CYTOPLASM - NUCLEUS RELATIONSHIPS IN THE CMS POLLEN FERTILITY RESTORATION IN FUNDULEA 1 (ANT-1) CMS OF SUNFLOWER

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Summary :

The Fundulea-1 (Ant-1) cytoplasmic male sterility, different to other CMS types, performs completely sterile plants and fully sterile generations. The pollen fertility restoration pattern in this case involved more than one dominant genes. The restorer genes for Ant-1 CMS were detected in the wild species H annuus ssp. texanus which is the origin of this CMS source.

The hybridization of total DNA from the two Ant-1 CMS inbreds , from their maintainers , and from the H annuus ssp texanus species with the sunflower pLT mitochondrial plasmid 32P labelled as a probe, revealed that there are no sequences homologous with this plasmid in the Ant-1 CMS, but a homologous plasmisd was detected in the maintainer carrying the *H. annuus* cytoplasm.

Introduction

The cytoplasmic male sterility (CMS) in sunflower revealed three patterns depending upon the relationship between the cytoplasm and the nucleus. They are : - autoplasmic, cytoplasm and nucleus come from the same species, - holoplasmic, cytoplasm and nucleus come from two populations of the same species and, - alloplasmic, when they come from two different species (9). In any case the mechanism of these patterns is very few elucidated. For the latter pattern frequently described in Helianthus, the interaction between cytoplasm and nucleus lead to sterile progenies after some backross generations only. There is no a pertinent explanation of the pollen abortion.

The disturbances in pollen development are unique to each species. Investigations of the underlying molecular events in several species and CMS have confirmed that a diversity of mitochondrial mutations is most likely involved in this phenotype (10).

The Fundulea 1 (ANT-1) CMS has been detected in the hybrid *H. annuus ssp texanus* x sunflower (12)). The genetic pattern of pollen fertility restoration in the case of ANT-1 CMS, is one dominant allele (3).

In *Helianthus annuus*, the circular 1,45 kb mitochondrial (pLT or P_1) has been discribed (1)(4)(5). This plasmid molecule was observed with male fertile cytoplasm, but cannot be detected by agarose gel electrophoresis, in nuclear isogenic lines with Leclercq CMS (7) . The presence of the pLT plasmid allows to recognize different cytoplasms (2) (6)-.

The present paper reports - the genetic analysis of the pollen fertility restoration pattern in the *H. annuus* ssp.*texanus* x sunflower offsprings, - the observations on the CMS stability, and - the molecular assay to look for pLT in the ANT-1 CMS source.

Material and methods

The plants of two ANT-1 cms lines (LC-1011 and R-10639) and their male fertile maintainers were grown in the green house at Dijon. The behaviour of ANT-1 CMS was studied in three backcrosses (BC) of [(*H. annuus ssp texanus* x sunflower) x sunflower] and in hundreds of sunflower inbreds crossed with ANT-1 CMS source. Pollen fertility restoration pattern was estimated by segregation ratio in BC1 and BC2 generations of two sunflower inbreds crossed with the restoring factor (Rf) for the ANT-1 CMS source. The Rf were identified in the hybrid H.*annuus* ssp.*texanus* x sunflower.

Molecular analysis of the ANT-1 CMS (A and B lines) and the wild species H.*annuus* ssp.*texanus* were performed on the total DNA, hybridized by the pLT plasmid ^{32}P labelled available at INRA-Dijon. The total DNA extraction were performed from 10-12g of fresh leaves (lC-1011 and R-10639) and from 0.2-1g of 8 day-old seedlings, following the protocols already described (8). The samples were loaded in 0.8% agarose gels, and electrophoresis was performed at 2V/cm for 1.5 hours. Gels were stained in ethidium bromide and photographied on HP-4 Ilford plan-film under UV light. The reference size markers were purchased from BRL. The DNA was transfered to the Nylon hybond N (Amersham) according to seller' instructions.

Labelling of the DNA and hybridization conditions.

The mitochondrial pLT plasmid was denaturated at 95°C for 10 min and was ^{32}P labelled (specific activity 3 000 Ci/mmol) with the Random Primed DNA labelling kit from Boehringer Mannheim. Nylon membrane was preincubated for two hours at 65°C in hybridization solution (5 x Denhardt, 6 x SSC,) and then incubated in the same solution containing labelled pLT, at 65°C by agitation overnight. The hybridized membrane was washed in 2 x SSC solution at 65°C for 15 minutes and in 0,5 x SSC for 15 minutes and exposed to the XOMAT film with intensifying screen at -70°C .

Results

The hybrid (H.*annuus* ssp.*texanus* x sunflower) showed some variability concerning the fertile / sterile segregation ratio. Table 1 presents a small number of fertile plants in offsprings of the crosses with three maintainer lines. Total sterile progenies were obtained in the next generation (BC2) and further thus leading to the Fundulea-1 CMS (ANT-1).

This ANT-1 cms source was crossed with hundreds of inbreds (Table 2). The results of these crosses reveal the stability of ANT-1 cms source and also that the pollen fertility restorer genes are very scarce in cultivated germplasms. Such restorer genes, as expected, are in the wild species from which this CMS type comes from.

