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DNA polymorphism in *Allium cepa* cytoplasms and its implications concerning the origin of onions

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Summary. Mitochondrial and chloroplast DNA was isolated from fertile and cytoplasmic male sterile cultivars of cultivated onions. Restriction fragment length polymorphism led to the distinction between cytoplasms S and M. Mitochondrial DNA patterns from S cytoplasms appeared identical and characterized mostly male sterile lines. An open-pollinated variety was found to bear this cytoplasm and thought to be the origin of S types. Mitochondrial DNA patterns from M cytoplasms were subdivided into four types, M_1 and M_2 corresponding to normal N cytoplasm, M_3 and M_4 probably corresponding to T cytoplasms. S and M cytoplasms were also distinguished by chloroplast DNA restriction patterns. Our results confirm previous genetic distinction between S, N and T cytoplasms.

Key words: Mitchondrial DNA – Chloroplast DNA – DNA polymorphism – Cytoplasmic male sterility – Onion

Introduction

Although onions are the second most important vegetable crop in the world, after tomatoes, very little genetic data is available. The cross-pollinating nature of Allium, their strong inbreeding depression and their long generation time are responsible for this lack of knowledge (Rabinowitch 1988). The result is that the breeding of onions is very backwards compared with many other less important vegetables.

The discovery of a cytoplasmic male sterile (cms) onion by Jones in 1925 was certainly the main event in modern breeding. He showed how to transfer the cytoplasm to inbred parent lines and proposed a system for commercial production of hybrid seed (Jones and Clarke 1943; Jones and Mann 1963). U.S. male sterile lines used in commerical seed production derive their sterile cytoplasm from Jones' original plant and are, therefore, often closely related. In a number of countries, although onion breeding was initially based on the U.S. male sterile material, local male steriles are now being used (McCollum 1976).

Cms has arisen either spontaneously in natural populations, following mutagenic treatment of seeds (Hanson and Conde 1985), or in plants regenerated from protoplast culture (Li et al. 1988), or also from intergeneric, interspecific or intraspecific crosses (Hanson and Conde 1985). It is well established that the maternally inherited male sterile phenotype is caused by interactions between nuclear and cytoplasmic factors (Clayton 1950). Substantial evidence indicates that the cms trait is encoded by the mitochondrial genome. Plant mitochondrial genomes are generally large and multicircular, containing sequences of different stoichiometry. Evolution of these complex genomes appears to occur via reorganization of sequences rather than by point mutation. Mitochondrial DNA (mtDNA) restriction analysis showed specific modifications in male sterile cytoplasms of maize, wheat, tobacco, sorghum, bean, rapeseed, sugar beet, sunflower, petunia, rye and rice (see the reviews by Leaver and Gray 1982; Levings 1983; Hanson and Conde 1985; Pring and Londsale 1985). While many of these differences probably have no phenotypic effect in cms lines, some have been shown to be due to the modification of existing genes or the creation of novel mitochondrial genes by recombination (Leaver et al. 1985; Dewey et al. 1986; Young and Hanson 1987). These chimeric genes are expressed as variant polypeptides which appear to be cau-

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sally related to the cms phenotype (Forde and Leaver 1980; Boutry et al. 1984; Dewey et al. 1987).

In this study we analysed several cultivated onion cultivars with fertile or male sterile cytoplasms which are used in the production of hybrid seeds. The purpose of this work is to compare mt restriction fragment patterns so as to characterize different cultivars and attempt to establish relationships between them which could be useful to breeders.

Materials and methods

Plant materials

The different onion cultivars with fertile or male sterile cytoplasms we have analysed are presented in Table 1.

