originated from W type ctDNA. The deleted region of the T type ctDNA is probably not concerned with gene-cytoplasmic male sterility.

Key words: *Solanum tuberosum* ssp. *tuberosum* – Chloroplast DNA – Deletion – *Solanum* – Potato

Introduction

The common potato (*Solanum tuberosum* ssp. *tuberosum*, designated only as *tuberosum* in this paper) has a unique chloroplast DNA (ctDNA) (Hosaka et al. 1984). The uniqueness of *tuberosum* cytoplasm has been reported in relation to various kinds of male sterility caused by the interaction between *tuberosum* cytoplasm and cytoplasmic sensitive genes encoded by nuclear DNA (Grun 1979), and also has been characterized by the electrophoretic pattern of its Fraction 1 protein (Gatenby and Cocking 1978). In a previous paper (Hosaka 1986), the ctDNA type for *tuberosum* was determined and designated as T type, and six other ctDNA types (W, W′, W″, C, S and A) were established for other cultivated species and their relatives by using the ctDNA restriction fragment patterns of five different restriction endonucleases. The W type ctDNA is presumed to be the most primitive, from which C, W′ and W″ were derived. Types S and A apparently were derived from C. These types were distinguished by a single different ctDNA change, whereas the T type ctDNA of *tuberosum* differs by five changes from W type, indicating that *tuberosum* might be relatively distantly related to its wild relatives. However, four of the five changes between T and W type ctDNAs were deletion type changes as seen in each different restriction fragment pattern of *tuberosum* ctDNA. Thus, some of these changes would seem to be caused by a physical deletion and should be corrected.

In this paper, the number of changes between T and W type ctDNAs are estimated, and the implication of this finding on the origin of *tuberosum* is discussed.

Materials and methods

S. chacoense (PI 472820) and *S. tuberosum* ssp. *tuberosum* cv. "Irish Cobbler" were used for W type and T type ctDNA sources, respectively. The ctDNA extraction method was described earlier (Hosaka 1986).

*Bam*HI restriction fragments of W type ctDNA were ligated into the *Bam*HI site of pBR322. *E. coli* strain HB101 was then transformed with this DNA. Transformants were selected on LB media as ampicillin-resistant and tetracycline-sensitive colonies. From these, plasmid DNA with the 2.32 kbp *Bam*HI fragment derived from W type ctDNA inserted, was isolated using the boiling method. A 10.0 kbp *Bam*HI fragment was electroeluted into a dialysis bag from *Bam*HI restricted W type ctDNA which was separated on an

* Reference to a specific brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned

