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Molecular analysis of the cms-inducing MAX1 cytoplasm in sunflower

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Abstract DNA from different male sterility-inducing sunflower cytoplasms was investigated in order to determine whether the cytoplasmic male sterility-inducing insertion of the PET1 mitochondrial DNA (mtDNA) is present in other cytoplasms. In one of these cytoplasms (MAX1) the mtDNA shows 93% sequence homology to the *orf*H522 of the PET1 mtDNA, which is probably responsible for cytoplasmic male sterility (cms) in the latter cytoplasm. In contrast to the situation in the PET1 mitochondrial genome, no transcription of the *orf*H522-related sequence could be detected in lines with the MAX1 cytoplasm. The organization of the MAX1 mtDNA and the mtDNA of a fertile line is shown to be widely different. In the study described here, homology to the mtDNA insertion was also detected in a fertile *Helianthus maximiliani* population, whereas DNA of four other *H. maximiliani* populations showed no hybridization signals.

Key words Sunflower · cms · *orf*H522 · MAX1-cytoplasm · *H. maximiliani*

Introduction

Male sterility is characterized by the inability of a plant to produce viable pollen while being female fertile. The male-sterile phenotype is inherited either as a Mendelian or a cytoplasmic trait. Male-sterile cytoplasm has long been of interest for its usefulness in hybrid seed production in a number of important crop species such as maize, sorghum, and sunflower. In sunflower, the first reliable cytoplasmic male-sterile (cms) system (PET1) was reported by Leclerq (1969) in descendants of an interspecific hybrid between *Helianthus petiolaris* Nutt. and the cultivated sunflower (*H. annuus* L.). This is still the only male-sterile cytoplasm commercially used in sunflower hybrid production world-wide (Friedt 1992). In the meantime other sources of cms have been found in the progenies of interspecific crosses, intraspecific crosses, or spontaneously in natural wild sunflower populations (Crouzillat et al. 1991). There is considerable evidence suggesting that cms is encoded by mitochondrial genes and controlled by nuclear dominant restorer genes (reviewed by Hanson 1991; Horn et al. 1993). The most thoroughly investigated cms type in sunflower is the PET1 cytoplasm. Mapping studies of mitochondrial DNAs (mtDNAs) from isonuclear fertile and PET1 lines have revealed that there is a much greater similarity between the two mtDNAs than has been observed in comparisons of fertile and cms lines of other plant species (Siculella and Palmer 1988). In the former 94% of the mtDNAs are colinear, and detectable alterations are restricted to a mtDNA region of about 16kb–a 11-kb inversion and a 5-kb insertion – immediately downstream of the *atp*A gene (Köhler et al. 1991; Laver et al.1991). The rearrangements seem to be the result of recombination events within an inverted repeat of 261 bp resulting in the creation of a new open reading frame (*orf*H522) that is cotranscribed with *atp*A in sterile sunflower (Köhler et al. 1991; Laver et al. 1991). The predicted translation product of *orf*H522 possibly encodes a 16-kD a protein linked with cms in PET1 (Horn et al. 1991).

To investigate whether the molecular origin of cms in other cytoplasms is alike, we used parts of the mtDNA insertion of the PET1 cytoplasm as probes against the mtDNA of other sources of cms in sunflower and detected one cytoplasm (MAX1) with a homology to this insertion. The correlation of this homologous mtDNA sequence with cms in the MAX1 cytoplasm and the origin of the insertion are discussed.

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Materials and methods

Plant material

The genetic constitution of the lines investigated is illustrated in Table 1. Lines with different cms cytoplasms were kindly provided by Dr. H. Serieys (INRA Breeding Station, Chemin de Mezouls, 34130 Mauguio, France). Fresh leaf material from *H. maximiliani* populations MAX30, MAX40, MAX42, MAX44, and MAX1674, grown at the research station of Rauischholzhausen near Giessen, was used.

