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Chloroplast DNA variation between the common cultivated potato (*Solanum tuberosum* ssp. *tuberosum*) and several South American relatives

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Summary. Chloroplast DNA (ctDNA) from the tuber-bearing *Solanum* species *tuberosum, vernei, phureja,* and *chacoense* has been compared by restriction endonuclease analysis. Digestion by Hind III or Xba I reveal no differences, but digestion with Bam HI and Eco RI reveals minor differences in the ctDNA among these species. The ctDNA restriction patterns of the tetraploid common cultivated potato of North America and Europe, *S. tuberosum* ssp. *tuberosum* and the South American tetraploid, *S. tuberosum* ssp. *andigena* are identical for all four restriction endonucleases. These data suggest that ssp. *tuberosum* and ssp. *andigena* contain similar ctDNA and therefore may share a common ancestor, or direct lineage. The ctDNA restriction patterns of *S. vernei* and *S. chacoense* are identical for all four restriction endonucleases, and *S. phureja* ctDNA, can be distinguished from the other diploid ctDNAs by digestion with Bam HI. None of the diploids analyzed contain ctDNA identical to the tetraploids and therefore either did not contribute their chloroplast genomes to the evolution of the tetraploids, or the ctDNA has diverged since this evolutionary event. The ctDNAs studied did not contain restriction polymorphisms which could be correlated to cytoplasmic male sterility in *Solanum.* This is the first demonstration of ctDNA diversity in the tuber-bearing *Solanum* species.

Key words: Potato-Chloroplast DNA-Restriction polymorphisms – Evolution

Introduction

The electrophoretic patterns of restriction endonuclease digested ctDNA are distinct in plants from different classes, subclasses, orders, and families, but within genera clear similarities or identities can be demonstrated (Rhodes et al. 1981).

Restriction endonuclease analyses of ctDNA have been used to investigate the phylogenetic relationship among the genera *Triticum, Secale, Hordeum,* and *Avena* (Vedel et al. 1980), and within several genera including *Triticum* (Vedel et al. 1978), *Zea* (Timothy et al. 1979), *Brassica* (Lebacq and Vedel 1981), *Lycopersicon,* and *Solanum* (Palmer and Zamir 1982). Zamir was limited to 3 non-tuber-bearing *Solanum* species which are closely related to the genus *Lycopersicon.* To date the restriction endonuclease analyses of the economically important tuber bearing *Solanum* species has been limited to the study of potato-tomato somatic hybrids (Schiller et al. 1982; Shepard et al. 1983), *S. tuberosum-S. nigrum* somatic hybrids (Gressel et al. 1984), and to the protoclones derived by protoplast regeneration (Kemble and Shepard 1984). In these studies no ctDNA variation was observed. Isoelectric focusing patterns of ribulose bisphosphate carboxylase suggest that variation exists in the chloroplast genome between the common cultivated potato of North America and Europe, *S. tuberosum* ssp. *tuberosum,* and the cultivated potato of South America, *S. tuberosum* ssp. andigena (Gatenby and Cocking 1978).

We present here a restriction endonuclease analysis of ctDNA from *S. tuberosum* ssp. *tuberosum* and several closely related South American species. The results are related to the origin of the chloroplast genome in *S. tuberosum,* cytoplasmic male sterility (CMS), and the identification of potential sources of chloroplast genome diversity of this important crop plant.

Materials and methods

Chloroplast DNA from the following *Solanum* species was analysed: *S. tuberosum* ssp. *tuberosum* cv 'Kennebec' (4N), *S. tuberosum* ssp. *andigena* (4N), *S. chacoense* f. *gibberulosum* (2N), *S. phureja* (2N), and *S. vernei* (2N).