** Present address: Department of Vegetable Crops, University of California, Davis, CA 95616, USA

*** To whom offprint requests should be addressed

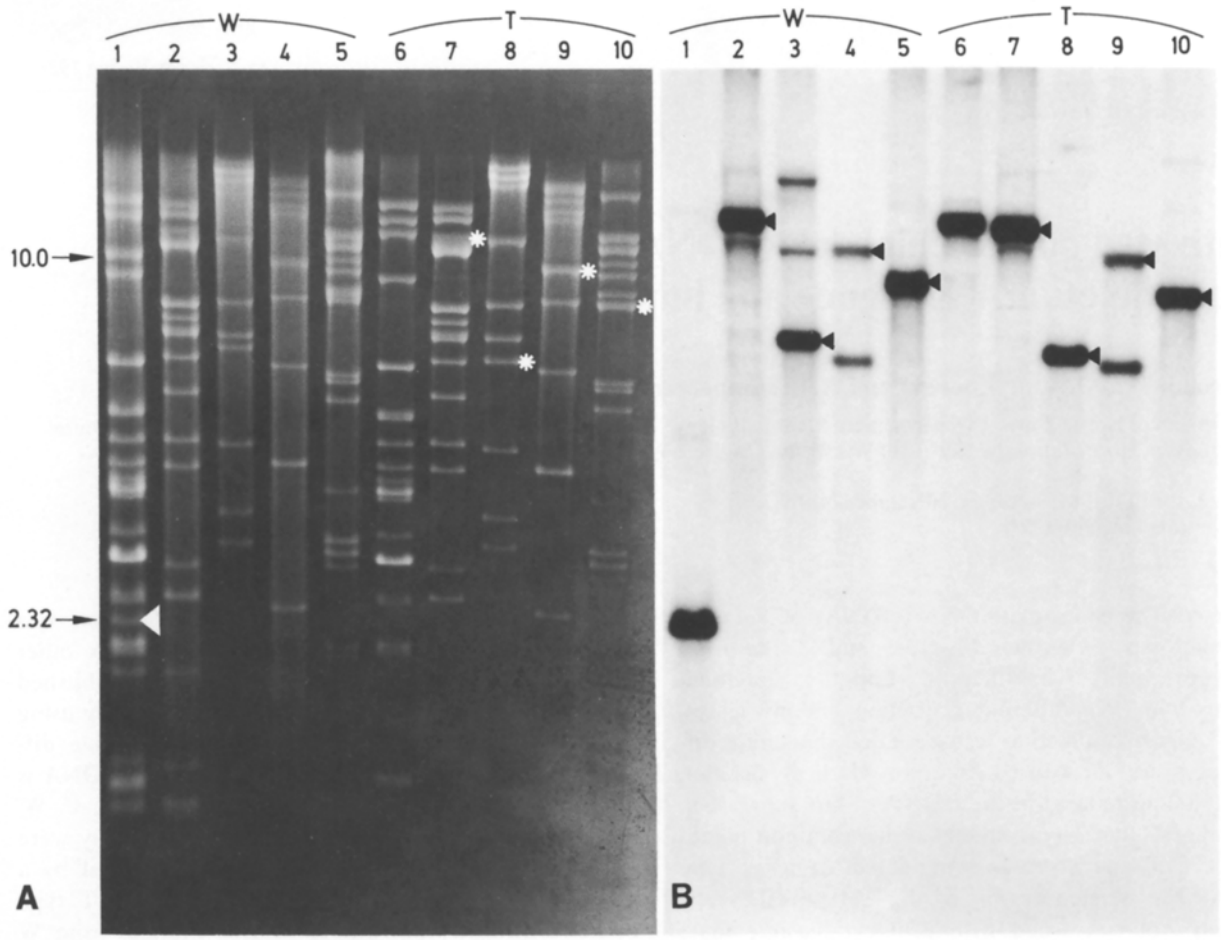

Fig. 1. Restriction fragment patterns (*A*) and hybridization with 2.32 kbp probe (*B*). The DNA probe was originally made from the fragment shown in *lane A (1)* by a *white triangle*. For *A* and *B: Lanes 1* or *6 Bam*HI; *lanes 2* or *7 Hind*III; *lanes 3* or *8 Kpn* I; *lanes 4* or *9 Pvu*II; *lanes 5* or *10 Xho*I restriction fragment patterns. *Lanes 1–5* are *W* type ctDNA, and *lanes 6–10* are *T* type ctDNA. Size-reduced fragments are shown by *white asterisks* in *A*. *Black triangles* indicated in *B* denote the portions of size-reduced fragments (*7–10*) and their original fragments (*2–5*)

agarose gel. Both the electroeluted 10.0 kbp DNA and the 2.32 kbp inserted plasmid DNA were labeled with ^{32}P by using a Nick Translation Reagent Kit (Bethesda Research Labs) and were used as 10.0 kbp and 2.32 kbp probes, respectively, for the Southern hybridization tests. *Bam*HI, *Hin*dIII, *Kpn*I, *Pvu*II and *Xho*I restriction fragment patterns of W and T type ctDNAs were probed (Southern-blot), after transfer to cellulose nitrate paper (Gene Plus, Du pont), with the ^{32}P-labeled DNA probes. The detailed procedures for cloning, electroeluting and hybridization used here are all described by Maniatis et al. (1982).

Results

Restriction fragment patterns of W and T type ctDNAs when digested by *Bam*HI, *Hin*dIII, *Kpn*I, *Pvu*II and *Xho*I, are shown in Figs. 1 A, 2 A and 3. Comparing T with W type ctDNAs, four of the five restriction fragment patterns differed by reduction in size of one fragment, viz. 12.2 kbp *Hin*dIII fragment to 11.7 kbp, 6.45 kbp *Kpn*I fragment to 6.15 kbp, 10.3 kbp *Pvu*II fragment to 9.88 kbp, and 8.6 kbp *Xho*I fragment to 8.2 kbp in size. The reduced size amounted to approximately 400 bp (Hosaka 1986).