DNA analysis

Total DNA of etiolated seedlings and green leaves was isolated using the slightly modified CTAB method. Aliquots of 2–4 g of plant material were homogenized in 1 µl extraction buffer per milligram plant material in a potter for 2–3 min at 1200 rpm and incubated for 5 min at 60 °C. Further isolation procedures were as described by Rogers and Bendich (1985, 1988). Restriction endonuclease-digested DNA was separated on 0.8% agarose gels. Mitochondria were purified according to Horn et al. (1991). Subsequent mtDNA isolation was performed as described by Crouzillat et al. (1987). MtDNA of sterile plants with the cytoplasm MAX1 was digested with *Sal*I and cloned in pUC18 according to Maniatis et al. (1982). Colony hybridization was performed with digoxigenin-labelled probes as described by Düring (1991). Plasmid isolation, electrophoresis, and Southern blotting were done as described by Maniatis et al. (1982). The DNA was fixed by UV cross-linking. Gene probes were labelled by random priming using α-[^{32}P] dATP (NEN) or digoxigenin (Boehringer) or were directly labelled using the ECL kit of Amersham. Hybridizations were done according to the manufacturers' instructions. MtDNA regions of interest were sequenced using the 'T7 Sequencing Kit' of Pharmacia.

RNA analysis

Total RNA was isolated using the guanidin extraction method described by Herdenberger et al. (1990). Formaldehyde gel electrophoresis was performed according to Davis et al. (1986). The RNA was transferred onto nitrocellulose (Schleicher and Schuell) or nylon membranes (Quiabrane) by capillary blot using 20 × SSC and fixed either by heat (80 °C, 2 h) or by UV cross-linking. Hybridization and subsequent washing conditions were according to the kit or membrane manufacturer's instructions.

Results

The probe I1, containing the central part of the PET1 mtDNA insertion, hybridized only with fragments of the DNA from the PET1 and MAX1 cytoplasms. The DNA of the other cytoplasms showed no hybridization signals (Fig. 1).

In order to ivestigate the relationship between *orf*H522 and the mtDNA of MAX1 we cloned the mtDNA of etiolated seedlings of MAX1 using the restriction enzyme *Sal*I. A 9.5-kb *Sal*I clone (MS4) showed hybridization singals to the insertion of PET1 by colony hybridization. Probes of the *atp*A, *cob*, *cox*I, and *cox*III genes did not hybridize to MS4 (not shown). MS4 was subcloned and the part with homology to *orf*H522 was sequenced (Fig. 2). The *orf*H522-related sequence of the MAX1 mtDNA was found to share 93% homology to the published sequence of *orf*H522 (Köhler et al. 1991), with the first nucleotide of the predicted translation start of *orf*H522 in PET1 missing in MAX1. Only the last 29 nucleotides of the PET1 261-bp inverted repeat are present in the MAX1 mtDNA. Upstream of the *orf*H522-related sequence, a region with high homology

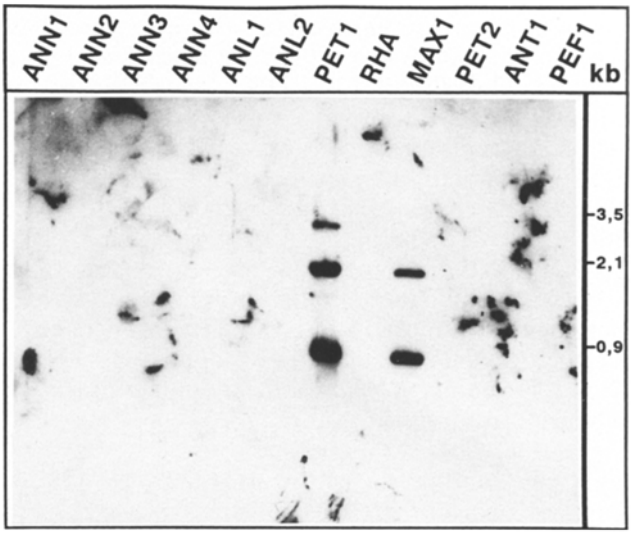

Fig. 1 Southern hybridization of total DNA of sunflower lines with the shown cytoplasms. Hybridization probe was the clone I1 from the central part of the cms-associated insertion of the PET1 mtDNA. The DNA was digested with *Bam*HI. *RHA* fertile sunflower line (*H. annuus* cytoplasm)

Table 1 Description of sunflower lines used in this study

Line	Cytoplasm	Phenotype
RHA 265	ANN1	Male sterile
RHA 265	ANN2	Male sterile
RHA 265	ANN3	Male sterile
RHA 265	ANN4	Male sterile
RHA 265	ANL1	Male sterile
RHA 265	ANL2	Male sterile
HA 89	PET1	Male sterile
RHA 265	Fertile	Fertile
RHA 265	MAX1	Male sterile
RHA 265	PET2	Male sterile
ANT1-B	ANT1	Male sterile
RHA 265	PEF1	Male sterile