Isolation of chloroplast DNA (ctDNA)

Bulbs were germinated in the dark before being placed in the light (16 h photoperiod at 18 °C) for 3–4 weeks. Chloroplasts were isolated in a medium of high ionic strength (Bookjans et al. 1984). Green shoots were homogenized at 4 °C in a Waring blender 5 s 3 times at low speed in 10 ml/g fresh weight NaCl buffer (50 m*M* TRIS-HCl, pH 8.0, 1.25 *M* NaCl, 25 m*M* EDTA, 0.1% BSA, 7 m*M* β-mercaptoethanol). The homogenate was filtered through a 35 μm nylon net and the filtrate centrifuged at 900 g, 10 min (Sorvall, GSA rotor). The pellet was discarded and the supernatant centrifuged at 4,000 g, 10 min (Sorvall, GSA rotor). The pellet (corresponding to 10 g fresh material) was lysed with 3 ml buffer containing 50 m*M* TRIS-HCl pH 8.0, 20 m*M* EDTA; 2% laurylsarcosinate and 1 mg/ml pronase (Boehringer Mannheim) for 1 h at 20 °C. To 1.3 ml aliquots of lysate were added 1.7 g CsCl and 20 μl of an ethidium bromide solution at 10 mg/ml. After careful mixing the solution was poured in a quickseal tube (Beckman, TLV rotor, TL100 ultracentrifuge). Each tube was filled with buffer 50 m*M* TRIS-HCl pH 8.0, 20 m*M* EDTA, sealed and centrifuged at 90,000 rpm for 3 h at 18 °C. ctDNA band was collected at 360 nm, diluted with TE buffer (10 m*M* TRIS, pH 8, 1 m*M* EDTA) and spun at 100,000 rpm, 90 min at 18 °C (Beckman, TLA 100.2 rotor, TL 100 ultracentrifuge). The ctDNA pellet was dissolved in 30–100 μl of TE.

Table 1. Description and origin of the genotypes used

Genotype	Description and origin
a	cms line on unknown Italian cytoplasm, distributed by INRA (France)
b	cms line found in a cultivar of open-pollinated variety Dorata di Parma from Italy
c	cms line on S cytoplasm (Jones). Line W 202 A released by the University of Wisconsin, USA
d	F_1 hybrid on cms line c cytoplasm (S cytoplasm)
e	F_1 hybrid on cms line a cytoplasm
f	Commercial F_1 hybrid Copra on cms cytoplasm of unknown origin
g	F_1 hybrid on cms line b cytoplasm
h	F_1 hybrid on cms line c cytoplasm (S cytoplasm)
i	cms line on T cytoplasm. Strigunowskij line from USSR, distributed by INRA (France)
j	Maintainer line of cms line a. Cultivar of German variety Stuttgarter Riesen, distributed by INRA (France)
k	Maintainer line of cms line c. Line W 202 B released by the University of Wisconsin, USA
l	F_1 hybrid on cms line Rff cytoplasm found in a cultivar of open pollinated variety Rosé de Roscoff distributed by INRA (France)
m	Fertile line on unknown cms cytoplasm of Polish origin
o	Cultivar of open-pollinated variety Dorata di Parma from Italy
p	Cultivar of open-pollinated variety Jaune paille des Vertus from France
q	Cultivar of open-pollinated variety Valenciana Grano from Spain
r	Fertile line on unknown cytoplasm from Italy
s	Maintainer line of a cms S line, selected in variety q

Isolation of mtDNA

Mitochondria were prepared as described previously (Vedel and Mathieu 1982), using deoxyribonuclease treatment and centrifugation in four-step discontinuous sucrose gradients. MtDNA was isolated from the last mitochondrial pellet as indicated with ctDNA and it was purified by CsCl centrifugation in the same manner as ctDNA.

ct and mtDNA restriction analysis

One to 3 μg of ct or mtDNA was digested in 30 μl reaction with sufficient enzyme to give complete digestion. The restriction fragments were separated by electrophoresis in 0.7% agarose gels (Vedel et al. 1976; Quétier and Vedel 1977). The 1-kb ladder (Bethesda Research Laboratories) and Hind III fragments from Lambda DNA (Boehringer Mannheim) were used as molecular weight standards.

In some instances, mtDNA restriction fragments were transferred from agarose to Pall biodyne A membranes according to the manufacturer's procedure.

