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Molecular analysis of cytoplasmic male sterility in chives (*Allium schoenoprasum* L.)

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Abstract. The mitochondria of chive plants with normal N or male-sterile S cytoplasms have been examined by restriction fragment analysis and Southern hybridizations of mitochondrial DNA (mtDNA) and in organello protein biosynthesis. Restriction fragment patterns of the mtDNA differed extensively between N- and S-cytoplasms. The percentage of fragments with different mobility varied between 44–48% depending on the restriction enzyme used. In contrast to mtDNA, the restriction fragment patterns of the chloropolast DNA from N- and S-cytoplasms were identical. The organization of the analyzed mitochondrial genes *cox*II, *cox*III, *nad*1 and *nad*3 was different in N- and S-cytoplasms. Comparison of mitochondrial proteins analyzed by in organello translation revealed an 18-kDa protein present only in S-cytoplasm. The restorer gene *X* suppressed the synthesis of that protein in S-cytoplasm. Thus, the 18-kDa protein seems to be associated with the cytoplasmic male-sterile phenotype.

Key words: *Allium schoenoprasum* L. – Cytoplasmic male sterility – Mitochondrial DNA – Mitochondrial proteins – Influence of restorer gene

Introduction

Cytoplasmic male sterility (CMS) is a maternally inherited trait that prevents the production of viable pollen. Since the maturation of female gametes is not disturbed in CMS plants, they can serve as seed parents in hybrid seed production. Thus, CMS represents the best genetic mechanism for breeding hybrids in many plant species; for example, maize, sunflower, sugar beet, rice, *Sorghum*, pearl millet and onion. However, in some plant species CMS has not been found or created as yet, e.g. spinach and leek, while in other plant species, such as rape, cole crops or wheat, a usable CMS system has not yet been established. Also, problems concerning the environmental influence on the expression of CMS and the unexpected occurrence of partially sterile and male-fertile plants in CMS populations have not been explained satisfactorily. Additional knowledge about the molecular and functional bases of CMS could contribute to more effective use of CMS in hybrid breeding.

Kobabe (1965) reported the spontaneous appearance of male-sterile plants in field populations of *Allium schoenoprasum*. The events resulting in pollen abortion are preceded by abnormal hypertrophy of the tapetum in meiosis, and the abortion of microspores occurs during tetrad formation (Ruge et al. 1993; Singh and Kobabe 1969). Tatlioglu (1982) found that male sterility in *Allium schoenoprasum* is inherited by a cytoplasmic factor, S. The dominant restorer gene *X* is able to restore fertility in S-cytoplasm independently of the environmental conditions. Thus, the male-sterile plants must be recessive at the restorer locus. Genotypes with normal cytoplasm N are male fertile, independent of the genetic constitution at the restorer locus. In contrast with other plant species, two other nuclear genes are involved in the CMS system of chives. Temperature-sensitive CMS plants are characterized by a reversible male fertility at a constant temperature of 24 °C if they carry the dominant temperature gene "*T*" (Tatlioglu 1985, 1987). Tetracycline-sensitive CMS plants possessing the recessive

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tetracycline gene "*a*" become male fertile after treatment with the antibiotic tetracycline (Tatlioglu 1986; Tatlioglu and Wricke 1988). The fertility caused by tetracycline is reversible.

In temperature- and tetracycline-sensitive CMS plants there is the possibility of "switching on and off" the male sterility in chives. Chive is perennial, and it can be easily propagated generatively as well as vegetatively. These are additional advantages when selecting and multiplying genetically defined CMS genotypes and maintaining plants by vegetative propagation. Thus, chives represent a favorable CMS system by which to investigate the molecular and functional bases of this trait.

Materials and methods

Plant material

Male-sterile plants and fertility-restored genotypes, both with S-cytoplasm, were selected in F_1, BC_1 and BC_2 populations originating from crosses between male-sterile and fertile plants. Male-fertile plants with N-cytoplasm were selected in the first and second selfed progenies of maintainers. The cultivation of the plant material was carried out in the greenhouse at 20 °C/12 °C, day/night and 16-h daylength. The genetic constitution of the investigated plants are listed in Table 1.

To study the influence of the temperature gene *T* on in organello protein biosynthesis shoots of temperature-sensitive CMS-genotypes, Sxx*TT*, were grown in vitro (Potz 1992). Some of the clone members of each genotype were kept as controls at 20 °C/12 °C, and the rest at 24 °C/24 °C, day/night. Mitochondria were isolated from shoots separately grown at 20 °C/12 °C and 24 °C/24 °C and subjected to in organello translation.