Fig. 2 Nucleotide sequence comparison of the mtDNA of MAX1(M) with the complementary sequence fo the 3′-flanking region of the *atp*A gene of the fertile line (*F*) and the 3′-flanking region of the *atp*A gene of the PET1 mtDNA (*P*). The *numbering* of the nucleotides relates to the predicted translation start of the *atp*A gene of the PET1 mtDNA. Sequence homologies between the lines indicated by *asterisks*. The *underlined* region represents a 24-bp inverted repeat; *boldface* bases represent a 25-bp inverted repeat. Regions of homology to *orf*B of *Oenothera* and *atp*6 of rapeseed are *overlined* with a *thin dashed line*; homologous sequences to the *atp*9 gene of sunflower and a cms-associated region of *Petunia* are *overlined*. The amino acid sequence of an open reading frame of 160 amino acids (*orf*M160), present in the MAX1 line, is indicated above the coding region

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M  GGCATTCCTGCCAGCATAAAAATGAAAAAAGACCTAGAGAAAGTAGCTAGTACGTTAGATTAGAATGAGAATTAC
   ******************************************** **  *** ***** **************
F  GGCATTCCTGCCAGCATAAAAATGAAAAAAGACCTAGAGAAAGTACATAACACG-TAGATGAGAATGAGAATTAC

M  GAAGAAAAGAAAAGAACTCTTTCTTTCTCTTCGAAACTCTCATTCGATAAACCAGGATGAGGGTAACTA-ACAAAA
   ********************************************** ***** ***********   ****
F  GAAGAAAAGAAAAGAACTCTTTCTTTCTCTTCGAAACTCTCATTCGATAAATCAGGAGGAGGGTAACTATTAAAAA

M  AAAGAAAGTAAATAGGGTGGAAAT-CCGGGGCAGCCCGGTCCTAGAAAACGTCCGAGTAGAATTGTGAAAAGCGG
   *** ***** ************** ************* ********************** **********  **
F  AAATAAAGTCAATAGGGTGGAAATCCCGGGGCAGCCCGTTCCTAGAAAACGTCCGAGTAGCATTGTGAAAATAGG

M  TAAAATAGCAAAGCGAAAGAAATAAGTGAATTTATCCAGTTGAGGCATCTTGATAAATTCACTTATTTCACACAA
    ******* ********************************************
F  CAAAATAGTAAAGCGAAAGAAATAAGTGAATTTATCCAGTTGAGGCATCTTGA**********************
                              orfH873   <----1699--|** **
P                                          ATGCCTCAACTGGATAAATTCACTTATTTCACACAA
                                           |-1699--->   orfH522

                 M  L  F  R  L  T  A  R  S  L  F  L  S  F  S  F  F   M  L  V  G
M  TTCTTCTGGTCATGCTTTTTCGACTTACAGCACGCTCTCTTTTTCTGTCTTTTTCTTTTTTT-ATGTTAGTAGGC
   *************** ********** * ** * ******************** ******* ** ******* *
P  TTCTTCTGGTCATGCCTTTTCGACTTGCGGCGCACTCTCTTTTTCTGTCTTTTTATTTTTTTGATATTAGTAGCC

    R  S  V  F  M  E  Q  I  T  P  Y  K  K  G  R  S  V  S  G  P  S  S  Q  K  N
M  CGTTCCGTGTTTATGGAACAGATCACGCCCTATAAAAAAGGGCGAAGTGTTTCTGGGCCGAGTTCTCAAAAAAAT
   ********************************   ***** ****** ******* ** * * ******  * *
P  CGTTCCGTGTTTATGGAACAGATCACGCCCTAT---AAAGGCCGAAGTATTTCTGGTCCAAATGCTCAAAGCATT

    L  P  L  P  G  G  S  G  D  D  P  D  K  R  K  K  V  P  V  S  K  D  T  A  N
M  CTCCCCCTCCCTGGTGGATCGGGCGATGACCCGGATAAGCGTAAGAAGGTCCCTGTGTCTAAGGACACTGCAAAC
   ******************** **********    **  * ***** ************************ *
P  CTCCCCCTCCCTGGTGGATCCGGCGATGACCC---AAATAGAAAGAAAGTCCCTGTGTCTAAGGACACTGCAACC