Table 1 : The composition of crosses between sterile hybrid plant (ANT-1 CMS) and three sunflower inbreds. C* restorers for classical CMS

		BC 1 plants	
female partner	male partner	sterile	fertile
H. annuus ssp	WF1706C*	85	3
texanus x sunflower	P1380C*	36	6
(sterile hybrid)	A1566C*	40	15

Table 2 : Results of crosses between ANT-1 CMS source and different genotypes (Fundulea 1986-1989)

Genotypes	Number of crosses	F1 Fertile	F1 Sterile	BC1 Fertile	BC1 Sterile
Inbreds	320	0	12800		
Varieties	21	2	840	0	51
Hybrids	38	1	1520		
Interspecific hybrid	6	0	230		
H. annuus ssp texanus	3	13	46		
Inbred pollen mixture	7	2	140	0	68
wild annual species	5	0	13		

We checked the inheritance of pollen fertility restoration in the case of ANT-1 cms source, (Table 3). The data are in agreement with at least two dominat complementary alleles.

The comparison of ANT-1 CMS, French CMS, *H. petiolaris* fallax CMS, *H giganteus* CMS source *H. annuus* ssp *lenticularis* CMS source, and *H. annuus* 2 and 3 (wild *H. annuus* CMS source) probed with the pLT, displays strong hybridization signals at 1.45kb in *H. giganteus* and in B maintainer while *H. petiolaris fallax* displays signals at 2.3kb. The other cytoplasms do not carry plasmid homologous to pLT, Fig. 1 as the wild species, Fig. 2.

Figure 1

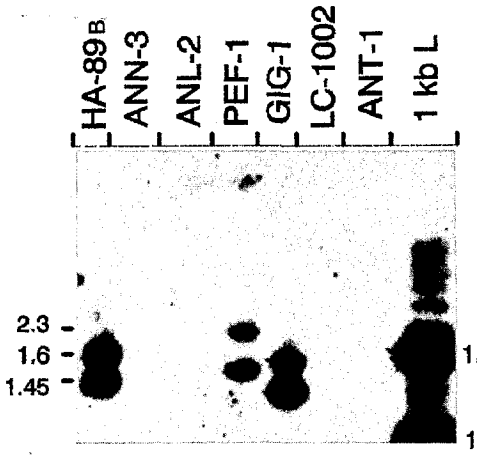

Figure 2

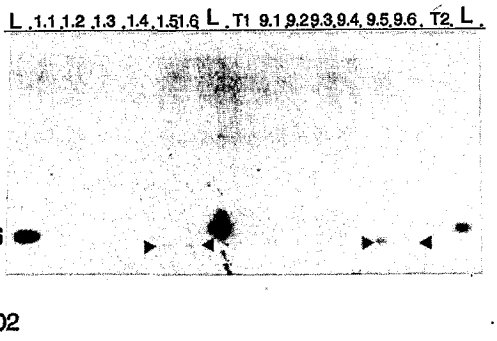

Fig. 1 : Autoradiogram of mitochondrial DNA from maintainer and CMS hybridized by pLT. B : normal cytoplasm from *H. annuus* ; ANN-3 : CMS from *H. annuus annuus* ANL-2 : CMS from *H. annuus lenticularis* ; PEF-1 CMS from *H petiolaris fallax* GIG-1 : from *H. giganteus* ; ANT-1 : from *H. annuus texanus* .

Fig . 2 : Autoradiogram of *Eco* RI restricted total DNA from *H. annuus texanus* and subsequent progenies obtained after 5 backcrosses with the maintainer, hybridized by pLT. 1.1 to 1.4 and 9.1 to 9.4 are sister CMS plants ; 1.5, 1.6, 9.5 and 9.6 are maintainer. T1 and T2 : *H. annuus texanus.* L : 1kb Ladder from BRL.

Table 3 : Plants segregation in case of Rf ANT-1 pollen fertility restoration by baccrosses (Fundulea 1990)

Inbred	Generation	Plant segregation fertile	partially fertile	sterile	Theoretical ratio
LC 1 Rf-ANT-1	BC1	63	2	80	1:1
LC 2 Rf-ANT-1	BC1	95	1	101	1:1
0-7069 RfANt1	BC2	110	0	45	2:1
SA-H55 RfANT1	BC2	38	1	15	2:1

Discussion and conclusion

The transfer of pollen fertility restoration genes in different valuable inbreds is under working, by backross and selection. The segragation ratio of these backcrosses is different than that previously obtained (3)(. In addition, partial fertile and partial sterile plants were registered and they suggest to us a more complex inheritance pollen fertility restoration pattern of ANT-1 cms.

In most cases of cytoplasmic male sterility specific nuclear genes, or sets of genes, can restore a normal fertile phenotype. The interaction of these nuclear genes, called restorer genes, with mitochondrial gene expression is still unknown.

The break sterility phenomenon discribed in case of petiolaris cms is not known in ANT-1 cms, though a lot of inbreds are in advanced generations of backrosses. The percent of 1-3 fertile plants between cms plants is explain by microgenes accumulation after many generations of cms maintenance (12)(). Any way, the ANT-1 CMS type is different than the classical french cms.

The segregation of pollen fertility restoration genes in three or even in four classes (sterile, partiel fertile, partial sterile, fertile) is difficult to be explain after three generation of backrosses. Selection of completely fertile plants is needed, less, till getting of additional informations on cytoplasm nucleus interaction.

Molecular techniques permit direct examination of cytoplasmic genomes, so, cytoplasms can now be differentiated further.

The fact that we cannot detect any hybridization with 1.45kb plasmid in our experiments suggest that there are no sequences homologous with this plasmid. Although many DNA fragments are common to all cytoplasms, each one has characteristic fragments. The mitochondrial plasmid pLT is a good marker to characterize fertile and sterile cytoplasms and is convenient for CMS source differentiation.

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