Isolation of chloroplast DNA (cpDNA)

Leaves (25 g) were homogenized in four volumes of chloroplast buffer (0.35 *M* sucrose, 50 m*M* Tris-HCl, pH 8.0, 5 m*M* EDTA, 0.1% bovine serum albumin (BSA), 1 ml/β-mercaptoethanol) according to Brears et al. (1986) in a "Braun Multimix". After filtration through two layers of miracloth (Calbiochem) and cheesecloth, the suspension was centrifuged for 10 min at 2000 *g*. The pellet was lysed in 100 ml chloroplast buffer and centrifuged again. Chloroplasts were resuspended in 50 ml DNase buffer (0.35 *M* sucrose, 50 m*M* Tris-HCl, pH 8.0, 15 m*M* $MgCl_2$, 1 mg DNase I/100 g tissue) and incubated at 4 °C for 1 h. They were then pelleted by centrifugation for 15 min at 2000 *g* and washed 3 times with 50 ml wash buffer (0.6 *M* sucrose, 50 m*M* Tris-HCl, pH 8.0, 20 m*M* EDTA). Lysis of purified chloroplasts was made in 3 ml lysis buffer (50 m*M* Tris-HCl, pH 8.0, 20 m*M* EDTA) with 0.5% SDS and 100 µg/ml Proteinase K for 2 h at room temperature. Proteins were extracted after the addition of 200 µl 5 *M* potassium acetate and incubation for 20 min on ice. After centrifugation for 5 min at 13 000 rpm in an Eppendorf centrifuge nucleic acids were precipitated with 5 *M* ammonium acetate and isopropanol.

Table 1. Genetic constitution of the plants used in the experiments and their response to different temperatures and to tetracycline

Type of plant	Plant number	Genetic constitution[a]	Fertility/sterility[b]		
			20°/12 °C	24°/24 °C	Tet
Maintainer	Gr.7	(N) *xx*	mf	mf	mf
	Gr.16	(N) *xx*	mf	mf	mf
	2025/3	(N) *xx*	mf	mf	mf
	2035/4	(N) *xx*	mf	mf	mf
Restored plants	133	(S) *Xx*	mf	mf	mf
	2018/3	(S) *Xx*	mf	mf	mf
	2018/18	(S) *Xx*	mf	mf	mf
	2023/1	(S) *XX*	mf	mf	mf
	2023/14	(S) *XX*	mf	mf	mf
	2023/16	(S) *XX*	mf	mf	mf
Temperature- and tetracycline-insensitive plants	5204/4	(S) *xx tt A-*	ms	ms	ms
	5222/44	(S) *xx tt A-*	ms	ms	ms
Temperature-insensitive, tetracycline-sensitive plants	5207/10	(S) *xx tt aa*	ms	ms	mf
	5223/73	(S) *xx tt aa*	ms	ms	mf
Temperature-sensitive, tetracycline-insensitive plants	5223/28	(S) *xx T-A-*	ms	mf	ms
	5232/5	(S) *xx T-A-*	ms	mf	ms
Temperature-sensitive, tetracycline-sensitive plants	2004/8	(S) *xx T-aa*	ms	mf	mf
	2022/10	(S) *xx T-aa*	ms	mf	mf

[a] *X/x*, Restorer gene; *T/t*, temperature gene; *A/a*, tetracycline gene
[b] Tet, Tetracycline treatment, ms, male sterile; mf, male fertile

Isolation of mitochondrial DNA

The method used for the mtDNA extraction represents a combination of differential centrifugation steps and DNase I treatment (Chase and Pring 1986). Twenty grams of umbels were picked and disrupted in a "Braun Multimix" in 200ml of buffer A (0.5 *M* sucrose, 50 m*M* Tris-HCl, pH 7.5, 5 m*M* EDTA, 5 m*M* DIECA, 5 m*M* β-mercaproethanol, 0.1% BSA). The suspension was filtered through miracloth and cheesecloth. Cell debris and most of the chloroplasts were removed by centifugation twice for 10 min at 1000 *g*. Mitochondria were pelleted from the supernatant by centrifugation for 15 min at 12000 *g*. A third low speed centrifugation step was inserted. DNase I was added at 40 µg/ml and $MgCl_2$ was added up to a concentration of 10 m*M*. After an incubation period of 1 h at 4 °C DNase was removed by centrifugation through 20 ml of shelf buffer (0.6 *M* sucrose, 20 m*M* EDTA, 10 m*M* Tris-HCl, pH 7.5) at 12000 *g* for 20 min. The mitochondrial pellets were resuspended in 20 ml shelf buffer and centrifuged again. A volume of 2.4 ml NN buffer (20 m*M* EDTA, 50 m*M* Tris-HCl, pH 8.0) with 0.5% SDS and 100 µg/ml Proteinase K was added to each pellet. Probes were incubated for 1 h at 37 °C. The mitochondria were lysed by the addition of 2.4 ml of extraction buffer (80 m*M* EDTA, 0.1 *M* NaCl, 0.15 *M* Tris-HCl, pH 8.0, 1.5% SDS) and incubation at 65 °C for 10 min. After the addition of 200 µl potassium acetate and incubation for 20 min at 0 °C, tubes were centrifuged for 5 min at full speed in an Eppendorf centrifuge. The supernatant was transferred to a new tube that contained 400 µl isopropanol and 40 µl 5 *M* ammonium acetate. MtDNA was pelleted after a 1-h incubation at 20 °C by spinning in an Eppendorf centrifuge for 5 min at 13000 rpm. After being washed twice with 70% cold ethanol the mtDNA was resuspended in 50 m*M* Tris-HCl, pH 8.0, 10 n*M* EDTA. MtDNA was reprecipitated in 75 µl 3 *M* sodium acetate and 500 µl isopropanol. After being washed in 70% ethanol the mtDNA was dried under vacuum and suspended for storage in 200 µl TE buffer (10 m*M* Tris-HCl, pH 8.0, 1 m*M* EDTA).