In digestion with *Bam*HI, the 2.32 kbp and 10.0 kbp fragment of W type ctDNA were replaced by a new fragment of 12.3 kbp in T type ctDNA (Figs. 1 A and 2 A, lanes 1 and 6).

These 2.32 kbp and 10.0 kbp *Bam*HI fragments were obtained by cloning and electroeluting, respectively, and were labeled with ^{32}P and hybridized with the five restriction fragment patterns of each ctDNA, as described in the "Materials and methods".

The 2.32 kbp probe hybridized with 12.3 kbp *Bam*HI fragment and all other size-reduced fragments of T type ctDNA (Figs. 1 B and 3). In *Pvu*II fragment patterns (Fig. 1 B, lanes 4 and 9), the probe hybridized

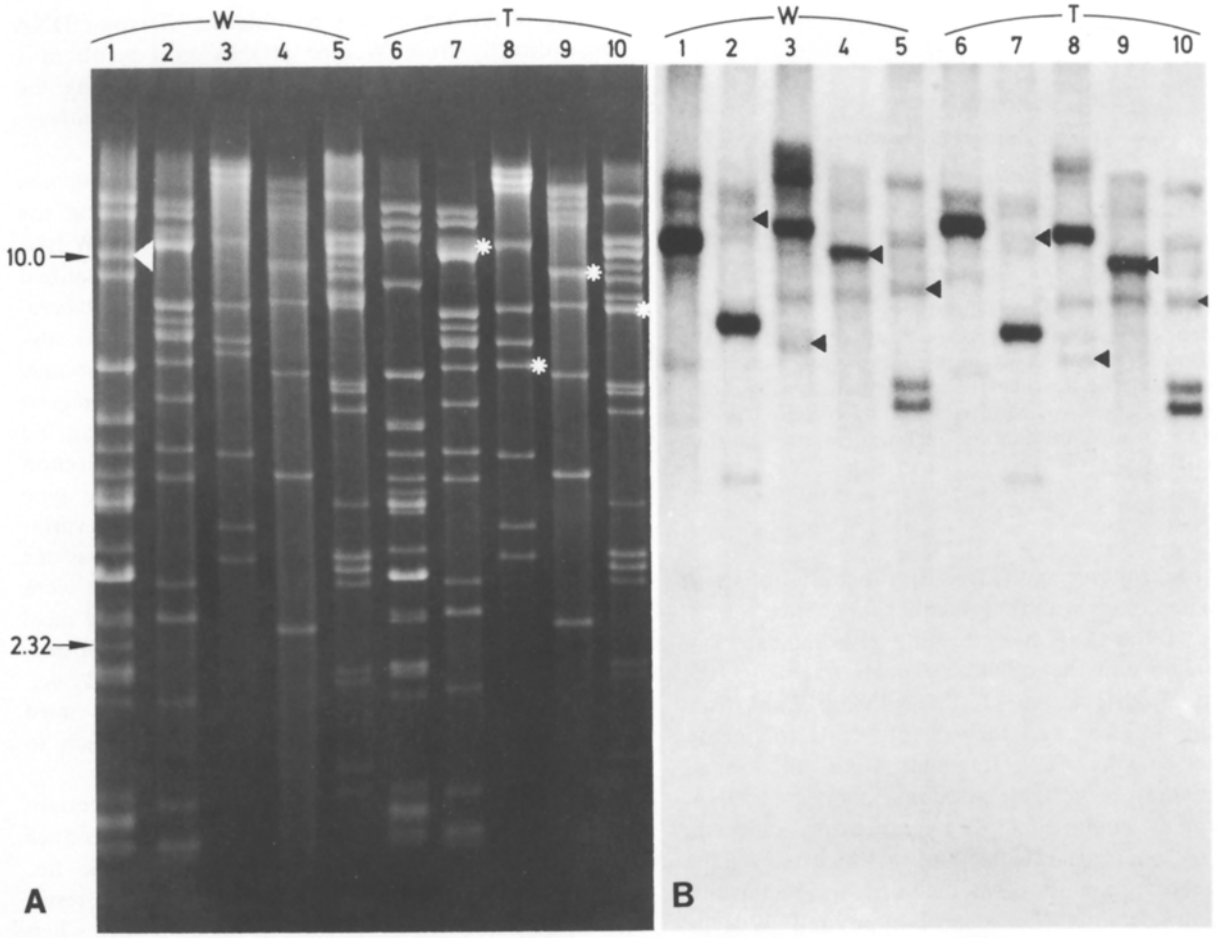

Fig. 2. Restriction fragment patterns (*A*) and hybridization with 10.0 kbp probe (*B*). See Fig. 1 for explanation of symbols and samples of each lane

