MOLECULAR ANALYSIS OF THE MITOCHONDRIAL DNA OF CYTOPLASMIC MALE STERILE SUNFLOWER CYTOPLASM MAX1

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Introduction

The ability to produce hybrid seed has been of fundamental importance for sunflower breeding, both, in terms of increasing yield and generating wealth. The genetic approach to the production of F_1-hybrid seed was made possible by exploitation of cytoplasmic male sterility (CMS), a widespread trait reported in over 140 species of higher plants, that is specified, in most cases, by the mitochondrial genome (HANSON 1991, LEAVER 1992).

In sunflower the 'classical' *Helianthus petiolaris* (PET1) cytoplasm (LECLERCQ 1969) has been exclusively used to produce commercial sunflower hybrids so far. However, the interest of plant breeders in other CMS materials is increasing in order to introduce cytoplasmic diversity into the crop. It seems that rearrangements of the mitochondrial DNA (mtDNA) are responsible for CMS. In order to contribute to a better understanding of this phenomenon, mtDNAs of different CMS-inducing cytoplasms were investigated in the present study.

Material and Methods

Helianthus maximiliani populations MAX30, MAX40, MAX42, MAX1654 and cytoplasms listened in table 1 were analyzed. Both, mtDNA and total DNA were isolated as previously described by KÖHLER *et al.* (1991).

Table 1: Origin of cytoplasmic male sterilities in sunflower (CROUZILLAT *et al.* 1991)

FAO code	Origin	FAO code	Origin
1. Spontaneous CMS		3. Interspecific Hybrids	
ANN1	*H. annuus* (California)	PET1	*H. petiolaris*
ANN2	*H. annuus* (Texas)	PET2	*H. petiolaris*
ANN3	*H. annuus* (Arizona)	PEF1	*H. petiolaris* ssp. *fallax*
ANN4	*H. annuus* (Australia)	GIG1	*H. giganteus*
2. Intraspecific Crosses		MAX1	*H. maximiliani*
ANL1	*H. annuus* ssp. *lenticularis*		
ANL2	*H. annuus* ssp. *lenticularis*		

Results

Only the mtDNA of the MAX1 cytoplasm shows homology to the *orfH522*, an open reading frame which is correlated with the CMS phenotype in the PET1 cytoplasm (fig. 1). The *orfH522* is cotranscribed with the *atpA* gene in male sterile lines carrying the 'Leclercq-cytoplasm'. However, opposite to the PET1 cytoplasm, the insertion showing homology to the *orfH522* is not located in the 3'-flanking region of the *atpA* gene in the MAX1 cytoplasm. But there exists a homology to the *orfH873* which is also present in

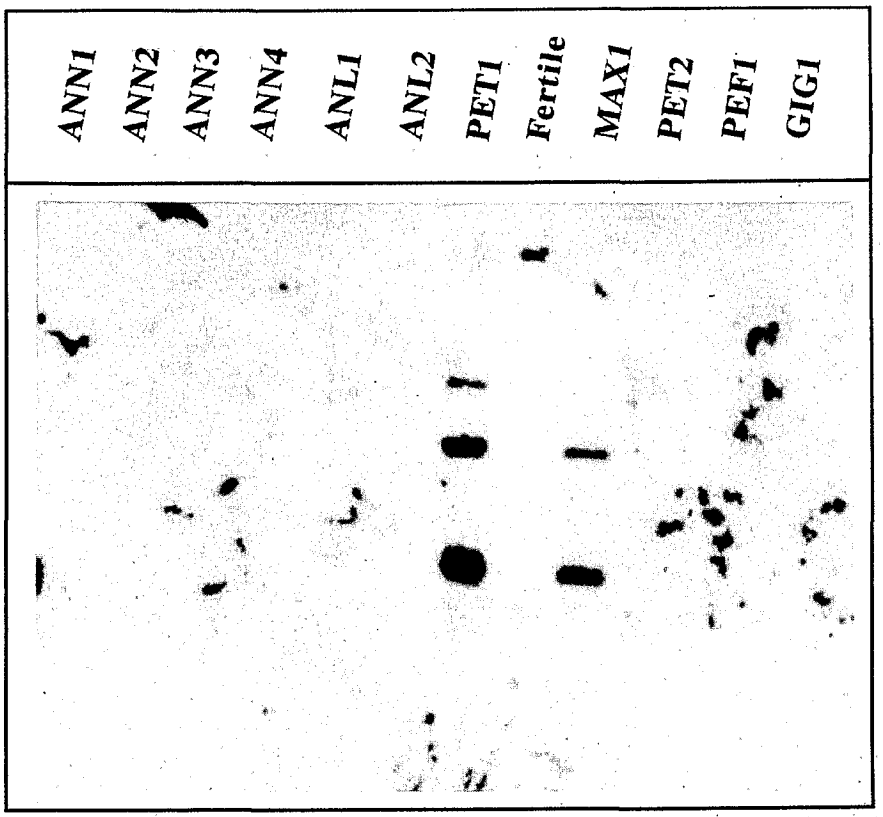

Fig. 1: Detection of homologies to the 5kb insertion of the PET1 cytoplasm in other cytoplasms. A 1.7kb fragment from the PET1 mtDNA, covering a part of the insertion (see Fig. 2) was used as a probe against BamHI digests of total DNA of sunflower lines, carrying different cytoplasms.