Digestion, electrophoresis and hybridization of nucleic acids

Aliuots of 10 µg mtDNA or cpDNA were digested with 10 units of restriction enzyme (*Bgl*I, *Bam*HI, *Hin*dIII, *Eco*RI, *Eco*RV, *Kpn*I) in a buffer system provided by Pharmacia. Additionally, 1 µg RNase A was added to the restriction mixture. For complete digestion, the reaction was carried out at 37 °C for 4–6 h. Electrophoresis was performed in 0.8% agarose gels (Biozyme) using TAE buffer (0.04 *M* Tris-acetate, 1 m*M* EDTA). Gels were run at 50 V for 14 h), stained with ethidium bromide and photographed over an UV light source. For Southern hybridizations the DNA was transferred with 0.4 *N* NaOH on Hybond N^+ membranes (Amersham). Filters were prehybridized for 2 h in 0.5 *M* $NaHPO_4$, pH 7.2, 0.5 *M* NaCl, 2 m*M* EDTA, 7% SDS (Church and Gilbert 1984). The heterologous gene probes were *cox*II from maize (provided by D. M. Lonsdale) and *cox*III, *nad*I and *nad*3 from *Oenothera* (provided by A. Brennicke). Radioactive labelling of the gene probes was done by "random priming" (Feinberg and Vogelstein 1983, 1984) with the Oligolabeling kit of Pharmacia using α-[^{32}P]dCTP (Amersham, 3000 Ci/mmol). Unincorporated nucleic acids were removed by chromatography through a Sephadex G50 column. Labelled DNA was heat denatured for 5 min at 95 °C. After addition of the labelled probe (500000 cpm/ml prehybridization buffer) hybridization was conducted overnight at 65 °C in a rotating glass cylinder using a hybridization oven. The membranes were washed twice in 2× SSC/0.5% SDS and 0.2× SSC/0.1% SDS at 65 °C. Wet membranes were wrapped in plastic foil and exposed to Fuji RX film for 2–7 days at −80 °C with intensifying screen.

In organello translation

Mitochondria used for in organello translation were isolated from 3-week-old shoots grown in vitro. Five grams of shoots were homogenized using a mortar and pestle in 100 ml homogenization buffer (0.4 *M* sucrose, 50 n*M* Tris-HCl, pH 7.5, 10 m*M* KH_2PO_4, 1 m*M* EGTA, pH 7.2, 5 m*M* β-mercaptoethanol, 1% BSA, 1% PVP-40). After differential centrifugation steps, the mitochondria were layered on a three-step Percoll density gradient (45%, 26%, 13.5% Percoll in 0.25% sucrose, 0.5% BSA). Purified mitochondria were collected from the 45%/26% interface, washed in 30 ml wash buffer (0.4 *M* mannitol, 10 m*M* KH_2PO_4, 0.5% BSA), pelleted by centrifugation at 16000 *g* for 20 min and immediately used for in organello translation as described by Leaver et al. (1983). Control incubations were carried out with Na-acetate as an energy source in order to check for bacterial contaminations of the mitochondria. Test incubations of mitochondria with erythromycin and chloramphenicol revealed that the radioactive-labelled proteins are products of the mitochondrial translation activity. After incubation for 90 min at 25 °C the incorporation of [^{35}S]methionine was stopped by the addition of cold stop buffer (0.4 *M* mannitol, 10 m*M* L-methionine, 10 m*M* tricine, pH 7.2, 1 m*M* EGTA). Mitochondria were pelleted by centrifugation at 14000 rpm for 5 min in an Eppendorf centrifuge. Pellets were stored at −80 °C until the gel electrophoresis.