    A  A  V  S  L  L  R  Q  V  I  L  E  I  L  A  R  A  R  D  P  S  L  R  E  G
M  GCAGCCGTGAGTTTACTCCGGCAAGTAATTCTAGAAATTTTAGCCCGAGCGCGGGATCCGTCCTTGCGTGAGGGT
   ************************ *  **** ****************************************
P  GCAGCCGTGAGTTTACTCCGGCAACTCGTTCTTGAAATTTTAGCCCGAGCGCGGGATCCGTCCTTGCGTGAGGGT

    L  H  N  P  T  T  Q  A  W  N  R  A  I  T  T  A  I  Q  E  R  S  G  N  Y  S
M  TTGCACAACCCAACCACCCAAGCGTGGAATAGGGCCATCACCACTGCCATTCAAGAGCGGTCCGGGAACTACTCT
   ********************************************************** ******* ********
P  TTGCACAACCCAACCACCCAAGCGTGGAATAGGGCCATCACCACTGCCATTCAAGAGCAGTCCGGGCACTACTCT

    I  S  T  L  G  A  I  Q  R  T  I  E  V  A  G  E  L  V  F  E  G  E  Q  S  A
M  ATATCCACCTTGGGTGCGATCCAAAGGACCATTGAGGTAGCGGGAGAATTAGTATTTGAGGGGGAGCAGAGTGCT
   ***************** ****** * ******* * * ******************  * ******* *** **
P  ATATCCACCTTGGGTGCAATCCAAGGAACCATTGCGCTCGCGGGAGAATTAGTATTTTCGAGGGAGCAAAGTCCT

    F  F  L  R  V  L  Q  L  V  R  E  R  Y  S
M  TTTTTTTTACGAGTACTTCAACTCGTGAGAGAGAGGTACTCATAGAGAGTTCGAGATGTTAGGAGGTGCAAAACT
   ********************************  ************* ************** ************
P  TTTTTTTTACGAGTACTTCAACTCGTGAGAGAACGGTACTCATAGAGGGTTCGAGATGTTAGAAGGTGCAAAACT

M  AATAGGGGCCGGAGCTGCTACATGATGCTTTCTTATCTTATTCGTTATTACGAGGCTAA-CCCCGAAAATGCCCG
   ****************************************** ***** ******** ***************
P  AATAGGGGCCGGAGCTGCTACATGATGCTTTCTTATCTTATTCG-TATTATGAGGCTAACCCCCGAAAATGCCCG
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to *orf* H873, present in fertile lines as well as in sterile lines with PET1 cytoplasm, is located in opposite orientation (Fig. 3). An incomplete inverted repeat of 24 bp was found at the breakpoint of the homologies to *orf* H522 and *orf* H873. The *orf* H873-related sequence covers an incomplete inverted repeat of 25 bp, also present in PET1 and fertile lines (Köhler et al. 1991). Alignments of these short inverted repeats revealed complete homology. At a position 48 bp downstream of the predicted translation start of *orf* H522 an ATG codon is present that marks the start of an open reading frame consisting of 480 nucleotides (*orf* M160). The 5′ part of the *orf* H522-related region shares homology to *orf* B of *Oenothera* (Hiesel et al. 1987) and the *atp*6 gene of rapeseed (Handa and Nakajima 1992). At a position 5 bp downstream of the predicted termination codon of *orf* M160 a region showing homology to the *atp*9 gene of *Pisum* (Morikami and Nakamura 1987) and a cms-associated region of petunia (Young and Hanson 1987) was identified.

Transcription analysis

Northern blots with probes covering the *atp*A gene of sunflower were performed to investigate whether in MAX1 the *atp*A gene is cotranscribed with other sequences as described for PET1, but only one transcript of 1600 nt was found in MAX1 just like in fertile lines (Fig. 4). The same and other blots were used in hybridization experiments with probes covering parts of the *orf* H522 to investigate the transcription of *orf* M160. These probes failed to detect any transcript of MAX1 (data not shown).