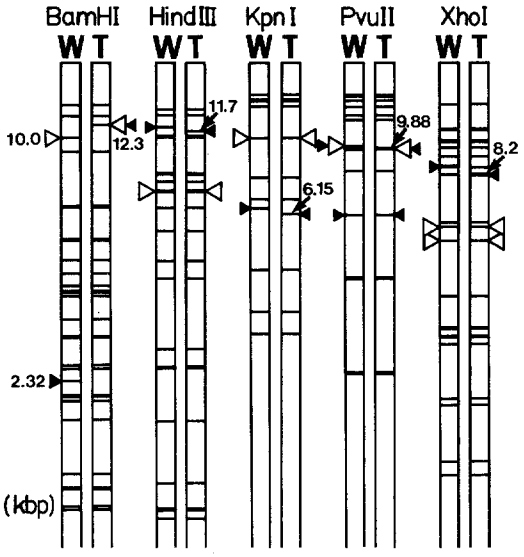

Fig. 3. Schematic presentation of restriction fragment patterns, in which main fragments that hybridized with the 2.32 kbp probe and with 10.0 kbp probe are indicated by *solid* and *empty triangles*, respectively. Size-reduced fragments are indicated by *arrows*

with the fragment related to the size reduction (10.3 kbp → 9.88 kbp) and also with the 5.9 kbp fragment with a similar hybridization activity (band density) as the former fragment. This indicates that the 5.9 kbp and the 10.3 kbp or the 9.88 kbp *Pvu*II restriction fragments are connected and that the *Pvu*II recognition site between these fragments exists near the midpoint of the 2.32 kbp *Bam*HI fragment. Additional hybridization bands that were less intense appeared when the 2.32 kbp probe was Southern-blotted with the *Kpn*I restriction fragment pattern of W type ctDNA (Fig. 1 B, lane 3), possibly because of hybridization with partially digested fragments.

The 10.0 kbp probe hybridized with the 12.3 kbp *Bam*HI fragment of T type ctDNA (Fig. 2 B, lane 6), indicating that the 10.0 kbp and 2.32 kbp *Bam*HI fragments are connected to form the 12.3 kbp fragment of T type ctDNA. The fragments that hybridized most strongly with 10.0 kbp probe are shown in Fig. 3. In the *Pvu*II pattern, the 10.0 kbp probe hybridized with the fragment related to the size reduction, but in the *Hin*dIII, *Kpn*I and *Xho*I patterns, it produced only a

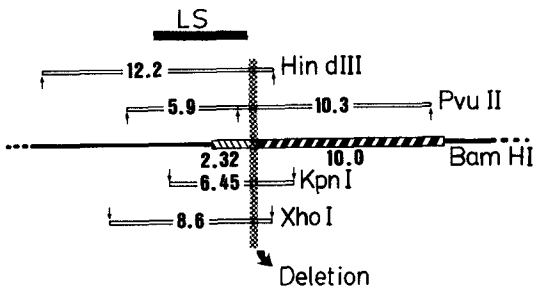

Fig. 4. Hypothetical arrangement of restriction fragments and the deleted region of W type ctDNA. Positions of the 2.32 kbp and 10.0 kbp fragments that were used to produce radioactive probes are shown on the *Bam*HI line. *LS* represents the coding region of the large subunit of ribulose 1,5-biphosphate carboxylase, the relative position of which was extrapolated from tomato ctDNA

faint hybridization band (Fig. 2 B), probably because it had less homology with these smaller fragments.

These DNA-DNA hybridization tests indicate that the 10.0 kbp and the 2.32 kbp *Bam*HI fragments, the 12.2 kbp *Hin*dIII fragment, the 6.45 kbp *Kpn*I fragment, the 10.3 kbp and the 5.9 kbp *Pvu*II fragments, and the 8.6 kbp *Xho*I fragment were all located approximately at the same place in the W type ctDNA. This strongly implies that, as hypothetically shown in Fig. 4, just one physical deletion, in which one of the *Bam*HI recognition sites was included, led to the five differences obtained between T type and W type ctDNAs.