Electrophoresis of proteins

The protein pellets were resuspended in loading buffer (10% glycerol, 60 m*M* Tris-HCl, 5% β-mercaptoethanol, 2% SDS, 0.1% bromphenol blue) and heat denatured at 95 °C for 2 min. Equal amounts of TCA-precipitable radioactivity were loaded in each lane of a 15% SDS-polyacrylamide gel. Non-radioactive marker proteins (Serva Proteinmarker 4+5) were loaded in parallel lanes. Electrophoresis was carried out at constant current of 4 mA over 14 h in a buffer system of Laemmli (1970). After fixation and staining with Coomassie Brilliant Blue G, the gels were dried and exposed for 14–21 days to Amersham βmax X-ray films.

Results

Analysis of cpDNA

Restriction enzyme profiles of the cpDNA from N- and S-cytoplasms did not show any difference after digestion with different restriction enzymes. Figure 1 shows the restriction fragment profiles of *Bam*HI and *Hin*dIII digestions. Thus, no variation of banding patterns was detected between the cpDNAs of male-sterile (S) and male-fertile (N) cytoplasms.

Analysis of mtDNA

Figure 2 shows the restriction fragment profiles of mtDNA from S-cytoplasm and from fertile genotypes with the N-cytoplasm after digestion with *Bgl*I, *Bam*HI and *Eco*RI. Several bands in both cytoplasms had the same mobility, but most of them were unique for each cytoplasm. The percentage of fragments with a different mobility in S- and N-cytoplasm varied between

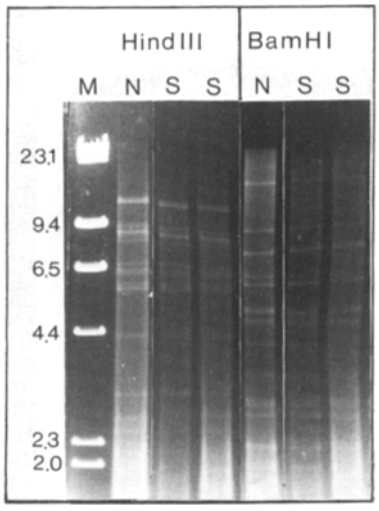

Fig. 1. Agarose gel of cpDNA from genotypes with N-cytoplasm (*N*) and S-cytoplasm (*S*). The cpDNA was digested with *Hin*dIII and *Bam*HI. The size of lambda DNA fragments restricted with *Hin*dIII (*M*) as a marker is given in kb

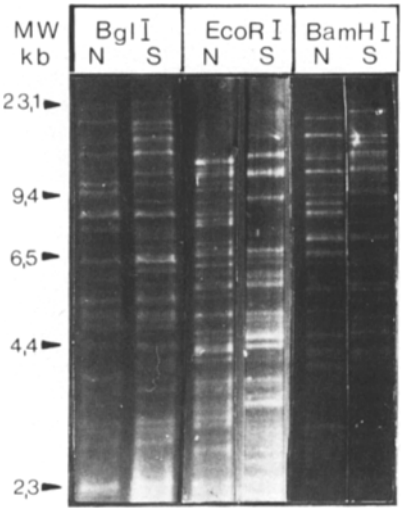

Fig. 2. Agarose gel of mtDNA from genotypes with N-cytoplasm (*N*) and S-cytoplasm (*S*). Digestion with *Bgl*I, *Eco*RI and *Bam*HI showed extensive fragment polymorphisms between the two cytoplasms. *MW*, Size of lambda DNA restricted with *Hin*dIII

44% and −48% depending on the restriction enzyme used. The mtDNA genome of the male-sterile cytoplasm was extensively altered relative to that of the N-cytoplasm. No alterations were detected among different genotypes carrying the N-cytoplasm as well as among different genotypes with the S-cytoplasm. Therefore, the plants with male-sterile (S) cytoplasm and fertile (N) cytoplasm could be identified by their individual mtDNA restriction patterns.

Southern hybridization experiments with mtDNA showed that all of the tested probes containing the coding sequences of the mitochondrial genes *cox*II, *cox*III, *nad*1 and *nad*3 had different organizations in N- and S-cytoplasms. Figure 3 gives an example of hybridization patterns with the enzyme/probe combinations *Bam*HI/*cox*II, *Eco*RV/*nad*3 and *Eco*RV/*nad*1. The few but strong hybridization signals reveal a clear characterization of N- and S-cytoplasms. As these results were reproduceable, the high-molecular-weight hybridization signals cannot be due to partially digested mtDNA.

The restorer gene *X* temperature gene *T* and tetracycline gene *a* had no influence on mtDNA organization. Also, after shoot regeneration in vitro no variation could be detected by Southern hydridizations with heterologous probes *cox*III, *nad*1 and *nad*3 (data not shown).