Chloroplast DNA was isolated from leaves by the method of Saltz and Beckman (1981) with the addition of a CsCl bisbenzimide gradient centrifugation purification step prepared as follows: gradients containing ctDNA, 4.40 g CsCl, and 1.0 mg bisbenzimide (Sigma) were made to 5 ml with buffer (10 mM Tris-HCl, 1 mM Na_2 EDTA, pH 7.8). The gradients were centrifuged at 35,000 rpm at 20 °C for 60 h in a Beckman Sw55 rotor. The single DNA band was removed from the gradient, extracted with n-butanol, dialyzed against the above buffer, and precipitated with ethanol.

The ctDNA was digested with restriction endonucleases for one hour under the conditions specified by the manufacturer (Bethesda Research Laboratories). The DNA was electrophoresed on 0.85% agarose gels in a BRL horizontal electrophoresis unit essentially as described by Schleif and Wensink (1981). The gels were stained for 1–2 h with 1 μg/ml ethidium bromide and illuminated with short wave length UV light using a transilluminator and photographed using polaroid type 55 positive/negative film in a Polaroid MP3 camera fitted with a Wratten red filter.

Results

CtDNA from *S. tuberosum* ssp. *tuberosum, S. chacoense* f. *gibberulosum, S. tuberosum* ssp. *andigena, S. vernei,* and *S. phureja* were digested with the following restriction endonucleases: Xba I, Hind III, and Bam HI. There are no differences in the Xba I (Fig. 1) or Hind III (Fig. 2) restriction patterns of the five potato ctDNAs analyzed. The Bam HI patterns of ctDNA from *S. tuberosum* ssp. *tuberosum* and *S. tuberosum* ssp. *andigena* are identical and contain 23 bands (Fig. 3, lanes 1 and 3). The Bam HI patterns of ctDNA from *S. chacoense* f. *gibberulosum* and *S. vernei* are identical and contain 24 bands (Fig. 3, lanes 2 and 4). The Bam HI pattern of *S. phureja* also contain 24 bands, however, the *S. phureja* pattern has a band of greater molecular weight DNA than any of the other species tested (Fig. 3, lane 5). CtDNA from all of the above species and subspecies except *S. phureja* were digested with the restriction endonuclease Eco RI (Fig. 4). Eco RI cuts the ctDNA frequently producing many short DNA fragments. Some of the smallest fragments migrated off the electrophoretic gel in Fig. 4. The Eco RI patterns of the tetraploid subspecies *tuberosum* and *andigena* are identical and contain a ca. 6.6 Kbp band not present in the diploid patterns (Fig. 4, lanes 1 and 2). The Eco RI patterns of the diploids *S. chacoense* f. gibberulosum and *S. vernei* are identical and contain a ca. 3.2 kbp band not present in the tetraploid patterns (Fig. 4, lanes 2 and 4).

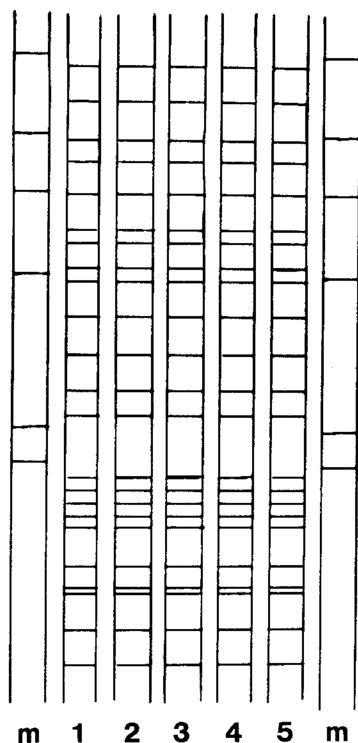

Fig. 1. Diagram of electrophoresis on 0.85% agarose gel of Xba I fragmented ctDNA: *lane 1 S. tuberosum* ssp. *tuberosum; lane 2 S. chacoense* f. *gibberulosum; lane 3 S. tuberosum* ssp. *andigena; lane 4 S. vernei; lane 5 S. phureja. Lanes m* contain size marker fragments of lambda DNA digested with Hind III