Hybridization

Total Bam HI restricted mtDNA was denatured at 95 °C in a boiling water bath, chilled quickly at 0 °C and labelled according to the Chemiprobe procedure (Orgenics Ltd). Hybridization was carried out in buffer containing 45% formamide for one night at 42 °C, after 2 h of prehybridization with heterologous DNA. Detection of hybridization bands was according to the Chemiprobe procedure.

Results

Onion mtDNAs show two very distinctive types of restriction patterns, whatever the enzyme used. The most widespread among fertile lines and open-pollinated varieties we called type M (Figs. 1 and 2, lanes i–m). Within type M we observed four patterns showing a few restriction fragment differences with enzyme Bam HI (Fig. 1, lanes i–m). M_1 (j) and M_2 (k) are similar except for one 4-kb fragment which is not found in M_2. M_3 (l) shows several variations for fragments at 6.5, 7.2, 8 and 9 kb. M_4 (i, m) has a 12-kb fragment missing when compared to other M types. Restriction patterns with enzyme Bcl I

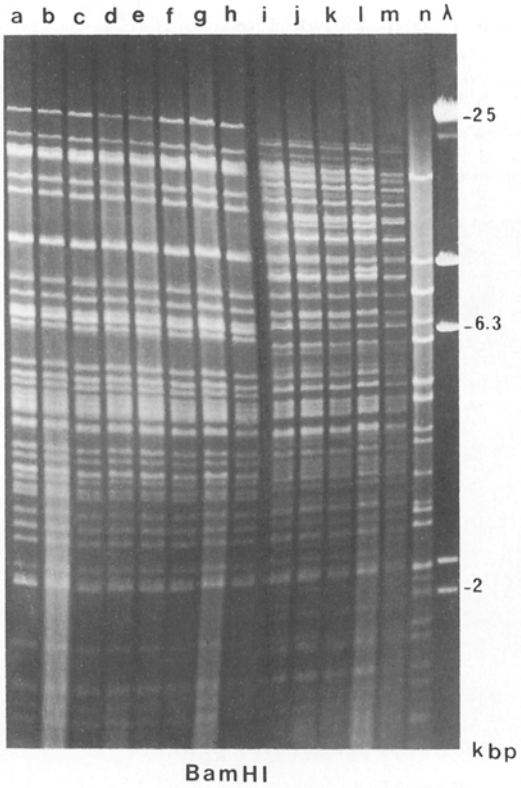

Fig. 1. Bam HI restriction patterns of mt DNAs from various cms or fertile cultivars of cultivated onion; *lanes a–m* are described in Table 1. *n*, Bam HI restriction pattern of ct DNA from line a (Table 1) indicating that mt DNA diagrams are not contaminated by ct DNA and vice versa. *Lambda*, molecular weight standard (Marker II, Boehringer Mannheim)

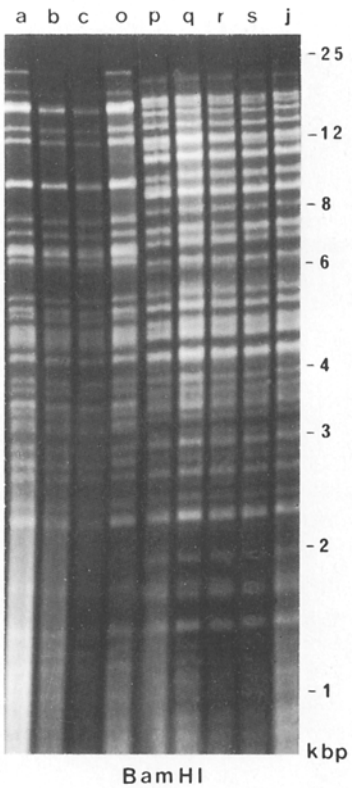

Fig. 3. Bam HI restriction patterns of mt DNAs from various cms or fertile lines of cultivated onion; *lanes a–j* as described in Table 1