In order to compare the organization of the MAX1 and fertile mitochondrial genomes hybridization experiments with different mitochondrial gene probes were conducted (Table 2). Differences could be found in the hybridization patterns of these two mitchondrial genomes with probes including the genes *atp*A, *cob*, *cox*III, and *atp*6, whereas no different patterns appeared after hybridization with the gene probes 5S + 18S and *cox*I (Table 3).

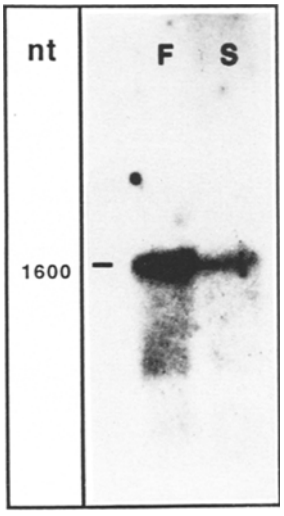

Fig. 4 Transcript analysis of total RNA isolated from fertile (*F*) *H. annuus* and male-sterile (*S*) MAX1 sunflower. RNA was hybridized to the *atp*A gene of sunflower

Table 2 Genes and clones used as probes

Gene/clone	Enzyme	References
*atp*A	*Pst*I/*Hin*dIII	Schuster and Brennicke 1986
cob	*Eco*RI	Schuster and Brennicke 1985
*cox*I	*Eco*RI	Hiesel et al. 1987
*cox*III	*Eco*RI/*Pst*I	Hiesel et al. 1987
*atp*6	*Nhe*I	Schuster and Brennicke 1987
5S + 18S rRNA	*Bam*HI	Brennicke et al. 1985
I1	*Asp*718/*Sma*I	J. Gerlach, personal communication
MS4	*Sal*I	

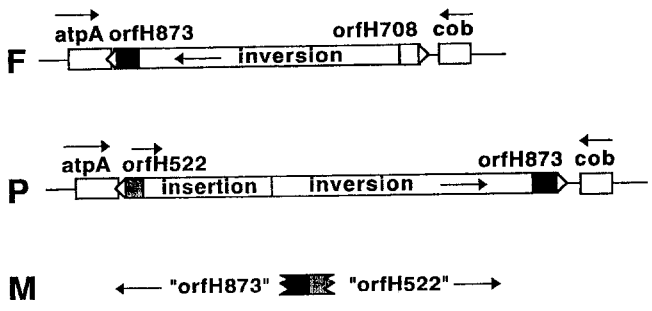

Fig. 3 Organization of the rearranged region of sunflower mtDNA in different cytoplasms. The mtDNA of the fertile line (*F*) and the male-sterile line carrying the PET1 cytoplasm (*P*) differ in an 11-kb inversion and a 5-kb insertion (Köhler et al. 1991). The rearranged mtDNA region is flanked by a 261-bp inverted repeat (>). For the MAX1 cytoplasm (*M*), the homologies to the PET1 cytoplasm are marked with " ". The *arrows above* the genes indicate the direction of transcription

Investigations on the origin of the *orf* H522-related sequence

Total DNA was isolated from leaves of *H. maximiliani* populations MAX30, MAX40, MAX44, and MAX1674 and hybridized with the probe I1. Only the population MAX30 showed hybiridization signals (Fig. 5). Because of the intensity of the signals it can be assumed that the homologous sequence is located in the mitochondrial genome of MAX30. By means of hybridization experiments we compared the mitochondrial genomes of the male-sterile cytoplasm MAX1 and *H. maximiliani* population MAX30. The following probe/enzyme combinations were tested: *cox*I/*Eco*RI, *cox*I/*Hin*dIII, *atp*A/*Eco*RI, *cox*III/*Eco*RI, *cox*III/*Hin*dIII, *cob*/*Eco*RI, *cob*/*Hin*dIII, I1/*Eco*RI, I1/*Bam*HI, MS4/*Eco*RI. No differences in the hybridization patterns were found (data not presented).