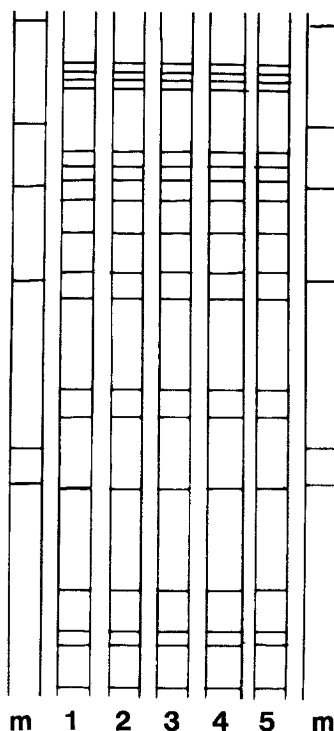

Fig. 2. Diagram of electrophoresis on 0.85% agarose gel of Hind III fragmented ctDNA: *lane 1 S. tuberosum* ssp. *tuberosum; lane 2 S. chacoense* f. *gibberulosum; lane 3 S. tuberosum* ssp. *andigena; lane 4 S. vernei; lane 5 S. phureja. Lanes m* contain size markers as in Fig. 1

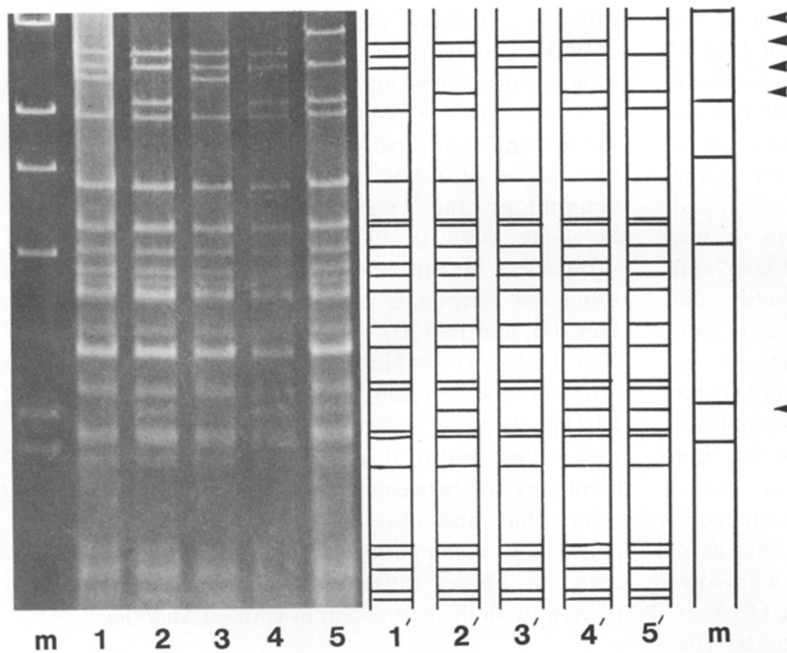

Fig. 3. Electrophoresis on 0.85% agarose gel of Bam HI fragmented ctDNA: *lane 1 S. tuberosum* ssp. *tuberosum; lane 2 S. chacoense* f. *gibberulosum; lane 3 S. tuberosum* ssp. *andigena; lane 4 S. vernei; lane 5 S. phureja; lanes 1′ to 5′* diagram of above, respectively. *Lanes m* contain size markers as in Fig. 1. The additional or missing fragments are indicated by *arrows*

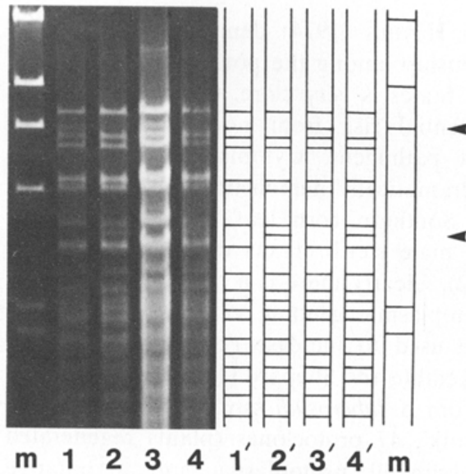

Fig. 4. Electrophoresis on 0.85% agarose gel of Eco RI fragmented ctDNA: *lane 1 S. tuberosum* ssp. *tuberosum; lane 2 S. chacoense* f. *gibberulosum; lane 3 S. tuberosum* ssp. *andigena; lane 4 S. vernei; lanes 1′ to 4′* diagram of above respectively. *Lanes m* contain size markers as in Fig. 1. The additional or missing fragments are indicated by *arrows*