In organello translation products

Similar to what is found in other plant species, autoradiographs of [^{35}S]-labelled mitochondrial proteins showed about 20 abundant translation products that range in size from 6 to 52 kDa. A comparison of in organello-synthesized proteins of fertile plants with

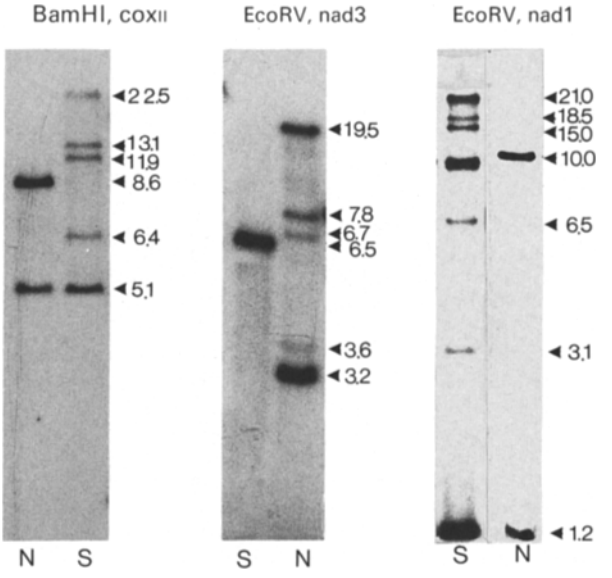

Fig. 3. Autoradiographs of Southern blot hybridizations of mtDNA from fertile genotypes with N-cytoplasm (*N*) and male-sterile genotypes with S-cytoplasm (*S*). Mitochondrial DNA was digested with *Bam*HI or *Eco*RV. Southern blots are probed with mt genes *cox*II, *nad*3 and *nad*1. All gene probes showed polymorphisms in the two cytoplasms. The two lanes *Eco*RV and *nad*1 were assembled from different blots. Hybridization patterns were reproducible from various restriction digests and hybridization experiments. Sizes of fragments in kb were estimated from lambda DNA restricted with *Hin*dIII

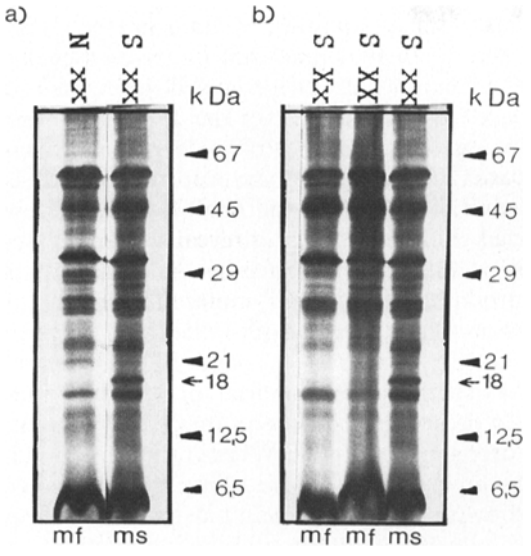

Fig. 4a, b. Autoradiograph of mitochondrial proteins synthesized in organello. **a** From a fertile genotype with N-cytoplasm and a male-sterile genotype with S-cytoplasm; the additional 18-kDa protein in the S-cytoplasm is indicated; **b** from two male-fertile, restored genotypes with S-cytoplasm and different constitutions at the restorer gene and a male-sterile genotype with *S*-cytoplasm. The additional 18-kDa protein is only visible in the Sxx-genotype, and it is completely suppressed in the two genotypes that are restored to fertility. Proteins were labelled with [^{35}S]methionine and separated on 15% SDS-polyacrylamide gels. Radioactive proteins were visualized by autoradiography of the gel for 20 days. *mf* Male fertile, *ms* male sterile

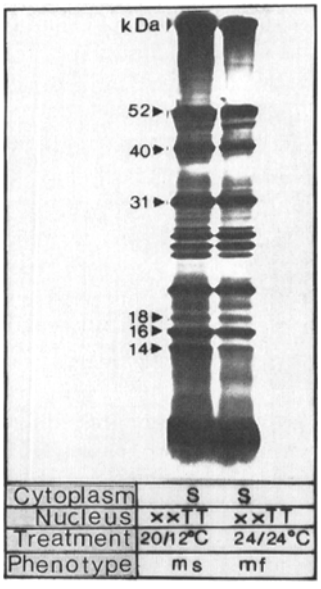

Fig. 5. Autoradiograph of mitochondrial proteins synthesized in organello of a temperature-sensitive male-sterile genotype with S-cytoplasm. Clones were grown in vitro under normal temperatures of 20 °C/12 °C day/night and 24 °C/24 °C. At 24 °C temperature-sensitive male-sterile genotypes revert to male fertility in the greenhouse. Isolated mitochondria from leaves were used for in organello protein synthesis. The autoradiograph shows no difference in protein patterns. The 18-kDa protein is not influenced by temperatures of 24 °C