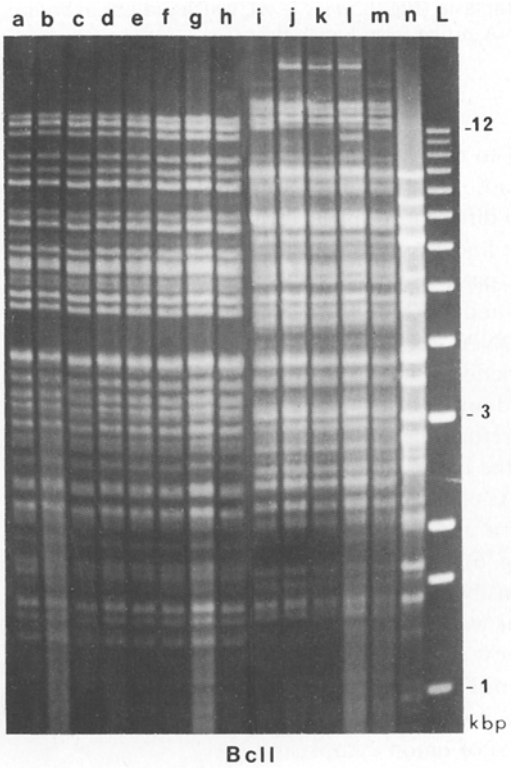

Fig. 2. BclI restriction patterns of mt DNAs from various cms or fertile lines of cultivated onion; lanes as in Fig. 1. *L*, 1-kb ladder (Bethesda Research Laboratory) was used as a molecular weight standard

(Fig. 2) show the same type of variations, except that M_1 and M_2 appear identical.

The second type includes mostly cms cytoplasms (Figs. 1 and 2, lanes a–h), amongst them line c bearing Jones' cytoplasm (Jones and Mann 1963). It was, therefore, called type S. Unlike type M, no variability was detected within S for enzymes Bam HI, Bcl I (Figs. 1 and 2) Eco RI, Bgl II, Eco RV, Pvu II and Hae III (results not shown).

Bam HI patterns of five fertile lines (Fig. 3, lanes o–s) confirm the presence of two mtDNA types in onions. Genotypes p, q, r and s have M-type patterns like control j, whereas genotype o is characterized by an S-type pattern identical to cms lines a, b and c. Comparison with lines i to m show that q and s have an M_1 cytoplasm type, p an M_3 cytoplasm type and r an M_4 cytoplasm type. The presence of an S-type cytoplasm in an open-pollinated variety such as *Dorata di Parma* (genotype o) was unexpected. Its mtDNA was further

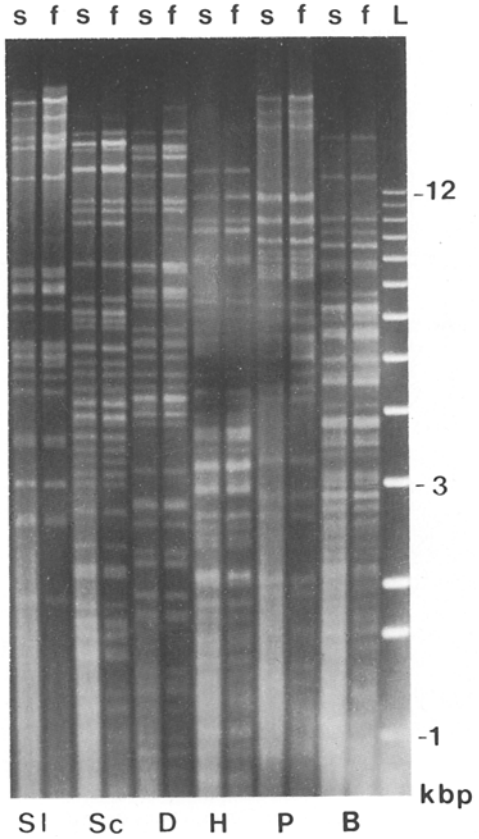

Fig. 4. Sal I (*SI*), Sac I (*Sc*), Dra I (*D*), Hind III (*H*), Pst I (*P*) and Bgl II (*B*) restriction patterns of mt DNA from: s, cms line a and f, fertile line o. *L*, molecular weight standard as in Fig. 2