Table 3 Comparison of the hybridization patterns of the mtDNAs of MAX1 and the fertile cytoplasm (+ differences detected, − no differences detected)

Probe	Enzyme	Differences	Probe	Enzyme	Differences
	*Bam*HI	−		*Ban*HI	+
*cox*I	*Eco*RI	−		*Eco*RI	−
	*Hin*dIII	−	*cob*	*Hin*dIII	+
	*Pst*I	−		*Pst*I	−
				*Bgl*II	+
	*Bam*HI	+		*Bam*HI	+
	*Eco*RI	+		*Eco*RI	−
*atp*A	*Hin*dIII	−	*cox*III	*Hin*dIII	+
	*Pst*I	+		*Pst*I	−
	*Bgl*II	+		*Bgl*II	+
	*Bam*HI	−	*atp6*	*Sac*I	+
	*Eco*RI	−			
5S + 18S	*Hin*dIII	−	*atp9*	*Bgl*II	−
rRNA	*Pst*I	−			
	*Bgl*II	−			
	*Sac*I	−			
	*Xba*I	−			

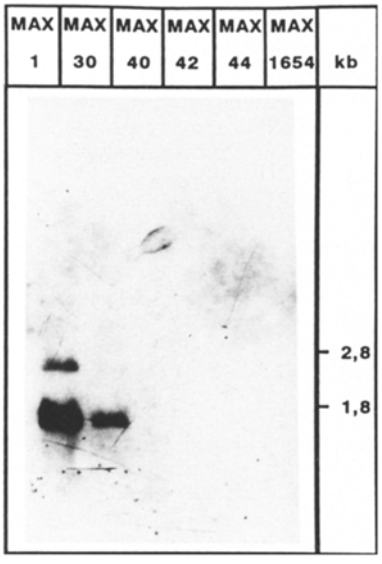

Fig. 5 PET1-insertion-specific Southern hybridization signals of total DNA in the *H. maximiliani* population MAX30. The DNA of the shown sunflower populations and the male-sterile line MAX1 was digested with *Eco*RI. Hybridization clone as in Fig. 1. The 2.8-kb signal of MAX30 is present but not visible on this blot

Discussion

In sunflower, the mtDNAs of fertile and PET1-cms lines are colinear over a contiguous region encompassing 94% of the genome (Siculella and Palmer 1988). Within a rearranged region of 11 and 16 kb, respectively, a 5-kb insertion is present in the mtDNA of the PET1 cytoplasm that is thought to play a role in the cms of sunflower (Köhler et al. 1991). Using a probe that includes the central part of this insertion we hybridized the DNAs of various sunflower lines chosen on the basis of different cms cytoplasms. The DNA of lines with the MAX1 cytoplasm showed hybridization signals; therefore, this mtDNA region must be present there. On the contrary, the whole insertion or at least the central part of the insertion of the PET1 cytoplasm is obviously absent in mtDNAs of other cytoplasms studied, provided that the insertion does not occur in sublimons (Leaver et al. 1988). This means that the molecular origin of cms in these cytoplasms must be different from PET1-cms. The same situation exists in other species as, for example, in maize, where the specific mechanisms of cms differ among cmsC, cmsS, and cmsT (reviewed in Braun et al. 1992). Spassova et al. (1992) used Southern analysis to investigate mtDNA in the region of the *atp*A gene of additional cms sources of sunflower. No differences appeared to the PET1 mtDNA in the hybridization patterns of the *atp*A gene. Therefore, the mtDNA of these cms lines obviously carry the insertion of the PET1 cytoplasm.

The sequence data of the mtDNAs of PET1 and MAX1 in the *orf* H522 region show a homology of 93%. In PET1, *orf* H522 is cotranscribed with the *atp*A gene (Köhler et al. 1991; Laver et al. 1991). The translation product of *orf* H522 is thought to code for a 16-kDa polypeptide that is present in PET1 but not detectable in fertile lines with *H. annuus* cytoplasm and therefore possibly involved in cms (Horn et al. 1991; Laver et al. 1991; Smart et al. 1992). However, we could neither detect a cotranscription of the *atp*A gene nor any transcripts of *orf* M160, and so it seems to be unlikely that *orf* M160 is directly involved in the MAX1-cms. However, we cannot exclude transcripts not detectable by hybridization assays (Finnegan and Brown 1990). Moreover, tissue-specific transcription of *orf* M160, e.g., in anthers, is conceivable because our investigations were made with etiolated seedlings. In petunia, the fused mitochondrial gene associated with cms is developmentally regulated (Young and Hanson 1987). An increase in the pcf transcript compared to other transcripts is observed in anthers, and in sunflower, tissue-specific effects on the transcription of mitochondrial genes were detected by Gerlach and Zetsche (1992).