N-cytoplasm and male-sterile plants with S-cytoplasm yielded a 18-kDa protein that was characteristic of the S-cytoplasm (Fig. 4a). The 18-kDa protein was absent in the N-cytoplasm. The synthesis of this mitochondrial protein was influenced by the restorer gene *X* in the S-cytoplasm (Fig. 4b). Comparisons of [^{35}S] methionine-labelled proteins of male-sterile genotypes (S) *xx* with restored genotypes carrying one or two dominant restorer alleles, (S) *Xx* or (S) *XX*, revealed that the 18-kDa protein was absent in restored genotypes. The restorer gene *X* appeared to suppress the synthesis of the 18-kDa protein in the mitochondria of the S-cytoplasm. A dose of one dominant restorer allele is sufficient for a complete suppression of the 18-kDa protein.

Figure 5 shows the autoradiograph of radioactive-labelled mitochondrial proteins of temperature-sensitive CMS plants grown at 20 °C/12 °C and 24 °C/24 °C (day/night). The 18-kDa protein characteristic of male-sterile plants was detectable in both cases. Neither the occurrence nor the absence of another protein was detected after the treatment at 24 °C/24 °C. Thus, at conditions conducive to male fertility (24 °C/24 °C) there was no detectable influence of the temperature gene *T* on in organello translation of mitochondria isolated from green shoots.

Discussion

No differences could be found in the restriction enzyme patterns of the cpDNA between S- and N-cytoplasms. However, the comparison of the mtDNA restriction enzyme pattern of S- and N-cytoplasms showed extensive alterations. The percentage of bands with different mobilities between the two cytoplasms was about 44–48%. Southern hybridizations with heterologous probes containing the mitochondrial genes *cox*II, *cox*III, *nad*1 and *nad*3 revealed polymorphisms in the male-sterile and male-fertile cytoplasm. These results indicate that in *Allium schoenoprasum* as in various other plants it is the mitochondria and not the chloroplasts that are probably involved in the inheritance of cytoplasmic male sterility. In the related species *Allium cepa* there are differences on the mitochondrial genome between male-sterile S and the male-fertile N cytoplasms comparable with those described for *A. schoenoprasum*. But in contrast to *A. schoenoprasum* there are also some differences between the two cytoplasms in *A. cepa* with respect to the chloroplast genome (De Courcel et al. 1989; Holford et al. 1991).

Similar degrees of mtDNA reorganization for male-sterile cytoplasms relative to fertile cytoplasms have also been described in some other plant species. In

Daucus carota, the portion of restriction enzyme bands with a different mobility is between 81% and 92.5% for the male-sterile (Sp) (petaloid CMS type) and male-fertile (N) cytoplasm and between 81% and 88% for the male-sterile (Sa) (brown anther CMS type) and N-cytoplasm (Pingitore et al. 1989). In Southern hybridization experiments on the mtDNA of *Daucus carota*, probes containing *cox*II, *cox*III, *nad*2, *nad*3, *atpA* and *atp*6 showed different organizations between the two male-sterile cytoplasms (brown anther and petaloid) and male-fertile cytoplasm (Scheike et al. 1992). For probes containing *nad*1 and *nad*5 no differences could be detected.

In comparison with *A. scheoenoprasum*, the differences in the restriction enzyme profiles of the mtDNA between male-sterile and male-fertile cytoplasms in *Sorghum* (Lee et al. 1989) and in sunflower (Crouzillat et al. 1987) are fewer, being restricted to 3–5 variable fragments in the latter two species. In sunflower the differences are limited to a region of 17 kb (size of the mitochondrial genome: 300 kb) containing the *atpA* gene. This region seems to be involved in mtDNA rearrangements (Siculella and Palmer 1988; Köhler et al. 1991). In such plant species it may be possible to find the mtDNA sequence responsible for the CMS trait by means of detailed investigations of the few fragments with different mobilities in male-sterile and fertile cytoplasms.