Discussion

Evolution of ctDNA in Solanum tuberosum

It is believed that *S. tuberosum* ssp. *andigena*, a tetraploid of the Andes Mountains, could have evolved from one of the diploids *S. phureja, S. stenotomum, S. sparsipilum,* or *S. vernei* (Grun et al. 1977; Hawkes 1967; Brücher 1964). The evolution of *S. tuberosum* ssp. *tuberosum* however is not as well understood. Juzepezuk and Bukasov (1929) postulated that ssp. *andigena* and ssp. *tuberosum* have separate origins. Others believe that ssp. *tuberosum* evolved in Europe by natural selection of ssp. *andigena* for long-day tolerance and blight resistance (Simmonds 1964).

Grun has reported that the cytoplasm of ssp. *tuberosum* and ssp. *andigena* are distinct. When ssp. *tuberosum* is crossed as the female parent to ssp. *andigena* nucleo-cytoplasmic incompatibilities cause the hybrid to be male sterile. To account for the differences in cytoplasms between the two subspecies Grun (1979) hypothesized that ssp. *tuberosum* evolved from ssp. *andigena* in several steps including the donation of cytoplasmic genes from a species such as *S. chacoense* f. *gibberulosum,* a diploid of Chile that is cytoplasmically compatible to ssp. *tuberosum.*

The isoelectric focusing patterns of ribulose bisphosphate carboxylase have been used to investigate the origin and evolution of the European potato *S. tuberosum* ssp. *tuberosum* (Gatenby and Cocking 1978). *S. tuberosum* ssp. *andigena* and *S. tuberosum* ssp. *tuberosum* have different large subunit banding patterns, but similar small subunit patterns. They conclude that the nuclear genomes of the subspecies are similar, but that the cytoplasmic genomes are distinct. They hpothesize that the *S. tuberosum* ssp. *tuberosum* cytoplasm may have been donated by a Chilian potato type.

The restriction patterns produced by XbaI (Fig. 1), Hind III (Fig. 2), Bam HI (Fig. 3) and Eco RI (Fig. 4) show no differences between ssp. *andigena* and ssp. *tuberosum* ctDNAs. This would suggest that the differences found in the banding patterns of the large subunit of ribulose bisphosphate carboxylase from the two subspecies are caused by small differences in their ctDNA not identified by this analysis, and/or by

differences in postranscriptional modifications of the large subunit gene product. Also the Bam HI (Fig. 3) and Eco RI (Fig. 4) restriction pattern of *S. chacoense* f. *gibberulosum* are different from *S. tuberosum* ssp. *tuberosum* and therefore suggests that *S. chacoense* f. *gibberulosum* did not contribute its cytoplasm to the evolving *S. tuberosum* ssp. *tuberosum* as was suggested by Grun (1979). Bam HI restriction patterns of two other *S. tuberosum* ssp. *tuberosum* cultivars ('Flava' and 'Alpha') were identical to the patterns of *S. tuberosum* ssp. *tuberosum* cv 'Kennebec' and *S. tuberosum* ssp. *andigena* in Fig. 3 (data not shown). These patterns are the same as those reported by Shepard et al. (1983) for cultivar 'Russet Burbank'. These data suggest that the ctDNA in *S. tuberosum* is highly stable and has not diverged significantly between the subspecies *tuberosum* and *andigena,* or between the varieties 'Kennebec', 'Flava', 'Alpha', and 'Russet Burbank'. Our data is in agreement with Kemble and Shepard (1984) who concluded that *S. tuberosum* ssp. *tuberosum* ctDNA is stable and resistant to sequence rearrangements. The identity of the ctDNA of ssp. *tuberosum* and ssp. *andigena* suggests either that the ctDNA was donated by the same source independently to each subspecies, the subspecies evolved from a common ancestor, or one subspecies evolved into the other.