The rearrangement of the mtDNA in the MAX1 cytoplasm might be traceable to the 24-bp incomplete inverted repeat, which could form a stem-loop structure, located at the breakpoints of homology to *orf* H522 and *orf* H873, respectively. Certain possibilities are plausible with respect to the origin of this mtDNA section because the 24-bp inverted repeat shares homology to other parts of the mtDNA of sunflower, for example, to the 5′-regions of *orf* H873 , *orf* H708, and *orf* H522 (Köhler 1991) and to different parts of mtDNA of other species, for example, to *orf* B of *Oenothera* (Hiesel et al. 1987), detected in sunflower in the 5′-flanking region of *cox*III (Quagliarello et al. 1990). Further investigations are necessary to show which regions are causally involved in the rearrangements of the MAX1 mtDNA.

The origin of the *orf* H522-related region

Orf H522 and the PET1 insertion as a whole seem to be composed of different parts, including sequences of mtDNA, the nuclear genome, and parts of yet unknown origin (Zetsche and Horn 1993). Hybridization experiments with probes covering parts of the insertion did not detect a related sequence in the mitochondrial genome of *H. petiolaris* (Horn 1991), the expected donor of the PET1 cytoplasm. This means that the insertion was created by the interspecific cross *H. petiolaris* × *H. annuus*. Regarding the MAX1 cytoplasm, we found a homologous region in the DNA of *H. maximiliani* population MAX30. In this context, it is especially interesting that other investigated *H. maximiliani* populations did not show hybridization signals with this probe. This suggests that the cytoplasm of MAX30 is closely related to the MAX1 cytoplasm, a conclusion confirmed by the fact that no differences in hybridization patterns were found using mitochondrial probes. The situation is possibly similar in the PET1 cytoplasm, and a search among other *H. petiolaris* populations will finally detect the insertion in one of these populations. This poses the question of whether the cms phenotype results from nuclear-cytoplasmic incompatibility (Hanson 1991) or rather from interaction with the 16-kDa polypeptide (Horn et al. 1991).

Whether the MAX1 mtDNA region related to the insertion in PET1 causes pollen sterility in sunflower lines carrying the MAX1 cytoplasm remains unknown. Relative to the PET1 mtDNA, additional differences between the MAX1 mtDNA and the mtDNA of the fertile *H. annuus* cytoplasm can be identified. However, differences in mitochondrial genome organization are well known in plants and mostly do not influence the function of the genes (Leaver 1989). The identification of fertile *H. maximiliani* population MAX30 as a possible ancestor of male-sterile cytoplasm MAX1 offers the opportunity now to elucidate whether the cms phenotype originated by recombination events in the mitochondrial genome of MAX1 or by nuclear-cytoplasmic interactions after substitution of the *H. maximiliani* nuclear genome by that of *H. annuus*.

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References

Braun JC, Brown GG, Levings CS III (1992) Cytoplasmic male sterility. In: Herrmann RG (ed) Plant gene research: cell organelles. Springer, Heidelberg Berlin New York, pp 219–245

Brennicke A, Möller S, Blanz PA (1985) The 18S and 5S ribosomal genes in *Oenothera* mitochondria: sequence rearrangements in the 18S and 5S genes of higher plants. Mol Gen Genet 198:404–410

Crouzillat D, de la Canal L, Perrault A, Ledoigt G, Vear F, Serieys H (1991) Cytoplasmic male sterility in sunflower: comparison of molecular biology and genetic studies. Plant Mol Biol 16:415–426

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

Davis LG, Dibner MD, Battey JF (1986) Basic methods in molecular biology. Elsevier Sci Publ, New York

Düring K (1991) Ultrasensitive chemiluminescent and colorigenic detection of DNA, RNA, and proteins in plant molecular biology. Anal Biochem 169:433–438

Finnegan PM, Brown GG (1990) Transcriptional and post-transcriptional regulation of RNA levels in maize mitochondria. Plant Cell 2:71–83

Friedt W (1992) Present state and future prospects of biotechnology in sunflower breeding. Field Crops Res 30:425–442

Gerlach J, Zetsche K (1992) Der Einfluss des Kernhintergrunds auf die gewebsspezifische Expression mitochondrialer Gene von cytoplasmatisch sterilen, fertilen und restorierten Sonnenblumen. Deutsche Botaniker-Tagung 1992, Berlin, p 197