Discussion

The DNA-DNA hybridization test indicates the strong possibility that T type ctDNA of *tuberosum* was derived from W type ctDNA by one physical deletion instead of five mutational changes reported in the previous paper (Hosaka 1986).

The T type ctDNA is limited to the common North American and European potato varieties and the Chilean *tuberosum* which has been suggested as a direct cytoplasmic donor of the common potato (Hosaka and Kamijima 1985). Hosaka (1986) estimated five mutational changes occurred between T and W type ctDNAs, based on ctDNA restriction endonuclease banding patterns, leading to the hypothesis that Chilean *tuberosum* was of hybridogenic origin between *S. tuberosum* ssp. *andigena* (hereafter designated as *andigena*) as a male and an unidentified species as a female which should have had T type ctDNA or intermediate type ctDNA between T and W. However, the present results eliminate the need for such a species having T or pre-T type ctDNA, because based on the finding of this report it is possible that T type ctDNA arose directly from W type ctDNA as a result of a physical deletion in the W type ctDNA during the evolutionary developmental process of Chilean *tuberosum*.

Most of the South American tuber-bearing *Solanum* species, particularly wild species thought to be the ancestral species of the cultivated species, have W type ctDNA (Hosaka 1986). Thus all of them are qualified as candidates as the female ancestor of Chilean *tuberosum* based on the ctDNA type. Hawkes (1956) suggested *andigena* as the ancestor based on taxonomic considerations. It is also known that among *andigena* seedling populations, *tuberosum*-like plants can be detected after several cycles of recurrent selection (Simmonds 1966; Glendinning 1975). The A type ctDNA is common in *andigena,* but tremendous variation in ctDNA exists. Indeed, some *andigena* accessions have W type ctDNA. These *andigena* accessions were collected in Argentina and Bolivia, which is the most likely region to contribute to origin of Chilean *tuberosum* if one assumes that it originated from *andigena.* These findings suggest that *andigena* is the most probable ancestor to have donated its cytoplasm to Chilean *tuberosum*.

Tuberosum cytoplasm can interact with certain specific genes encoded in nuclear DNA to produce gene-cytoplasmic male sterility (Grun 1979). So far, seven nuclear genes have been identified, which result in various kinds of expression of male sterility when combined with *tuberosum* cytoplasm (Grun 1979). It could be that the deletion associated with T type ctDNA is related to gene-cytoplasmic male sterility. However, *S. chacoense* f. *gibberulosum* cytoplasm responds with the same male sterility induction effect as *tuberosum* cytoplasm (Grun 1979), but it does not have this deletion in its ctDNA (Hosaka 1986). Therefore, as indicated by Buckner and Hyde (1985), it is less probable that the gene-cytoplasmic male sterility induction effect in the potato is caused by ctDNA, but rather that it might be associated with mitochondrial DNA as is the case in other crops (Levings and Pring 1976; Quetier and Vedel 1977; Belliard et al. 1979; Kemble and Bedbrook 1980; Powling 1981; Boutry and Briquet 1982; Pring et al. 1982; Yamaguchi and Kakiuchi 1983; Brown et al. 1986).

It is questionable whether subspecies differentiation from *andigena* to *tuberosum* is associated with ctDNA differences, particularly with the deletion of 400 bp. The location of the deleted region has not been determined exactly, but based on the *Pvu*II or *Kpn*I restriction map of tomato ctDNA (Palmer and Zamir 1982; Phillips 1985), the restriction fragment patterns of which are comparable to those of the wild potato (W type ctDNA), the deleted region is presumed to be very

close to or within the region for the large subunit of ribulose 1,5-biphosphate carboxylase (Fig. 4), which is one of the most important enzymes for photosynthesis. Gatenby and Cocking (1978) found that *andigena* and *tuberosum* differed in the isoelectrofocusing pattern of the large subunit. The finding of a physical deletion in the ctDNA of *tuberosum* could have important implications for understanding the evolution of the cultivated potato as well as how it differs from its wild relatives.

Acknowledgements. K. Hosaka thanks S. M. Daniels-Waterman, S. A. Demler, C. A. Kitzinger, D. E. Kolner, K. J. Reinke and R. A. Welliver for their technical advice. This work is a cooperative investigation of the U. S. Department of Agriculture, Agricultural Research Service and the Wisconsin Agricultural Experiment Station.