It is very unlikely that all of the characterized differences on the mtDNA level found for *A. schoenoprasum* are correlated with the CMS trait. Since the number of proteins produced in plant mitochondria by in organello protein biosynthesis is small, it may be possible to find CMS-specific differences between S- and N-cytoplasms at the level of mitochondrial encoded proteins. By means of in organello translation we were able to show that the mitochondria of S-cytoplasm isolated from male-sterile plants (S) *xx* synthesize a novel 18-kDa protein that is absent in the mitochondria of N-cytoplasm isolated from different maintainer plants. In the mitochondria of fertile plants with S-cytoplasm but with dominant restorer gene *X*, (S) *Xx* or (S) *XX*, the 18-kDa protein could not be detected. Thus, the restorer gene is able to suppress the synthesis of the 18-kDa protein and to restore fertility. One dose of the dominant allele is sufficient for complete suppression of the 18-kDa protein (Fig. 4b) and, as the breeding experiments show, for the complete restoration of fertility (Tatlioglu 1982). Thus, the 18-kDa protein seems to be correlated with the CMS phenotype.

Additional proteins synthesized in the mitochondria of male-sterile cytoplasms can also be found in many other plant species including maize (Forde and Leaver 1980), *Vicia faba* (Boutry and Briquet 1982; Boutry et al. 1984), *Sorghum bicolor* (Dixon et al. 1982; Fu 1983; Bailey-Serres et al. 1986a, b), wheat (Boutry et al. 1984), petunia (Nivison and Hanson 1989), sunflower (Horn et al. 1991) and *Daucus carota* (Scheike et al. 1992). Dixon et al. (1982) showed that there is a critical level for the amount of the 13-kDa protein synthesized in mitochondria of male-sterile T-cytoplasm in maize above which male sterility is expressed. In *Sorghum* it was found that the 42-kDa protein synthesized in mitochondria of 9E male-sterile cytoplasm is an altered form of the cytochrome oxidase subunit I that has a molecular weight of 38 kDa in its normal form (Bailey-Serres et al. 1986b).

An influence of the restorer genes on the synthesis of a CMS-specific mitochondrial protein has been previously demonstrated only in maize and petunia. The restorer gene *Rf*1 reduces the synthesis of the 13-kDa mt protein in T-cytoplasm of maize, which could be found by in organello protein biosynthesis (Dewey et al. 1987; Leaver 1989). By probing immunoblots of mitochondrial proteins with an antibody produced to synthetic peptides specified by portions of the CMS-specific reading frame *urf-S* Nivison and Hanson (1989) found that the synthesis of the CMS-specific 25-kDa protein is much lower in fertile petunia plants carrying the male-sterile cytoplasm and a single restorer gene *Rf*. Thus, *A. schoenoprasum* represents the third example in which an influence of the restorer gene on the synthesis of a CMS-specific protein can be shown.

In contrast to our expectation, we were unable to detect an influence of the temperature gene causing male fertility of CMS-plants at 24 °C/24 °C on the expression of the 18-kDa protein. The mitochondria used in this experiments were isolated from leaves. It is possible that the gene *T* is tissue specific, and expressed only in flowers or anthers or that its product is not stable enough to survive the procedure during the isolation of mitochondria and in organello protein biosynthesis. The isolation of mitochondria from the flowers of temperature-sensitive plants could provide further clarification.

Our further investigations are concentrated (1) on verification of the correlation between the 18-kDa protein and CMS by use of tetracycline-sensitive CMS genotypes, (2) on the isolation and characterization of the 18-kDa protein and (3) on the detection of the mtDNA sequences coding for the 18-kDa protein.

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References

Bailey-Serres J, Dixon L, Liddell AD, Leaver CJ (1986a) Nuclear-mitochondrial interactions in cytoplasmic male sterile *Sorghum*. Theor Appl Genet 73:252–260

Bailey-Serres J, Hanson DK, Fox TD, Leaver CJ (1986b) Mitochondrial genome rearrangement leads to extention and relocation of the cytochrome c oxidase subunit I gene in *Sorghum*. Cell 47:567–576

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

Boutry M, Faber AM, Charbonnier M, Briquet M (1984) Microanalysis of plant mitochondrial protein synthesis products. Plant Mol Biol 3:445–452

Brears T, Schardl CL, Lonsdale DM (1986) Chloroplast genome organisation in sugar beet and maize. Plant Mol Biol 6:171–177

Chase CD, Pring DR (1986) Properties of the linear N1 and N2 plasmid-like DNAs from mitochondria of cytoplasmic male-sterile *Sorghum bicolor*. Plant Mol Biol 6:53–64

Church GM, Gilbert W (1984) Genomic sequencing. Proc Natl Acad Sci USA 81:1191–1995

Crouzillat D, Leroy P, Perrault A, Ledoigt G (1987) Molecular analysis of the mitochondrial genome of *Helianthus annuus* in relation to cytoplasmic male sterility and phylogeny. Theor Appl Genet 74:773–780

Dewey RE, Timothy DH, Levings CS III (1987) A mitochondrial protein associated with cytoplasmic male sterility in the T cytoplasm of maize. Proc Natl Acad Sci USA 84: 5374–5378