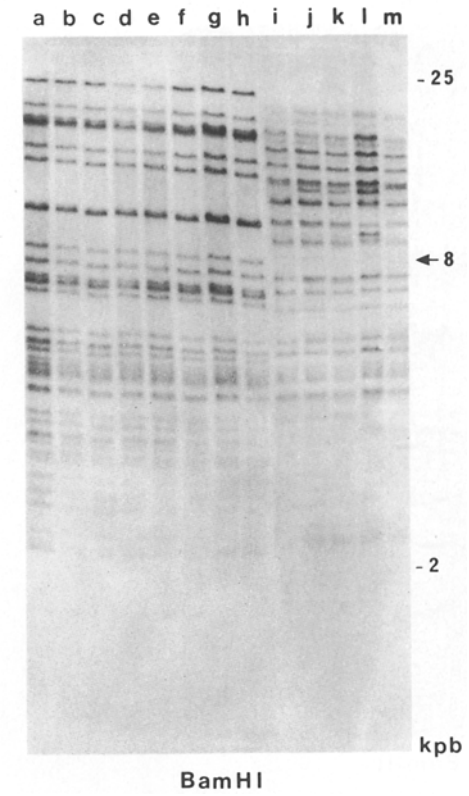

Fig. 5. Hybridization of total Bam HI restricted mt DNA isolated from line a (Table 1) and used as a non-radioactive probe ('Materials and methods') with Bam HI digests of mt DNAs from Fig. 1. The lane corresponding to Lambda marker in Fig. 1 was cut away before gel blotting. The *arrow* indicates the position of an 8-kb fragment, normally present in mt DNA digests from M cytoplasm (Fig. 1, *lanes i–m*), which did not hybridize with mt DNA probe from S cytoplasm

Table 2. Summary of results showing relationship between mitochondrial type and genotype

Mitochondrial type (Bam HI patterns)	Genotype	
S	a	cms S-like
	b	cms S-like
	c	cms S (Jones)
	d	cms S (Jones)
	e	cms S-like
	f	cms S-like
	g	cms S-like
	h	cms S (Jones)
	o	open-pollinated variety
M_1	j	a maintainer line
	q	open-pollinated variety
	s	maintainer line of a cms S line
M_2	k	c maintainer line
M_3	l	cms T-like
	p	open-pollinated variety
M_4	i	cms T-(Berninger)
	m	fertile line on cms T-like
	r	fertile line

compared to cms line a. Restriction patterns for Sal I, Sac I, Dra I, Hind III, Pst I and Bgl II are shown in Fig. 4. No differences appear between fertile genotype o and sterile line a.

S-type patterns, with a total molecular weight between 250 and 300 kbp, seem less complex than M-types. Total mtDNA of g was used as a probe and hybridized with restriction patterns in Fig. 1. An 8-kbp fragment (Fig. 5) did not hybridize in any M-types. This fragment must, therefore, be missing in S-type mtDNA, which confirms the lesser complexity of these mtDNAs.

ctDNAs of S- and M-type lines were compared using genotypes a and j with several different restriction enzymes (Fig. 6). Although both ctDNAs are very similar, they can easily be distinguished by enzymes Bam HI and Eco RV, as well as Eco RI and Dra I. Hind III and Bgl I did not show any differences.

Thus, mtDNA and ctDNA restriction patterns appear to be useful molecular markers to distinguish M- and S-types of onion cytoplasms.