Handa H, Nakajima K (1992) Different organization and altered transcription of the mitochondrial *atp6* gene in the male-sterile cytoplasm of rapeseed (*Brassica napus* L.). Curr Genet 21:153–159

Hanson MR (1991) Plant mitochondrial mutations and male sterility. Annu Rev Genet 25:461–486

Herdenberger F, Evrard JL, Kuntz M, Tessier LH, Klein A, Steinmetz A, Pillay DTN (1990) Isolation of flower specific DNA clones from sunflower. Plant Sci 69:111–122

Hiesel R, Schobel W, Schuster W, Brennicke A (1987) The cytochrome oxidase subunit I and subunit III genes in *Oenothera* mitochondria are transcribed from identical promotor sequences. EMBO J 6:29–34

Horn R (1991) Untersuchungen zu den molekularen Ursachen der cytoplasmatischen Pollensterilität bei der Sonnenblume (*Helianthus annuus*). Phothesis, Univ Giessen

Horn R, Köhler RH, Zetsche K (1991) A mitochondrial 16-kDa protein is associated with cytoplasmic male sterility in sunflower. Plant Mol Biol 7:29–36

Horn R, Hahn V, Friedt W (1994) Recombination: Effects on structure and function of the mitochondrial genome. Prog Bot 55: 219–235

Köhler RH (1991) Molekulare Analyse der cytoplasmatischen Pollensterilität bei der Sonnenblume (*Helianthus annuus*). PhD thesis, University of Giessen, Giessen, FRG

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 *atp*A gene. Mol Gen Genet 227:369–376

Laver HK, Reynolds SJ, Moneger F, Leaver CJ (1991) Mitochondrial genome organization and expression associated with cytoplasmic male sterility in sunflower (*Helianthus annuus*). Plant J 1:185–193

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

Leaver CJ, Isaac PG, Small ID, Bailey-Serres J, Liddel AD, Hawkesford MJ (1988) Mitochondrial genome diversity and cytoplasmic male sterility in higher plants. Philos Trans R Soc London Ser B 319:165–176

Leclercq P (1969) Une stérilité mâle cytoplasmique chez le tournesol. Ann Amél Plant 19:99–106

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Morikami A, Nakamura K (1987) The pea mitochondrial ATPase subunit 9 gene is located upstream of the ATPase alpha-subunit gene. Nucleic Acid Res 15:4692

Quagliariello C, Saiardi A, Gallerani R (1990) The cytochrome oxidase subunit III gene in sunflower mitochondria is cotran-

scribed with an open reading frame conserved in higher plants. Curr Genet 18:355–363
Rogers SO, Bendich AJ (1985) Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissues. Plant Mol Biol 5:69–76
Rogers SO, Bendich AJ (1988) Extraction of DNA from plant tissues. Plant Mol Biol Manual A6:1–10
Schuster W, Brennicke A (1985) TAG-termination codon in the apocytochrome *b* gene from *Oenothera* mitochondria. Curr Genet 9:157–163
Schuster W, Brennicke A (1986) Pseudocopies of the ATPase alpha-subunit gene in *Oenothera* mitochondria are present of different circular molecules. Mol Gen Genet 204:29–35
Schuster W, Brennicke A (1987) Nucleotide sequence of the *Oenothera* ATPase subunit 6 gene. Nucl Acid Res 15:90–92
Sicullela 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 *atp*A gene. Nucleic Acids Res 16:3787–3798
Smart CJ, Moneger F, Leaver CJ (1992) Correlation between cytoplasmic male sterility and the expression of a noval reading frame in sunflower. In: DFG-Workshop Interactions Three Genomes. Berlin, p 129 (Abstr)
Spassova M, Christov M, Bohorova N, Petrov P, Dudov K, Atanassov A, Nijkamp HJJ, Hille J (1992) Molecular analysis of a new cytoplasmic male sterile genotype in sunflower. FEBS 297: 159–163
Young EG, Hanson MR (1987) A fused mitochondrial gene associated with cytoplasmic male sterility is developmentally regulated. Cell 50:41–49
Zetsche K, Horn R (1993) Molecular analysis of cytoplasmic male sterility in sunflower (*Helianthus annuus*). In: Kück U, Brennicke A (eds) Plant mitochondria, Springer, Berlin Heidelberg New York, (in press)