De Courcel AGL, Vedel F, Boussac JM (1989) DNA polymorphism in *Allium cepa* cytoplasms and its implications concerning the origin of onions. Theor Appl Genet 77:793–798

Dixon LK, Leaver CJ, Brettell RIS, Gengenbach BG (1982) Mitochondrial sensitivity to *Drechslera maydis* T-toxin and the synthesis of a variant mitochondrial polypeptide in plants derived from maize tissue cultures with texas male-sterile cytoplasm. Theor Appl Genet 63:75–80

Feinberg AP, Vogelstein B (1983) A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Ann Biochem 132:6–13

Feinberg AP, Vogelstein B (1984) Addendum: a technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Ann Biochem 137:266–267

Forde BG, Leaver CJ (1980) Nuclear and cytoplasmic genes controlling synthesis of variant mitochondrial polypeptides in male-sterile maize. Proc Natl Acad Sci USA 77:418–422

Fu HY (1983) Variation in mitochondrial translation products and cytoplasmic male sterility in *Sorghum*. Acta Genet Sinica 10:471–476

Holford P, Croft JH, Newbury HJ (1991) Differences between, and possible origins of, the cytoplasms found in fertile and male-sterile onions (*Allium cepa* L.). Theor Appl Genet 82:737–744

Horn R, Köhler RH, Zetsche K (1991) A mitochondrial 16 kDa protein is associated with cytoplasmic male sterility in sunflower. Plant Mol Biol 17:29–36

Kobabe G (1965) Möglichkeiten zur züchterischen Verbesserung von Schnittlauchsorten. Gemüse 1:171–172

Köhler RH, Horn R, Lössl A, Zetsche K (1991) Cytoplasmic male sterility in sunflower is correlated with the co-transcription of a new open reading frame with the *atpA* gene. Mol Gen Genet 227:369–376

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685

Leaver CJ (1989) Structure and function of the mitochondrial genome in relation to cytoplasmic male sterility. Vortr Pflanzenzuecht 16:379–390

Leaver CJ, Hack E, Forde BG (1983) Protein synthesis by isolated plant mitochondria. Methods Enzymol 97:476–484

Lee SH, Muthukrishnan SM, Sorensen EL, Liang GH (1989) Restriction endonuclease analysis of mitochondrial DNA from *Sorghum* with fertile and male sterile cytoplasms. Theor Appl Genet 77:379–382

Nivison HT, Hanson MR (1989) Identification of a mitochondrial protein associated with cytoplasmic male sterility in *Petunia*. Plant Cell 1:1121–1130

Pingitore M, Matthews B, Bottino PJ (1989) Analysis of the mitochondrial genome of *Daucus carota* with male sterile and male fertile cytoplasms. J Hered 80:143–145

Potz H (1992) Interaktion zwischen Chondriom und Korngenom im CMS-System bei Schnittlauch (*Allium schoenoprasum* L.) und Variabilität des Chondrioms unterschiedlicher Provenienzen. PhD thesis, Fachbereich Gartenbau, University of Hanover, Germany

Ruge B, Potz H, Tatlioglu T (1993) Influence of different cytoplasms and nuclear genes involved in the CMS-system of chives (*Allium schoenoprasum* L.) on microsporogenesis. Plant Breed 110:24–28

Scheike R, Gerold E, Brennicke A, Mehring-Lemper M, Wricke G (1992) Unique patterns of mitochondrial genes, transcripts and proteins in different male-sterile cytoplasms of *Daucus carota*. Theor Appl Genet 83:419–427

Siculella L, Palmer JD (1988) Physical and gene organization of mitochondrial DNA in fertile and male sterile sunflower. CMS-associated alterations in structure and transcription of the atpA gene. Nucleic Acids Res 16:3787–3799

Singh VP, Kobabe G (1969) Cyto-morphological investigation on male sterility in *A. schoenoprasum* L. Indian J Genet 29:241–247

Tatlioglu T (1982) Cytoplasmic male sterility in chives (*Allium schoenoprasum* L.). Z Pflanzenzuecht 89:251–262

Tatlioglu T (1985) Influence of temperature on the expression of cytoplasmic male sterility in chives (*Allium schoenoprasum* L.). Z Pflanzenzuecht 94:156–161

Tatlioglu T (1986) Influence of tetracycline on the expression of cytoplasmic male sterility (cms) in chives (*Allium schoenoprasum* L.). Plant Breed 97:46–55

Tatlioglu T (1987) Genetic control of temperature-sensitivity of cytoplasmic male sterility (cms) in chives (*Allium schoenoprasum* L.) Plant Breed 99:65–76

Tatlioglu T, Wricke G (1988) Genetic control of tetracycline sensitivity of cytoplasmic male sterility (cms) in chives (*Allium schoenoprasum* L.). Plant Breed 100:34–40