the 3'-flanking region of the *atpA* gene in fertile lines and in the 3'-flanking region of the *cob* gene in male sterile lines with PET1 cytoplasm (Fig. 2). A 24bp inverted repeat can be identified in the rearranged area of the MAX1 mitochondrial genome.
The mtDNA of the MAX1 cytoplasm shows additional differences in comparison to the mtDNA of the fertile line RHA265. There are at least differences in the regions of the *cob*, *cox*III, *atp*6 and *atpA* genes, whereas no differences could be found in the region of the *cox*I gene

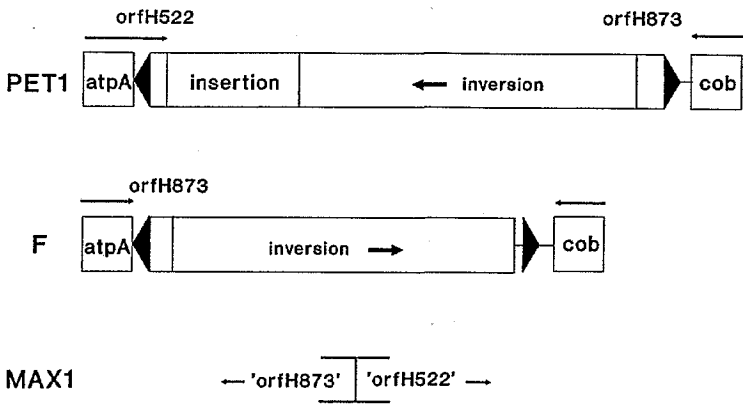

Fig. 2: Comparison of the rearranged region of sunflower mtDNA in different cytoplasms. The mtDNA of the fertie line and the male sterile PET1 line differ in an 11kb inversion and a 5kb insertion. The rearranged mtDNA region is flanked by a 261-bp-inverted repeat (solid arrows) (KÖHLER *et al.* 1991). A clone (9kb) covering a part of the MAX1 mtDNA shows homology to *orf*H873 as well as to *orf*H522; this clone shows no homology to the *atpA* gene and the *cob* gene, respectively.

Only one of the investigated *H. maximiliani* populations (MAX30) shows homology to a probe of the insertion (fig. 2) of the PET1 mtDNA by hybridizing against total DNA (fig. 3). This *H. maximiliani* population differs from the other *H. maximiliani* populations investigated with regard to the organization of the mtDNA in the region of the *atpA* gene.

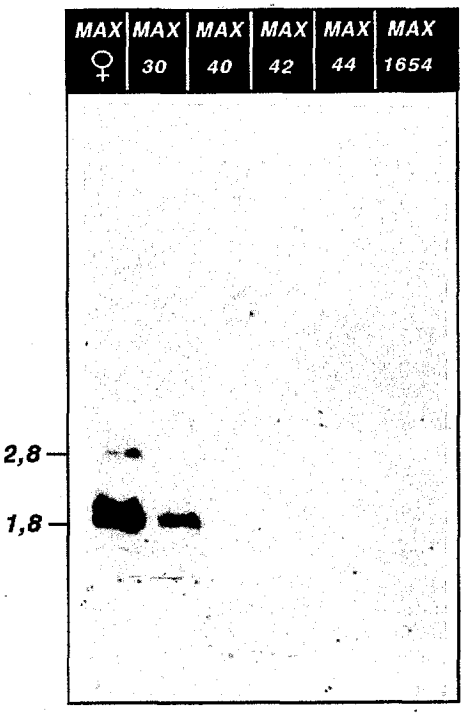

Fig. 3: Detection of homologies to the 5kb insertion of the PET1 cytoplasm in the total DNA of *H. maximiliani* populations. The same clone as in fig. 1 was used as a probe against EcoRI digests of total DNA.

Discussion

The sunflower species *H. petiolaris* and *H. maximiliani* are taxonomically classified in different sections (SCHILLING und HEISER 1981). However, the CMS-inducing cytoplasms derived from these species show homology of their mtDNAs in the area of the CMS-correlated 5kb insertion of the PET1 cytoplasm (fig. 2), whereas no homology on the mtDNA of the investigated CMS-inducing plasms could be detected by this probe (fig. 1).

The physical organization of the mtDNA of plants with MAX1 cytoplasm is different from the organization of the PET1 mtDNA reported by SICULLELA and PALMER (1988).

Sequence analysis revealed a rearrangement of the mtDNA region of MAX1 homologous to *orfH522* and *orfH873* as compared to the organization of the mtDNA of fertile plants or plants with PET1 cytoplasm. It seems that in the MAX1 cytoplasm a recombination event occurred in between the homologous region of *orfH522* and *orfH873*; the 24bp inverted repeat might be involved in this recombination. It is not clear yet, whether this recombination is involved in generating the CMS phenotype in MAX1 or if other rearrangements in the mtDNA play an important role.

The origin of the 5kb insertion of the PET1 mtDNA is unknown so far. However, the total DNA of one of the investigated *H. maximiliani* populations shows homology to a probe containing parts of this insertion. It seems to be likely, that the male sterile cytoplasm MAX1 arose from this population. The total DNA of this population shows the same hybridization patterns as the mtDNA of male sterile plants carrying the MAX1 cytoplasm, but distinct patterns than the other investigated *H. maximiliani* populations when using the *atpA* gene as probe. Further investigations of the mtDNAs of MAX1 and *H. maximiliani* (MAX30) are underway in order to finally clarify the molecular origin of the male sterility in the MAX1 CMS-system.

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