Cytoplasmic male sterility in Solanum

Restriction endonuclease analysis has been widely used to investigate CMS. Mitochondrial and chloroplast DNA from normal and CMS lines of maize compared by this technique show little variation in ctDNA, but significant variation in the mtDNA (Pring and Levings 1978). Therefore mtDNA is believed to be associated with CMS in maize. Similar studies have shown that CMS in wheat may be associated with mtDNA, however CMS in *Nicotiana* has been linked to both ctDNA (Frankel et al. 1979) and mtDNA (Belliard et al. 1979).

CMS is common in the genus *Solanum* (Grun 1979). When *S. tuberosum* ssp. *tuberosum* or *S. chacoense* f. *gibberulosum* are crossed as the female parent to *S. tuberosum* ssp. *andigena, S. vernei,* or *S. phureja* male sterile hybrids are produced. The reciprocal cross produces fertile hybrids. (Grun et al. 1962; Grun 1974). Grun has identified 8 cytoplasmic factors which, when present with the incompatible nuclear background, give rise to cytoplasmic male sterile hybrids. Grun has also shown that ssp. *tuberosum* from North America, Europe, and Chile are compatible with *S. chacoense* f. *gibberulosum,* an uncultivated diploid of Chile, for all of the cytoplasmic factors tested (5 tested) (Grun 1979). Since it is the organelle DNA that will determine the difference between the CMS hybrids and the fertile hybrids, produced by reciprocal crosses, one should look for differences in the ctDNA and mtDNA of the parent plants as possible evidence for that organelle's DNA being associated with CMS. An attempt to locate the origin of CMS in *Solanum* can be made by determining the extent of the differences in the organelle DNA of the above 5 potato species and subspecies. In this study the ctDNA was compared using the restriction endonucleases Xba I, Hind III, Bam HI, and Eco RI. No differences were found in the Xba I (Fig. 1) or Hind III (Fig. 2) and only minor differences were found in the Bam HI (Fig. 3) or Eco RI (Fig. 4) patterns for the *Solanum* species and subspecies analyzed in this study. The differences that are identified are between plants that are cytoplasmically compatible, and also between plants that are cytoplasmically incompatible. Therefore we are unable to correlate these differences in ctDNA restriction patterns with the expression of CMS in *Solanum.*

CtDNA variation in *Solanum*

Mendoza and Haynes (1974) demonstrated that the genetic relationship among the potato cultivars grown in the United States is very close. Genetic uniformity creates a potential risk from newly introduced or evolved plant pathogens. Cytoplasmic genetic uniformity was dramatically demonstrated when the susceptibility to Southern corn leaf blight disease was linked to the male sterile Texas cytoplasm in maize (Ullstrup 1972). Clearly there is a need for the identification of cytoplasmic genome varients which could potentially be used to increase genetic diversity. To such an end Kemble and Shepard (1984) have analyzed the ctDNA from *S. tuberosum* ssp. *tuberosum* cultivar 'Russet Burbank', 47 protoclones (plants regenerated from leaf mesophyll protoplasts), and a putative plastome mutant. They observed no ctDNA variation. In this study variation in the ctDNA from *S. tuberosum* ssp. *tuberosum* and *S. tuberosum* ssp. *andigena* was not observed, however three South American tuber-bearing diploids were identified which contain ctDNA different from that of the cultivated potato *S. tuberosum* (Figs. 3 and 4). Therefore, these or other cultivated and wild South American *Solanum* species are potential sources of cytoplasmic variation which could be introduced into *S. tuberosum.*

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