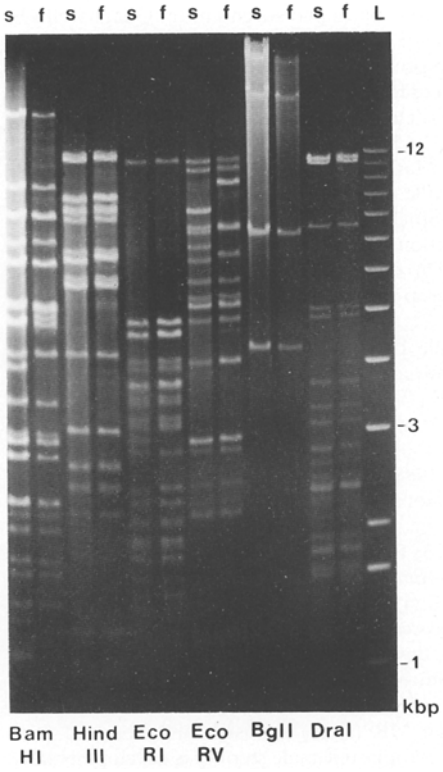

Fig. 6. Bam HI, Hind III, Eco RI, Eco RV, Bgl I and Dra I restriction patterns of ct DNA from: s, cms line a (S cytoplasm) and f, fertile line j (M cytoplasm). *L*, as in Fig. 2

Discussion

Table 2, in which results are summarized, shows that all S cytoplasms observed belong to cms lines or F_1 hybrids on cms cytoplasms, except a cultivar of the open-pollinated variety *Dorata di Parma* (genotype o). It is interesting to note that cms line b was found by French breeders in another cultivar of *Dorata di Parma*. It is also known that Jones' cms occurred in an imported population of *Italian red*, in the United States. Moreover, cms line a was found in France by Berninger in a commercial lot of onions from Rovigo (Italy). Several open-pollinated varieties from Italy must, therefore, bear the S cytoplasm. As these varieties are normally fertile, the Ms nuclear restorer allele must be present at a high allelic frequency. Sterility could be the result of accidental or voluntary crosses with onions of a different origin, bearing the recessive allele ms. This would account for the spontaneous occurrence both in the U.S.A. and in France of cms plants within these Italian populations.

Differences in both mtDNA and ctDNA between S-type lines and M-type lines bring us to the question of the origin of these Italian varieties. McCollum (1976) refers to Central Asia as the primary center of origin for onions, followed by the Near East and the Mediterranean as secondary centers of origin. The data shown here suggest that two distinct species could be at the origin of cultivated onions' cytoplasms, one of which could be particular to the Mediterranean centre and could have led to the Italian varieties, as an example *Dorata di Parma*. An extensive survey of related species from the Mediterranean area should allow this hypothesis to be confirmed and the original species to be identified.

Unlike S cytoplasm patterns, M patterns can be divided into several types. It seems that M_1 and M_2, which have only been found in S sterility maintainer lines (j, k, s) or in a Spanish open-pollinated variety (q), are characteristic of fertile type N cytoplasms recently described (Holford et al. 1988). M_3 includes open-pollinated variety *Jaune paille des Vertus* (p), within which Berninger (1965) described a new T cms plant and an F_1 hybrid (1) bearing a cms cytoplasm distributed by INRA in France. M_4 includes a fertile line (r) and two cms lines (i, m) of Eastern European origin, one of which bears a T cytoplasm.

These results confirm previous genetical analyses (Schweisguth 1973; Holford et al. 1988) describing at least three types of cytoplasms in onions, namely N, S and T. They also bring additional information to recently published results by Holford et al. (1988), who could not detect any differences within the N-group and did not precisely distinguish cms T and N cytoplasms.

Differences shown here within the M-type require further studies on a larger number of genotypes to reach a proper classification of these cytoplasms. It must be emphasized that when two restriction patterns show no differences, this does not prove that the two cytoplasms are identical.

Conclusion

Cultivated onion cytoplasmic DNAs show a high degree of polymorphism. Differences between S, T and N lines have been clearly shown, as have differences within the N-type.

The hypothesis of two distinct cytoplasmic origins must be checked and could lead to a better understanding of nuclear-cytoplasmic interactions in the appearance of cms. Female-maintainer couples a–j and c–k are particularly interesting. These isogenic lines bear the two types of cytoplasms. A comparative molecular study including op variety *Dorata di Parma* could provide a valuable approach to the mechanisms of cms.

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