References

Belliard G, Vedel F, Pelletier G (1979) Mitochondrial recombination in cytoplasmic hybrids of *Nicotiana tabacum* by protoplast fusion. Nature 281:401–403

Boutry M, Briquet M (1982) Mitochondrial modifications associated with the cytoplasmic male sterility in faba beans. Eur J Biochem 127:129–135

Brown GG, Bussey H, DesRosiers LJ (1986) Analysis of mitochondrial DNA, chloroplast DNA, and double-stranded RNA in fertile and cytoplasmic male-sterile sunflower (*Helianthus annuus*). Can J Genet Cytol 28: 121–129

Buckner B, Hyde BB (1985) Chloroplast DNA variation between the common cultivated potato (*Solanum tuberosum* ssp. *tuberosum*) and several South American relatives. Theor Appl Genet 71:527–531

Gatenby AA, Cocking EC (1978) Fraction 1 protein and the origin of the European potato. Plant Sci Lett 12:177–181

Glendinning DR (1975) Neo-tuberosum: new potato breeding material. 2. A comparison of Neo-tuberosum with unselected Andigena and with Tuberosum. Potato Res 18: 343–350

Grun P (1979) Evolution of the cultivated potato: a cytoplasmic analysis. In: Hawkes JG, Lester RN, Skelding AD (eds) The biology and taxonomy of the *Solanaceae*. Academic Press, London, pp 655–665

Hawkes JG (1956) Taxonomic studies on the tuber-bearing Solanums. 1. *Solanum tuberosum* and the tetraploid species complex. Proc Linn Soc London 166:97–144

Hosaka K (1986) Who is the mother of the potato? – Restriction endonuclease analysis of chloroplast DNA of cultivated potatoes. Theor Appl Genet 72:606–618

Hosaka K, Kamijima O (1985) On the cytoplasmic genome of common potato. Rep Soc Crop Sci Breed, Kinki 30:16–20

Hosaka K, Ogihara Y, Matsubayashi M, Tsunewaki K (1984) Phylogenetic relationship between the tuberous *Solanum* species as revealed by restriction endonuclease analysis of chloroplast DNA. Jpn J Genet 59:349–369

Kemble RJ, Bedbrook JR (1980) Low molecular weight circular and linear DNA in mitochondria from normal and male-sterile *Zea mays* cytoplasm. Nature 284:565–566

Levings CS III, Pring DR (1976) Restriction endonuclease analysis of mitochondrial DNA from normal and Texas cytoplasmic male-sterile maize. Science 193:158–160

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning, a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, p 545

Palmer JD, Zamir D (1982) Chloroplast DNA evolution and phylogenetic relationships in *Lycopersicon*. Proc Natl Acad Sci USA 79:5006–5010

Phillips AL (1985) Localisation of genes for chloroplast components in tomato plastid DNA. Curr Genet 10: 153–161

Powling A (1981) Species of small DNA molecules found in mitochondria from sugarbeet with normal and male sterile cytoplasms. Mol Gen Genet 183:82–84

Pring DR, Conde MF, Schertz KF, Levings CS III (1982) Plasmid-like DNAs associated with mitochondria of cytoplasmic male-sterile sorghum. Mol Gen Genet 186: 180–184

Quetier F, Vedel F (1977) Heterogenous population of mitochondrial DNA molecules in higher plants. Nature 268: 365–368

Simmonds NW (1966) Studies of the tetraploid potatoes. 3. Progress in the experimental re-creation of the Tuberosum Group. J Linn Soc Bot 59:279–288

Yamaguchi H, Kakiuchi H (1983) Electrophoretic analysis of mitochondrial DNA from normal and male sterile cytoplasms in rice. Jpn J Genet 58:607–611

Note added in proof

Very recently, a complete physical map of potato ctDNA was reported by Heinhorst et al. (1988, Theor Appl Genet 75:244–251). Their P6b, X7a and B3 fragments correspond to our 10.3 → 9.88 kbp *Pvu*II, 8.6 → 8.2 kbp *Xho*I and 2.32 + 10.0 → 12.3 kbp *Bam*HI fragments, respectively. Our hypothetical arrangement of these fragments corresponds very well with their reported physical map, although the coding region for the large subunit of ribulose 1,5-biphosphate carboxylase is far from the deleted region.